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Technical, Physical, Chemical and Microbiological Analyses of Siirt Herby Cheese

Murat GÜLMEZ¹✉, Kübranur Yıldız BAYHAN¹, Sefa ÜNER¹

¹Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Siirt University, Siirt/TURKEY

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Gülmez M, Bayhan KY, Üner S. Technical, Physical, Chemical and Microbiological Analyses of Siirt Herby Cheese. Bozok Vet Sci (2022) 3, (2): 33-39.

Abstract: A total of 19 production and sales points were inspected for their technical and hygiene status. Hygienic inadequacy and labeling deficiencies were determined in the relevant workplaces and products. A total of 20 herby cheese samples taken from the sales points were also analysed for their quality control. The mean±standard deviation (%) values of the samples were determined to be 1.9±0.7 for lactic acid, 49.6±4.8 for dry matter, 46.8±9.2 for fat in dry matter, 5.6±1.5 for ash, 2.9±0.7 for salt in dry matter and 65.7±6.1 moisture in cheese fat-free mass. The mean (log₁₀ cfu/g) values of the samples were determined as for total mesophilic aerobic bacteria; 8.7±0.99, *Enterobacteriaceae*; 6.8±2.7, coliform group bacteria; 4.6±2.8, coagulase positive staphylococci; 5.0±1.3 and yeasts- moulds; 8.1±1.3. The cheese has economic importance for the province of Siirt. But, production and sales have been remained at the traditional scale. The contribution of Siirt herby cheese to the economy of the city could be increased by applying standard and hygienic procedures for the cheesemaking.

Keywords: Siirt herby cheese, Economy, Regulations, Hygiene, Quality

Siirt Otlu Peynirinin Teknik, Fiziksel, Kimyasal ve Mikrobiyolojik Analizleri

Özet: Toplam 19 üretim ve satış noktası teknik ve hijyen açısından denetlendi. İlgili işyerlerinde ve ürünlerde hijyenik yetersizlikler ve etiketleme eksiklikleri gözlemlendi. Satış noktalarından alınan toplam 20 adet otlu peynir numunesi de kalite kontrolleri için analiz edildi. Örneklerin ortalama±standart sapma (%) değerleri laktik asit için 1.9±0.7, kuru madde için 49.6±4.8, kuru maddede yağ için 46.8±9.2, kül için 5.6±1.5, kuru maddede tuz için 2.9±0.7 ve peynir yağsız kitlesinde nem için 65.7±6.1 olarak belirlendi. Örneklerin ortalama (log₁₀ cfu/g) değerleri toplam mezofilik aerobik bakteriler için 8.7±0.99, *Enterobacteriaceae* için 6.8±2.7, koliform grubu bakteriler için 4.6±2.8, koagülaz pozitif stafilokoklar için 5.0±1.3 ve mayalar-küfler için 8.1±1.3. Bu peynir Siirt ili için ekonomik öneme sahiptir. Ancak üretim ve satışlar geleneksel ölçekte kalmıştır. Siirt otlu peynirinin şehir ekonomisine katkısı, peynir yapımında standart ve hijyenik prosedürler uygulanarak artırılabilir.

Anahtar Kelimeler: Siirt otlu peyniri, Ekonomi, Mevzuat, Hijyen, Kalite

1.Introduction

Many varieties of cheese are produced in Turkey. White pickled cheese, kashar cheese and tulum cheese are the most produced ones among them. It has been reported that the consumption of herby cheese per capita is 14.74 kg/year and the Turkey average is 3.2 kg/year in the Eastern and Southeastern regions, where herby cheese consumption is intense (1). There are Siirt, Batman, Bitlis, Hakkari, Erzurum and Trabzon herby cheeses in Turkey. Among them, only Van herby cheese has geographical indication certificate (2). The herbs sirmo, mendo, heliz, wild mint, thyme and siabo and 13 other herbs are mostly used for herby cheese production. The cheese should be left in ripening for at least 4 m when raw milk is used in production and 30-60 d when pasteurized milk is used. Siirt herby cheese, which is an indispensable dairy product on the tables of the people of Siirt, is a type of cheese similar to

Van herby cheese which is a semi-hard cheese. It is produced from raw sheep's milk or from a mixture of sheep, cow and goat milk. While the main herb added to cheese is sirmo (*sirik*, *Allium* sp.), heliz (*Ferula orientalis*) and çiriş (*Eremurus spectabilis*) are also used (2). Differences among Siirt herby cheese and other herby cheeses produced in Turkey has not been investigated yet extensively. Nevertheless, also, chemical composition and hygienic quality of the Siirt herby cheeses have not been investigated extensively yet (3). The minimum technical and hygienic criteria for cheeses are set by official documents and official inspections are carried out accordingly (4-8).

Numerous studies have been conducted on Van herby cheese (9-14). However, very little research has been done on Siirt herby cheese. Due diligence on field samples is necessary in terms of providing basic data for scientific studies. In this study, Siirt herby cheese samples taken from

sales points were analyzed to the economic and public health aspect of Siirt herby cheese was evaluated. In addition, observations were conducted at production and sales points. The Turkish Standards Institute (TSE) Herby Cheese Standard (TS 13205) (15), Turkish Patent Institute (TPE) Van Herby Cheese Geographical Indication (No: 405) (2) and Turkish Food Codex (TFC) Cheese Communiqué (No. 2015/6) was benefited for evaluations (4).

2. Materials and Methods

2.1. Status of production and sales points

The findings obtained by observations and interviews with the officials of 14 small production and/or sales points and 5 chain market branches in Siirt city center were evaluated for their compliance with the Turkish Food Codex (4, 5, 7, 8) and related Turkish Standard (6).

2.2. Analysis of Siirt Herby Cheese samples taken from sales points

At least 250 g sample from each of 20 randomly selected sales points in Siirt province, were brought and transferred to the lab within 2 hours. After each sample was mixed thoroughly in a sterile sample bag and turned into a homogeneous sample, it was used in analyzes. Each analyze was repeated 3 consecutive times.

The pH was measured by using a digital pH meter (Milwaukee AZ8686, USA). Titratable acidity (lactic acid, %) was determined by using 0.1 N NaOH and phenolphthalein indicator. The dry matter and ash were determined gravimetrically. The fat content was determined by using Van Gulik method. The salt was determined titrimetrically (15).

For microbiological analysis, reference methods were applied as mentioned in the book of Pouch and Iko (16). Briefly, 10 g of each sample was used for making ten-fold serial dilutions in diluted in 90 ml of sterile physiological saline (PS). For mesophilic aerobic bacteria (MAB), Plate Count Agar (PCA, Oxoid CM 0463) was used and the petri dishes were incubated at 30±2°C for 72 h. For *Enterobacteriaceae*, Violet Red Bile Glucose Agar (VRBGA, Oxoid-CM0485) was used for the enumeration of the coliforms, and procedures were performed as instructed on the medium. For coliforms, Violet Red Bile Lactose Agar (VRBLA, Oxoid CM0107) was used. Petri dishes were incubated for 24 hours at 30 °C, the growing pink-red colonies with a pink precipitation ring around were counted. For coagulase positive staphylococci, Baird Parker Agar plates including Baird Parker Agar Base (BPA, Oxoid CM1127) and Egg Yolk Tellurite Emulsion (Oxoid SR0054) were used. The plates were incubated at 37°C for 48 hours. Black shiny coagulase positive colonies with a diameter of 1.5-2.5 mm

with a transparent zone around were counted. For yeast – moulds, Yeast Extract Glucose Chloramphenicol Agar (YGCA, Merck 1.16000) was used and the petri dishes were incubated at 25°C for 5 d. Colonies formed on the medium were counted.

2.3. Statistical analysis

The mean values of the samples and the standard deviation values between the samples were calculated by using the Microsoft Excel program.

3. Results

3.1. Status of production and sales points

Local herby cheese production and sales points (14 businesses): During the visits, we personally have inspected some inconveniences at the production and sales points. There were no adequate hygienic preconditions from production places. The cheese making staff had no adequate hygiene preconditions during cheese making. Cheese curd and prepared cheese bags were mostly left at room temperature because of unavailability of adequate refrigerators. There were no labels on the prepared cheese bags. We could not demonstrate the sales points as hygienic as required.

Chain Market Branches (5 businesses): One branch of each of 5 different national chain markets operating in the city center of Siirt was visited and the sales department managers were interviewed. It has been observed that the cheeses are the products of local producers, they are kept in plastic bins of 1, 2, 5, 10 and 20 kg, on the cold shelf or in the refrigerator of the market, there are letters and/or numbers on the products with a felt-tip pen, but there is no label. Although there is Van herby cheese in these businesses, its sales are reported to be at most 5% of Siirt herby cheese. In these enterprises, it was observed that the market aisles were cleaner than the above-mentioned production areas and the products were kept under the cold chain. It was understood that these markets bought and sold these products from the herby cheese production points mentioned above, and they did not produce their own. Siirt herby cheese is sold in most of the grocery stores and markets in the city center.

3.2. Analysis of Siirt Herby Cheese samples taken from sales points

The pH, acidity, dry matter, fat, ash and salt levels in the samples are shown in Table 1. The mean values of the analyzes were 5.3±0.5 for pH, 1.9±0.7 % lactic acid, 49.6±4.8 for % dry matter, 46.8±9.2 for % fat in dry matter, 5.6±1.5 for ash and 2.9±0.7 for salt in dry matter.

Table 1: Physical and chemical analysis results of Siirt herby cheese samples taken from sales points in Siirt.

Sample No.	pH	St. deviation	Lactic acids. %	St. deviation	Dry matter. %	St. deviation	Fat in dry matter. %	St. deviation	% Ash	St. deviation	Salt in dry matter. %	St. deviation
1	5.9	0.1	1.9	0.1	54.6	2.8	43.0	4.9	4.9	0.2	2.6	0.0
2	5.6	0.0	1.6	0.2	55.6	2.8	55.8	3.8	7.6	0.1	2.9	0.0
3	5.2	0.0	1.6	0.2	49.0	1.9	63.3	2.2	7.0	0.2	3.4	0.4
4	5.5	0.0	4.1	0.1	54.3	0.2	52.9	3.3	6.2	0.4	2.9	0.5
5	5.2	0.0	1.7	0.1	48.5	3.2	43.9	0.8	6.3	0.0	2.6	0.1
6	5.8	0.0	0.8	0.2	34.6	1.7	63.5	2.2	7.4	0.8	4.5	0.2
7	5.1	0.0	2.3	0.2	49.4	1.6	42.5	3.3	1.2	0.3	1.1	0.1
8	5.1	0.0	2.0	0.3	48.7	3.4	51.9	0.7	4.9	0.2	2.1	0.0
9	4.8	0.0	2.8	0.3	57.9	6.1	51.4	0.7	8.1	0.4	2.8	0.0
10	5.4	0.0	1.7	0.2	47.1	1.3	57.8	0.6	5.7	0.3	2.8	0.2
11	5.0	0.0	1.9	0.2	43.5	0.5	43.1	0.8	3.7	0.5	2.1	0.0
12	4.4	0.0	2.0	0.3	50.6	1.2	47.9	0.7	5.3	0.8	2.7	0.1
13	5.2	0.0	1.8	0.2	47.7	7.7	49.8	0.7	5.1	0.1	3.5	0.1
14	4.6	0.0	2.5	0.2	50.2	1.9	32.9	0.0	6.1	0.6	3.9	0.1
15	5.6	0.0	2.3	0.2	52.3	0.9	36.8	6.7	4.8	0.5	3.6	0.3
16	6.0	0.0	1.3	0.3	48.2	0.2	48.7	4.4	5.5	0.3	3.7	0.1
17	5.3	0.0	2.1	0.2	51.3	3.5	31.2	6.8	5.1	0.1	2.8	0.1
18	4.9	0.0	1.9	0.3	47.7	3.2	38.7	0.0	5.1	0.3	2.4	0.1
19	5.9	0.0	1.2	0.2	51.5	1.9	48.0	3.7	6.3	0.0	3.0	0.1
20	6.4	0.0	1.1	0.2	49.2	1.9	37.1	1.0	5.2	0.0	2.5	0.1
Mean	5.3	0.5	1.9	0.7	49.6	4.8	46.8	9.2	5.6	1.5	2.9	0.7
Least	4.4	0.0	0.8	0.2	34.6	1.7	31.2	6.8	1.2	0.3	1.1	0.1
Most	6.3	0.0	4.1	0.1	57.9	6.1	63.5	2.2	8.1	0.4	4.5	0.2

Microbiological analysis results of each sample are shown in Table 2. The mean numbers (\log_{10} cfu/g) of mesophilic aerobic bacteria, *Enterobacteriaceae*, coliform group bacteria, coagulase positive staphylococci and yeasts- moulds were determined to be 8.7 ± 0.99 , 6.8 ± 2.7 , 4.6 ± 2.8 , 5.0 ± 1.3 and 8.1 ± 1.3 , respectively.

Table 2: Microbiological analysis results of Siirt herby cheese samples taken from sales points in Siirt (\log_{10} cfu/g).

Sample No.	Mesophilic aerobes	St. deviation	<i>Enterobacteriaceae</i>	St. deviation	Coliforms	St. deviation	Coagulase positive staphylococci	St. deviation	Yeasts- moulds	St. deviation
1	7.6	0.0	1.0	0.0	1.0	0.0	3.4	0.0	7.2	0.0
2	8.8	0.1	7.4	0.0	6.8	0.2	5.1	0.0	8.4	0.1
3	8.6	0.2	1.0	0.0	1.0	0.0	3.7	0.1	5.7	0.2
4	7.4	0.1	1.0	0.0	1.0	0.0	3.2	0.1	6.1	0.0
5	9.7	0.1	8.7	0.1	7.9	0.3	5.3	0.2	9.4	0.0
6	8.0	0.1	5.7	0.4	4.8	0.1	5.6	0.2	7.8	0.2
7	8.9	0.0	6.1	0.2	6.7	0.1	5.4	0.2	8.4	0.2
8	10.4	0.2	9.9	0.1	7.3	0.4	5.6	0.2	10.1	0.3
9	8.5	0.0	7.5	0.0	1.0	0.0	4.4	0.0	7.4	0.0
10	8.7	0.1	8.3	0.2	3.6	0.1	3.8	0.0	8.5	0.1
11	9.9	0.0	9.7	0.2	5.5	0.1	3.4	0.1	9.6	0.0
12	9.5	0.0	7.3	0.3	3.4	0.1	4.3	0.1	6.6	0.1
13	7.9	0.0	7.8	0.0	6.2	0.0	5.7	0.3	7.9	0.1
14	8.5	0.0	7.3	0.1	1.0	0.0	3.5	0.1	7.4	0.2
15	8.7	0.0	7.1	1.0	1.0	0.0	5.0	0.1	7.0	0.0
16	7.7	0.0	7.7	0.1	4.7	0.0	7.0	0.0	7.8	0.1
17	9.8	0.1	9.2	0.0	6.9	0.1	6.2	0.1	9.1	0.0
18	9.1	0.1	7.3	0.1	9.0	0.1	7.3	0.0	9.8	0.0
19	8.3	0.1	7.3	0.0	5.1	0.2	5.6	0.1	8.5	0.1
20	7.7	0.0	7.7	0.1	7.5	0.1	7.2	0.1	10.0	0.1
Mean	8.7	0.9	6.8	2.7	4.6	2.8	5.0	1.3	8.1	1.3
Least	7.4	0.1	1.0	0.0	1.0	0.0	3.2	0.1	5.7	0.2
Most	10.4	0.2	9.7	0.2	9.0	0.1	7.3	0.0	10.1	0.1

4. Discussion and Conclusion

It has been reported that raw milk and raw milk cheeses are responsible for 96% of food poisonings caused by consumption of dairy products in the USA (17). Çetin and Durlu Özkaya (18) have demonstrated that cheese is playing an important role in food poisoning cases, and health risks of some dairy products in Turkey between the years 2014 and 2018.

Minimum requirements for production, packaging, labeling and sales points for cheese are stated in the Turkish Food Codex Cheese Communiqué (4). In the codex (4), packaging (Article 13/1), labelling (Article 14/1), product names (Article 4) or local/national names (Article 14/1a) have been regulated as standards for retail cheese. Cheeses produced from raw or thermized milk are ripened under cold storage for at least 4 months (Article 5/c). The ripening time period of the cheeses is stated as “has been ripened at least ...d or mo” on their labels. Our inspection results have demonstrated that the regulations mentioned above have not fully followed as minimum requirements for production and sales process by the relatives.

In many studies, it is concluded that herby cheeses sold in Turkey are unhealthy, poor quality, risky for public health (19-23). Kurt and Akyüz (24) reported in 1984 that primitive conditions should be abandoned and production should be carried out in modern factories. Likewise, the results of this study and formerly made studies demonstrate that the total amount of Siirt herb cheese produced under primitive conditions, and thus the hygienic quality and chemical parameters are variable as seen in Tables 3 and 4.

According to the Codex on the Supply of Raw Milk (8), only the supply of raw milk produced in a milk producing livestock enterprise with permission from the competent authority is allowed (Article 5/1). Farms must have a disease-free certificate (Article 5/2). The Siirt herby cheese is made from raw sheep's and/or goat's milk. We don't have information about the control and certification of herds and raw milk used for Siirt herby cheese production. Also, we could not find any production time or ripening time period for the cheese at the retail.

In the Herby Cheese Standard (6), it is stated that the amount of milk fat in dry matter should be at least 45%, humidity at most 60% and salt amount at most 7.5%. The minimum, (average) and maximum values (%) for the cheese are listed in the Geographical Indication Certificate as such: dry matter (%) 46.78 (43.81) 47.78, protein (%) 22.17 (20.60) 25.52, fat (%) 17.29 (16 .75) 19.21, ash (%) 6.85 (5.07) 7.45, salt (%) 5.73 (4.60) 6.9. When it is classified in terms of % Moisture in Cheese Fat-Free Mass in cheese standards, herby cheese has been reported to be from the semi-hard cheese group, which falls between 57-64% values (6, 20). The % of Moisture in Cheese Fat-Free

Mass of cheese samples analyzed was not less than 57%. The mean percentage was determined as $65.7\pm 6.1\%$ (data not given elsewhere).

According to the herby cheese standard (6), herby cheeses should contain at least 45% milk fat in dry matter, that is, it should be in the full-fat cheese category. It was observed that the average findings of the samples were compatible with the value specified in the Standard as shown in Table 1, and 15 samples were in the full-fat cheese category. However, 6 samples from the brined herby cheese samples and seven samples from the dry salted samples did not comply with the Standard as shown in Table 3. It has also been revealed in other studies that herby cheeses sold in the market have not been standardized in terms of fat contents. The results of this and formerly made studies were comparatively given in Table 3. The non-standardization of the production process and the variables from milk used in the production during traditional production has been shown to cause this situation (25). We also demonstrated in this study non-standardized fat contents from the cheese samples.

The findings of previously made studies and this study were evaluated in the light of the Turkish Herby Cheese Standard (6). As shown in Table 3, the most of the samples analyzed was out of the Standard values. Only dry matter and results obtained from this study were in compliance with the Herby Cheese Standard (6). Nevertheless, dry matter rates of the two (10%) samples dry matter were minimum 45%), and did not comply with the Standard. Dry matter values found in these two samples are also below the value (at least 43.81%) reported in the Van herby cheese geographical indication certificate (2). Although the production methods of pressed herby cheese are different, their chemical compositions are similar to Erzincan tulum cheese. The humidity rate for Erzincan Tulum cheese has been reported to be 45% at most (50% in low-fat and fat-free tulum cheeses) in the Standard (20). However, this value was reported to be higher (at most 60%) in herby cheese. This value is similar to the value of white cheese. In addition, the minimum 45% dry matter and maximum 60% moisture values in the herb cheese standard do not complement each other. As shown in Table 3 and Table 4, many of the samples analyzed in previously made studies and in this study have not been in compliance with the standard regulations.

Salt values have been reported as maximum 7.5% in Herby Cheese standard (6), and maximum 6.9% in Van Herby Cheese Geographical Indication Certificate (2). However, we suggest that the maximum value allowed in other tulum cheeses, which is 6%, is also in non-brined herby cheeses, which we prefer to express as herby Tulum cheese in this study. In the Cheese Communiqué (4), a maximum of 7.5% salt rate in dry matter is allowed in brined cheeses. As shown in Table 3, the values given belong to herby tulum

cheeses and there are samples containing 2-3 times more salt than allowed. In order to produce herby cheese with salt content in accordance with the standards, first of all, its technology must be developed and then its production must be carried out in licensed enterprises that have a technological competence certificate. Officially inspected licensed production facilities are needed

In the Turkish Food Codex Microbiological Criteria Regulation (9), it is stated that, as a hygiene criterion, the number of coagulase-positive staphylococci in cheese should not exceed the 104 cfu/g. The values of other microorganisms analyzed (total mesophilic aerobic bacteria, In a study, it was reported that the staphylococcal numbers were within standard values (29). When examined in terms of coagulase-positive staphylococci, which is the only value included as a hygiene criterion in the legal document, it was seen that the herby cheeses on sale did not comply with the standard as shown in Table 4.

Herby cheeses made after pasteurization of different milks were vacuum packed and their chemical changes have been investigated for 90 d (30). No significant changes have been observed in the average values of 50% for dry matter and 28% for fat. Although it is not possible to compare the findings of our study with the study, the findings of the study may be useful for industrial production models to be developed using pasteurized milk. High numbers of coliform bacteria and yeast-mold numbers were found to be high in our study and in same other studies as shown in Table 4. Herby cheese produced from raw milk poses a great risk in

coliforms and yeast-mold) were used only in comparison with the findings of other researchers as shown in Table 4. Since these values were not included in the communiqué, they were not evaluated according to the Communiqué (4). Thirteen (60%) of the 20 samples examined in our study contained coagulase positive staphylococci more than 4 log₁₀ cfu/g. As shown in Table 4, staphylococcal analysis was not performed in 5 of the 9 studies in which microbiological analysis was performed (19, 24, 26, 27). In the other 3 studies in which staphylococcal analysis was performed, it was reported that there was contamination above the values reported in the Communiqué (21, 22, 28).

terms of pathogenic microorganisms, and although the antimicrobial activities of herbs are known, they carry the same risk, and pasteurization of milk and herbs is necessary for hygiene reasons (31-34). Although pathogenic bacteria analysis was not performed in our study, it can be predicted that the number of bacteria accepted as hygiene criteria is quite high, and pathogenic bacteria may also be present in the samples we examined.

When Tables 3 and 4, in which the findings of this study and previous studies are presented comparatively, are examined, it is understood that the sale of these cheeses should be controlled because they do not comply with the minimum chemical and microbiological criteria. It has been reported that pickled herbs are also unhygienic and carry health risks (32). Antibiotic residues have also been found in herby cheeses(21).

Table 3: Comparison of the results of physical and chemical analysis of Siirt herb cheese samples taken from sales points (as given in the Table 1) with the results of previous researches.

Reference No.	pH	Lactic acids. %	Dry matter %	Fat in dry matter %	Ash. %	Salt in dry matter %
4	4.2-5.8	NA*	NA	NA	NA	NA
8	4.22-5.19	NA	45.24-57.38	35.33-59.37	NA	4.3-17.7
21	5.02-6.83	NA	NA	NA	NA	NA
22	5.09-5.93	NA	51.05-62.57	NA	NA	NA
23	4.33-4.90	0.87-1.36	50.10-56.69	18.93-21.09	5.21-5.77	6.08-7.28
24	4.32-5.8	0.24-1.45	37.32-58.24	NA	NA	3.62-7.3*
25	NA	0.18-1.75	29.1-61.57	22.74-56.28	NA	2.7-18.64
26	NA	NA	50.81-58.43	37.87-51.56	4.03-7.92	6.44-21.76
27	NA	0.31-1	42.52-52.31	28.12-51.06	3.28-15.21	2-13.81
29		0.11-0.72	36.26-46.8	26.8-48.76	NA	2.78-16.31
31	4.78-5.32	1.37-1.47	44-46	19.5-21.75	5.08-5.3	4.3-4.5
33	4.28-5.54	1.93-2.42	51.2-60.59	21.25-28.75*	5.32-12.51	4.24-14.4*
34	5.45	NA	44.37	NA	8.98	7.58
A	4.4-6.3	0.8-4.1	34.6-57.9	31.2-63.5	1.2-8.1	1.1-4.5
A*	5.3	1.9	49.6	46.8	5.6	2.9
B	NA	NA	En az 45	NA	NA	7.5
C	NA	NA	43.81-47.78	16.78-19.21	5.07-7.45	4.6-6.9
C*	NA	NA	46.78	17.29	6.85	5.73

NA: Not analyzed. A: The values are taken from Table 1. A*: The mean values are taken from Table 1. B: The values are taken from the Turkish Standards - Herby Cheese Standard (15). C: The values are taken from Van Herby Cheese Geographical Indication Certificate (2). C*: The mean values are taken from Van Herby Cheese Geographical Indication Certificate (2).

Table 4: Comparison of the results (log₁₀ cfu/g) of microbiological analysis of Siirt herb cheese samples taken from sales points (as given in the Table 2) with the results of previous researches.

Reference No.	Mesophilic aerobes	Enterobacteriaceae	Coliforms	Coagulase positive staphylococci	Yasts - moulds
4	3.5-8.1	<1-6	NA*	NA	1.7-7.5
8	4-8.2	<2-4.49	NA	NA	NA
11	NA	NA	0-3	2-6	1-6
22	6.47	NA	NA	NA	4.85
23	7.04-10.49	3.03	<1-4.68	NA	3.03-5.71
24	5.6-9.31	NA	<1-5.89	1-8.71	2.6-8
25	4.3-9.6	1.3-6.86	1-6.2	1-1.64	2.04-6.68
27	8-10	NA	3-5	NA	5-7
28	6.14-8.55	NA	2.32-5.86	2.43-4.29	2.2-6.86
A	7.4-10.4	1-9.7	1-9	3.2-7.3	5.7-10.1
A*	8.7	6.8	4.6	5.0	8.1

NA: Not analyzed. A: The values are taken from Table 1. A*: The mean values are taken from Table 1.

In conclusion, the production and trade of Siirt herby cheese should be produced and sold in more hygienic places by expert staff. When this progress is achieved, it is certain that the contribution of this cheese to the provincial economy will increase even more. Considering the goat presence and transhumance in Siirt province, the production process should be determined by scientific studies in order to produce Siirt herby cheese from a mixture of sheep and goat milk. Modern Siirt herby cheese production facilities should be established in the province. The brand value of the product should be created and this value should be established as a wide market network and used as a tourism-developing product. For this, the industry-appropriate technology of this cheese should be developed and the possibilities of using pasteurized milk and starter culture in production should be provided.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Hayaloglu AA, Fox PF. Cheeses of Turkey: 3 varieties containing herbs or spices Dairy Sci and Technol 2008; 88: 245—325.

- Anon. Van otlu peyniri menşe tescili. Van Sanayi ve Ticaret Odası, Van (article in Turkish with an English abstract).
- Doğan N. Siirt ilinde üretilen "Siirt otlu peynirinin" bazı özelliklerinin belirlenmesi. Yüksek Lisans Tezi, Harran Üniversitesi Fen Bilimleri Enstitüsü, Şanlıurfa 2012 (thesis in Turkish with an English abstract).
- Türk Gıda Kodeksi-Peynir tebliği (Tebliğ No: 2015/6). Resmî Gazete Tarih/Sayı: 08.02.2015/29261 Gıda Tarım ve Hayvancılık Bakanlığı, Ankara.
- Türk Gıda Kodeksi-Mikrobiyolojik kriterler yönetmeliği (3 mükerrer). Resmî Gazete Tarih/Sayı: 29.12.2011/28157.Gıda Tarım ve Hayvancılık Bakanlığı, Ankara.
- Türk Standartları- Otlu peynir standardı (Standart No 13205). Türk Standartları Enst, Ankara (article in Turkish).
- Türk Gıda Kodeksi- Hayvansal gıdaların resmi kontrollerine ilişkin özel kuralları belirleyen yönetmelik. Resmî Gazete Tarih/Sayı: 17.12.2011/28145. Gıda Tarım ve Hayvancılık Bakanlığı, Ankara.
- Türk Gıda Kodeksi - Çiğ sütün arzına dair tebliğ (Tebliğ No: 2017/20). Resmî Gazete Tarihi/Sayı: 27.04.2017/30050. Gıda Tarım ve Hayvancılık Bakanlığı, Ankara (article in Turkish).
- Şenel E, Yildiz F, Yetişemiyen A, Durlu-Özkaya F, Öztekin FŞ, Şanlı E. Evaluation of the biogenic amine content and some chemical and microbiological properties of Urfa and Van herby cheeses. Kafkas Univ Vet Fak Derg 2012; 18 (4): 537-544. doi: 109775/kvfd20115475.
- Özbek H, Aksoy H, Uğraş S, Öztürk G, Türkdogan K, Tuncer İ. Van otlu peynirinin sıçan sindirim sistemi ve bazı kan parametreleri üzerine etkisi. Genel Tıp Derg 2015; 15 (1): 5-10 (article in Turkish with an English abstract).
- Ocak E, Tunçtürk Y, Javidipour I, Köse Ş. Farklı süt türlerinden üretilen Van otlu peynirlerinde olgunlaşma boyunca meydana gelen değişiklikler: Mikrobiyolojik değişiklikler, lipoliz ve serbest yağ asitleri. Yüzüncü Yıl Ü Tarım Bil Derg 2015; 25 (2): 164-173 (article in Turkish with an English abstract).
- Ekici K, Okut H, İşleyici Ö, Sancak YC, Tuncay RM. The determination of some microbiological and chemical features in

- herby cheese. *Foods* 2019; 11; 8(1): 23. doi: 10.3390/foods8010023.
13. Andic S, Gençelep H, Kose S. Determination of biogenic amines in herby cheese. *Int J Food Properties* 2010; 13 (6): 1300-1314.
 14. Alemdar S, Ağaoğlu S, Şahin S. Otlu Peynir. Atasever M. (Editor) *Süt ve süt ürünleri*. 1 Baskı, Ankara: Türkiye Klinikleri, 2019; p. 186-194 (article in Turkish).
 15. Sadler GD, Murphy PA. pH and titratable acidity. in: *food analysis*. Springer New York, 1984; p. 207-225.
 16. Pouch DF, Ito K. *Compendium of methods for the microbiological examination of foods*. American Health Association Washington DC, 2001.
 17. Costard S, Espejo L, Groenendaal H, Zagmutt FJ. Outbreak-related disease burden associated with consumption of unpasteurized cow's milk and cheese United States 2009–2014. *EID* 2017; 23 (6): 957-964. doi:103201/eid2306151603.
 18. Çetin M, Durlu-Özlaya F. Medyada gıda zehirlenmeleri. *Türk Hij Den Biyol Derg* 2019; 76 (3): 285-296. doi: 105505/TurkHijyen201983604 (article in Turkish with an English abstract).
 19. Akkoç Z. Otlu peynirlerde histamin düzeyi ve mikrobiyolojik kalitenin araştırılması. Yüksek Lisans Tezi, Fırat Üniversitesi Sağlık Bilimleri Enstitüsü, Elazığ 2016; (thesis in Turkish with an English abstract).
 20. Ocak E, Köse Ş. Van Otlu peynirinin üretimi ve mineral madde içeriği. *Gıda* 2015; 40 (6): 343-348 (article in Turkish with an English abstract).
 21. Özer E, Bostan K, Sağun E. Van otlu peynirlerinde mikrobiyolojik kalite ve küf florası Yüzüncü Yıl Ü Vet Fak Derg 1992; 3 (1-2): 53-59 (article in Turkish with an English abstract).
 22. Hallaç B. Bitlis otlu peynirlerinin bazı önemli fizikokimyasal ve biyokimyasal özelliklerinin gıda güvenliği yönünden incelenmesi. *EJONS International J Math Eng Natural Sci* 2021; 5 (17): 139-147. DOI: 1038063/ejons386 (article in Turkish with an English abstract).
 23. İşleyici Ö, Akyüz N. Van ilinde satışı sunulan otlu peynirlerde mikrofloranın ve laktik asit bakterilerinin belirlenmesi. *Yüzüncü Yıl Üniv Vet Fak Derg* 2009; 20 (2): 59-64 (article in Turkish with an English abstract).
 24. Tunçtürk Y, Ocak E, Köse Ş. Farklı süt türlerinden üretilen van otlu peynirlerinin fiziksel ve kimyasal özellikleri ile proteoliz profillerinde olgunlaşma sürecinde meydana gelen değişimler. *Gıda* 2014; 39 (3): 163-170 (article in Turkish with an English abstract).
 25. Kurt A, Akyüz N. Van Otlu peynirinin yapılışı ve mikrobiyolojik fiziksel ve kimyasal nitelikleri. *Gıda* 1964; 9 (3): 141-146 (article in Turkish).
 26. Oğur S, Duruk M. Bitlis otlu peynirinin besin kompozisyonunun ve kimyasal bileşiminin standartlara göre değerlendirilmesi. *Food and Health* 2021; 7 (2): 91-102 doi: 103153/FH21011 (article in Turkish with an English abstract).
 27. Hallaç B, Güçer Y, Kılınççeker O, Poyrazoğlu ES. Geleneksel siirt peynirlerinin mikrobiyolojik kimyasal ve fiziksel özelliklerinin belirlenerek halk sağlığı açısından değerlendirilmesi. *ADYÜTAYAM* 2021; 9 (1): 61-72 (article in Turkish with an English abstract).
 28. Ektiren D, Güneş S, Vardın H. Siirt ve çevresinde üretilen otlu peynirlerin fizikokimyasal mikrobiyolojik ve duyuşal özelliklerinin belirlenmesi. *Harran Üniv Müh Derg* 2020; 5 (3): 260-267 (article in Turkish with an English abstract).
 29. Coşkun H, Öztürk B. Otlu peynirler adı altında üretilen peynirler üzerinde bir araştırma. *Gıda Müh Derg* 2001; 10: 19-23 (article in Turkish with an English abstract).
 30. Tekinşen KK. Hakkâri ve çevresinde üretilen otlu peynirlerin mikrobiyolojik ve kimyasal kalitesi. *Vet Bil Derg* 2004; 20 (2): 79-85 (article in Turkish with an English abstract).
 31. Emirmustafaoglu A, Coşkun H. Keçi sütü inek sütü ve bu sütlerin karışımından yapılan otlu peynirlerde olgunlaşma boyunca meydana gelen değişimler. *Gıda* 2012; 37(4): 211-218 (article in Turkish with an English abstract).
 32. Yaman H, Yaşlı B, Çakır İ, Tunçtürk Y, Coşkun H. Otlu peynir üretiminde kullanılan salamura otların bazı kimyasal ve mikrobiyolojik özellikleri. *Akademik Gıda* 2012; 10 (1): 53-56 (article in Turkish with an English abstract).
 33. Kara S, Köse Ş. Geleneksel yöntemle üretilen otlu peynirlerin bazı kalite özelliklerinin ve biyoaktivitesinin belirlenmesi. *Gıda* 2020; 45 (5): 942-953 (article in Turkish with an English abstract).
 34. Sağun E, Tarakçı S, Sancak H, Durmaz H. Salamura otlu peynirde olgunlaşma süresince mineral madde değişimi. *Yüzüncü Yıl Ü Vet Fak Derg* 2005; 16 (5): 21-25 (article in Turkish with an English abstract).



Distribution and Identification of the Parasitic Nematode *Contraecum spp.* in Planiliza Abu (Heckel, 1843: Mugiliformes, Mugilidae) From Razzaza Lake, Karbala Province, Iraq

Marwa JAWAD¹, Zahraa Mohammed RIDHA², Firas ALALI³, Saeed EL-ASHRAM⁴, Asaad Sh. M. ALHESNAWI⁵

¹University of Kerbala, College of Sciences, Department of Biology, Karbala/IRAQ

²Ministry of Agriculture, Karbala Veterinary Hospital, Veterinary Department, Karbala/IRAQ

³University of Kerbala, College of Veterinary Medicine, Department of Veterinary Microbiology and Parasitology, Karbala/IRAQ

⁴Kafrelsheikh University, Faculty of Science, Kafr El-Sheikh/EGYPT

⁵University of Kerbala, College of Applied Medical Sciences, Karbala/IRAQ

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Abstract: The Anisakidae family's most important genus, *Contraecum*, contains various host species participating in their life cycles and considerably negatively influencing humans' health. *Planiliza abu* specimens from the local market in Karbala, Iraq, were examined for *Contraecum spp.* in the abdominal cavity. A survey on parasites of *Planiliza abu* of Razzaza Lake was carried out over three years, from November 2019 to December 2021. A total of 148 fish were collected and tested for infection in 2019, 277 in 2020, and 577 in 2021. The prevalence in November and December 2019 was 48.73%, 65.08% in 2020, and 9.6% in 2021, respectively. Microscopically, it revealed larval type *Contraecum L3* (only in all fish). Baghdad University's Iraq Natural History Research Center and Museum confirmed the parasite as *Contraecum spp.* infection rates were greatest in January and February and lowest in July and August 2021. Regarding third-stage larvae, female fish (n=25) had a higher infection rate than male fish (n=8), with 40 and 18, respectively. This study showed that industrial pollution, human activity, and seasonal patterns all influenced infection rates. The molecular diagnosis of *Contraecum spp.* in different hosts is very important for studying their population ecology and biology, and this should be looked into in future studies

Keywords: *Contraecum spp.*, Light microscopic examination, *Planiliza abu*, Risk factors

Irak, Kerbela Eyaleti Razzaza Gölü'ndeki Planiliza Abu'da (Heckel, 1843: Mugiliformes, Mugilidae) Parazitik Nematod *Contraecum spp.*'nin Dağılımı ve Tanımlanması

Özet: Anisakidae familyasının en önemli cinsi olan *Contraecum*, yaşam döngülerine katılan ve insan sağlığını önemli ölçüde olumsuz etkileyen çeşitli konukçu türleri içerir. Irak Kerbala'daki yerel pazardan alınan *Planiliza abu* örnekleri *Contraecum spp.* karın boşluğunda. Razzaza Gölü'ndeki *Planiliza abu* parazitleri üzerine Kasım 2019'dan Aralık 2021'e kadar üç yıl boyunca bir anket yapıldı. 2019'da 148, 2020'de 277 ve 2021'de 577 balık toplandı ve enfeksiyon için test edildi. Prevalans sırasıyla, Kasım ve Aralık 2019'da %48.73, 2020'de, %65.08 ve 2021'de %9.6 idi. Mikroskopik olarak, larva tipi *Contraecum L3*'ü ortaya çıkardı (sadece tüm balıklarda). Bağdat Üniversitesi Irak Doğa Tarihi Araştırma Merkezi ve Müzesi, parazitinin *Contraecum spp.* olduğunu doğruladı. Enfeksiyon oranları Ocak ve Şubat aylarında en yüksek, Temmuz ve Ağustos 2021'de en düşüktü. Üçüncü aşama larvalarla ilgili olarak, dişi balıklarda (n=25), erkek balıklardan (n=8) (sırasıyla 40 ve 18) daha yüksek bir enfeksiyon oranına sahipti. Bu çalışma endüstriyel kirlilik, insan faaliyetleri ve mevsimsel kalıpların hepsinin enfeksiyon oranlarını etkilediğini gösterdi. *Contraecum spp.*'nin moleküler teşhisi farklı konaklarda popülasyon ekolojilerini ve biyolojilerini incelemek için çok önemlidir ve gelecekteki çalışmalarda incelenmelidir.

Ahtar Kelimeler: *Contraecum spp.*, Işık mikroskopik inceleme, *Planiliza abu*, Risk faktörleri

1.Introduction

Contraecum spp. is a parasitic nematode of the Anisakidae family. They have been shown to have extremely pathogenic effects on animals (fish, birds, and marine mammals) and humans(1). Anisakiasis is a parasitic infection caused by nematode larvae from the genera *Anisakis*, *Pseudoterranova*, and *Contraecum* of the Anisakidae family (2). For the first time, *Contraecum spp.* larvae were found in ten different fish species from various locations in Iraq(3). Adult worms of such larvae have been found in several aquatic birds in

Iraq (4). *Anisakis* species have a complicated and heteroxenous life cycle (the ultimate definitive hosts of these nematodes are marine mammals with a complex life cycle), which includes a variety of marine creatures at various levels of the trophic web in the marine environment. They are parasites that live in the larval stages of many fish species across the globe, some of which are commercially important (5). Anisakidosis has largely been documented in areas where uncooked fish consumption is common. The third-stage larvae of anisakid worms are primarily devoured

when fish feed on intermediate crustacean hosts or teleostean transit hosts (6). These nematode parasites exploit various crustacean and fish species as intermediate or paratenic hosts, with humans serving as accidental hosts (7). The parasitic Anisakidae family comprises eight unique parasite genera and 46 distinct parasite species. The Anisakidae parasites mostly infect marine animals, although they may also infect paratenic hosts such as molluscs, small crustaceans, and fish (8). There are around 100 species in this genus. *Contracaecum spp.* is comprised of 142 species (9,10). Anisakidosis is a unique disease that affects humans and is caused by members of the Anisakidae family. Since the discovery of these parasites in humans in the early 1960s, anisakid nematodes have spread globally and infected many invertebrates and vertebrates throughout their life cycles (11). The presence of anisakid larvae in fish endangers people and discourages consumers from purchasing infected products (12). Fish-borne parasitic zoonoses are a significant component of food-borne parasitic zoonoses. In several Asian nations, the number of fish-borne nematodiasis seems to be increasing (13). No studies have been conducted to determine the prevalence of *Contracaecum* larvae in *Planiliza abu* collected from different locations in Karbala and across Iraq. Given the above facts, this research aimed to determine the general prevalence and key risk factors related to the occurrence of *Contracaecum spp.* collected from the viscera of *Planiliza abu* in the local market of Karbala, Iraq.

2. Materials and Methods

2.1. Location

Karbala, also known as Kerbala, is the capital of the Karbala Governorate in central Iraq, about 100 kilometers southwest of Baghdad, and has an estimated population of 1 million pilgrims that come each year. Milh Lake, also known as Razzaza Lake, can be found in western Iraq, just west of Karbala (32°41'N, 43°40'E). It is Iraq's second-biggest freshwater lake and was previously a major source of fish. The lake, which covers an area of 1810 square kilometers and is 40 meters above sea level, can store 26 billion cubic meters of water. Some of the water from Habbaniyah Lake is sent into Razzaza Lake through a controlled exit route or canal from the Euphrates River (Fig. 6).



Figure 6: Map of Iraq, Karbala province and Razzaza Lake location.

2.2. Fish collection and inspection

The specimens were obtained fresh from Karbala's local market. Count, measure, and weigh all the fish. Following traditional dissection, each specimen was examined for anisakid larvae. Each specimen's viscera and flesh were investigated individually in two different Petri dishes. After a visual examination, the viscera and flesh were dissected under a stereoscopic microscope, and the worms were counted for each sample.

2.3. Morphological identification

Using a light microscope, examine the morphology of isolated larvae. The larvae of each fish species were rinsed in saline solution for 30 min, briefly fixed in alcohol, and then inspected under a light microscope. All the larvae were morphologically evaluated. Nematode larvae were collected and cleaned in physiological saline from fish body cavities, and lactophenol was used to clear the nematodes for morphological evaluation. In all fish, light microscopic inspection showed a larval type compared with the *Contracaecum* type (Figs. 1-5). The fish parasite was confirmed as *Contracaecum spp.* by the Iraq Natural History Research Center and Museum at Baghdad University.

2.4. Morphological characteristics of *Contracaecum*

As the genus name implies, these worms have two oppositely oriented ceca as part of their digestive system (Fig. 1). They also have an excretory orifice at the front of their bodies. Because they are the most consistent at all developmental stages, they should be regarded as the most relevant morphological traits for distinguishing *Contracaecum* species from the rest of the anisakid nematodes (1).

Table 1: Size range, number of infected and uninfected fish, and infection rate in the *Planiliza abu* sampled in 2021 in Karbala, Iraq.

		Fish length (range cm)	Total no. of fish	No. of infected fish	Infection rate	No. of nematodes in fish
2019	November	10-18	71	18	25.35	64
	December	5-18	77	18	23.38	95
2020	January	8-15	40	13	32.50	115
	February	9-15	68	15	22.06	92
	March	*	*	*	*	*
	April	*	*	*	*	*
	May	*	*	*	*	*
	June	*	*	*	*	*
	July	*	*	*	*	*
	August	*	*	*	*	*
	September	8-15	49	14	28.57	46
	October	10-13	40	4	10.00	5
	November	10-15	45	10	22.22	31
	December	12-19	35	15	42.86	37

Table 2: Size range, number of infected and uninfected fish, and infection rate in the *Planiliza abu* sampled in 2019 and 2020 in Karbala, Iraq.

	Fish length (range cm)	No of non-infected fish	No. of infected fish	Infection rate	Fish sex			
					Male	%	Female	%
January	6.5-11	43	6	12.2	11	22.4	38	77.6
February	8-12	41	5	10.9	9	19.6	37	80.4
March	7.5-11	41	3	6.8	8	18.2	36	81.8
April	6.5-10	59	4	6.3	16	25.4	47	74.6
May	5.8-10	39	3	7.1	7	16.7	35	83.3
June	7.5-10.5	45	2	4.3	4	8.5	43	91.5
2021 July	8-11	38	0	0	4	10.5	34	89.5
August	7.5-11	42	0	0	2	4.8	40	95.2
September	8-11	45	3	6.3	11	22.9	37	77.1
October	8-11	50	2	3.8	10	19.2	42	80.8
November	7.5-11	55	2	3.5	10	17.5	47	82.5
December	7.5-11	46	3	6.1	5	10.2	44	89.8
Total	6.5-12	577	33					

3. Results

In Planiliza abu, only *Contracaecum* spp. third-stage larvae (L3) were discovered, and there were no *Contracaecum* spp. fourth-stage larvae (L4) found. The bodies of the third-stage larvae were rather substantial. The cuticle becomes annular and forms a collar at the front end, and an excretory orifice opens at the anterior end. The esophagus ends in a short, sub-globular ventriculus. The intestinal cecum is much shorter than the ventricular appendix; the intestine is large and fills the rest of the body. The tail is highly pointed and has a spine. January and February had the highest infection rates, while July and August had the lowest. Female (n=25) fish had a greater infection rate than male (n=8) fish, with 40 and 18 third-stage larvae collected, respectively (Table 2).

A survey on nematode parasites of Planiliza abu of Razzaza Lake was completed for three years, from November 2019 to December 2021. Because of the COVID-19 pandemic, no data was obtained between March and August 2020. A total of 148 fish were collected and tested for nematode infection in 2019, 277 in 2020, and 577 in 2021. The infection rate in November and December 2019 was 48.73%, 65.08% in 2020, and 9.6% in 2021, respectively (Tables 1 and 2).

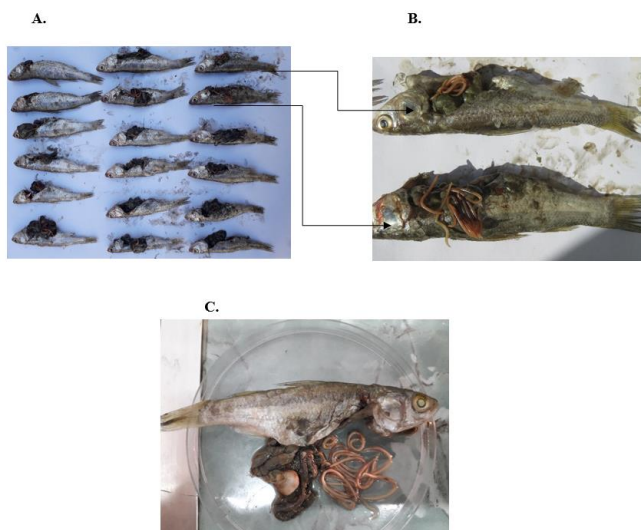


Figure 1: A, B, C. Counting the third-stage larvae of *Contracaecum* spp. in the viscera of dissected Planiliza abu.

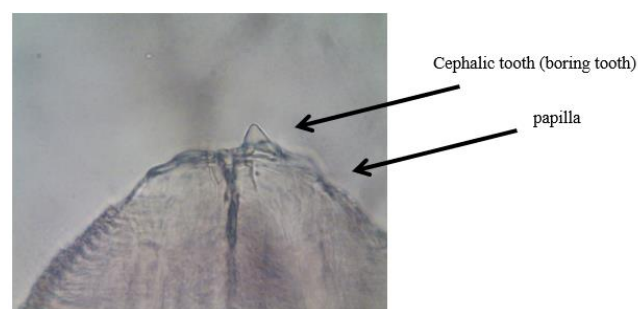


Figure 2: The anterior part of a third-stage larva of *Contracaecum* spp. provided with a ventral cephalic tooth (arrow (Scale bar= 0.10 mm).

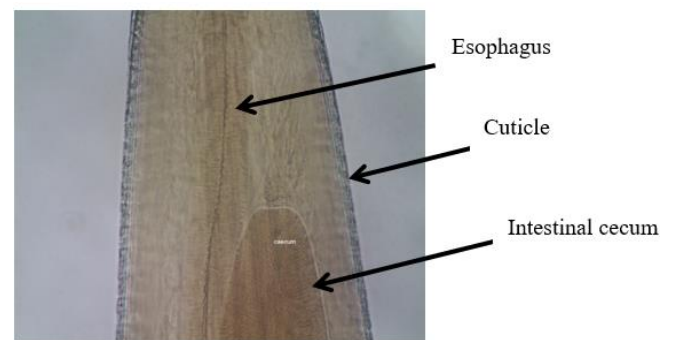


Figure 3: The anterior region showing the esophagus (OE), intestinal cecum (IC), and cuticle (C) (Scale bar = 0.10 mm).

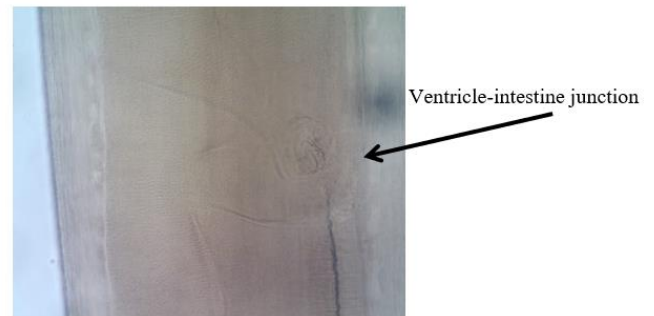


Figure 4: The middle region showing the ventricle-intestine junction (Scale bar = 0.10 mm).

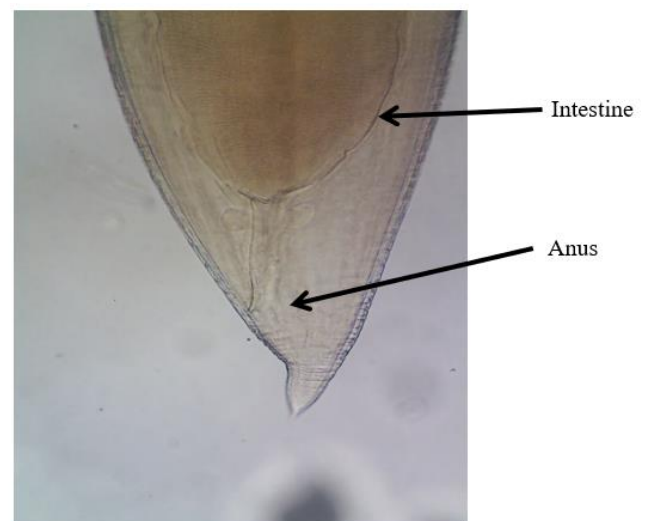


Figure 5: The posterior part of a third-stage larva of *Contracaecum* spp. showing conical tail, intestine, and anus (arrow (Scale-ar= 0.10 mm).

4. Discussion and Conclusion

Contracaecum, the most important nematode genus in the Anisakidae family, has a wide range of host species, a huge number of species, and a significant detrimental impact on human health. *Contracaecum* species in the abdominal cavity of Planiliza abu specimens from a local market in Karbala, Iraq, was studied. Over three years, from November 2019 to December 2021, researchers studied the nematodes that parasitize the Planiliza abu of Razzaza Lake.

A three-year survey on nematode parasites of *Planiliza abu* of Razzaza Lake was conducted from November 2019 to December 2021. In 2019, 148 fish were gathered and tested for nematode infection; in 2020, 277 fish were collected and tested; and in 2021, 577 fish were collected and tested for nematode infection. According to light microscopy, all the fish had the larval type of *Contracaecum*. The parasite was confirmed as *Contracaecum* spp. by the Iraq Natural History Research Center and Museum at Baghdad University. *Planiliza abu* contained only *Contracaecum* spp. L3 larvae. Infection rates were the highest in January and February 2021 and the lowest in July and August. Female fish (n=25) showed a greater infection rate than male fish (n=8), with 40 and 18, respectively, in third-stage larvae. The prevalence of all species was higher in Atlantic fish than in Mediterranean fish. The parasite was more abundant in the viscera than in the flesh, although in the most parasitized species, the parasite was more prevalent in the flesh (14). In the present investigation, the parasitic nematodes were found in the viscera rather than in the flesh. The infections were found in the Tigris, Euphrates, and Shatt Al-Arab rivers, some of their tributaries, lakes, marshes, drainage networks, and many fish ponds and floating cages across Iraq (15). Razzaza Lake is a suitable home for many aquatic birds (both resident wading and migratory) since it provides ample food and warm water. Aquatic vegetation in the area offers an ideal habitat for such birds. Aquatic birds are susceptible to various parasite diseases, which may cause significant mechanical and chemical damage to host critical organs and a drop in host weight and egg production. Some aquatic birds are the final hosts for parasitic worms that utilize fish as intermediate hosts. During the development of their larval stages, nematodes of the Anisakidae family may infect a broad range of aquatic hosts, primarily marine mammals, aquatic birds, such as pelicans, and freshwater fish, such as crucian carp, which are the hosts where the life cycle is completed (8,16). The Anisakidae family's tolerance to diverse hosts and environmental circumstances enables a global distribution. Six adult *Contracaecum* species, as well as some unidentified species of this genus, were isolated from 17 different bird species in Iraq, with the Eurasian bittern, *Botaurus stellaris*, and the pygmy cormorant, *Microcarbo pygmaeus*, infected with a maximum of three *Contracaecum* species and unidentified species of this genus (15). An overview of the numerous parameters influencing parasite assemblages in fish hosts is provided. These variables are roughly classified as biotic or abiotic. Host age and size, host, and parasite size, host specificity, host nutrition, and host sex, as well as their effect on parasite abundance and dispersion, are all studied and emphasized. Seasonality and other environmental conditions may favor parasite establishment and growth in host populations (17). We found that industrial waste, human activities, and seasonal changes all affected infection rates. The largest infection rate occurred during the winter, coinciding with migrating birds arriving in early October and departing in early March. However, no infection was detected during July and August 2020. The average high temperature in

Karbala in August is similar to that of July, at 44.4°C. Additionally, birds are drawn to fishing because fishermen throw out a lot of dead fishes. The utilization of invertebrates as paratenic and/or intermediate hosts is a core component of larval transmission in most anisakid species. Anthropogenic factors may increase *Contracaecum* parasite populations (1). This might be because of the fish's lower eating activity at cold temperatures, which reduces the possibility of infection by copepods (18). In natural environments, this guarantees that the parasite does not kill the intermediate host and makes it to the final host to complete the life cycle. These conditions may limit the probability of *Contracaecum* L3 larvae being transferred to their final host, reducing the overall infection (18).

In Chile, the most common endoparasite species observed were *Contracaecum rudolphii* s. l. (72/80, 90%) in the neotropic cormorant *Nannopterum* (*Phalacrocorax brasiliensis*) (Suliformes: Phalacrocoracidae) (19). Based on morphological descriptions in the proventriculus, recovered nematodes were identified as *Contracaecum* spp. 100(8.0% to 81.8%) piscivorous birds (Black Night Herons, *Nycticorax*) perished in their habitats at the Wild Duck Lake in Yanqing, northwest of Beijing, China (20). Birds are considered a necessary element of the life cycle. Many wading and migratory birds were affected by nematodes, particularly in Razzaza Lake in Karbala. These areas were found during an investigation of helminth infections in 663 aquatic birds in the Bahr Al-Najaf depression in mid-Iraq. These collected and examined birds from 11 different species were infected with nematodes. *Contracaecum* spp. were found in the proventriculus of six aquatic bird species: *E. alba*, *E. garzetta*, *A. ralloides*, *B. stellaris*, *A. purpurea*, and *C. rudis*, with incidences of 14.5%, 15.6%, 20%, 13.1%, 16.7%, and 3.4%, respectively (21). In the current study, the infection rate of *Contracaecum* spp. in *Planiliza abu* (Heckel, 1843) in November and December 2019 was 25.35% and 23.38%, respectively. In January and February 2020, the percentages were 32.50% and 22.06%, respectively, while in November, it was 22.22%, and in December, it was 42.86%. In addition, in 2021, January and February were 12.2% and 10.9%, respectively, while July and August were 0%, November 3.5%, and December 6.1%. The low parasite prevalence might be attributed to a disturbance in the infection cycle since piscivorous birds, the parasite's ultimate hosts, do not feed on *C. gariepinus* in Chivero Lake. In terms of ranges, the findings agree with prior studies. Only one study has described the endoparasites of fish in Al-Husainia Creek, Karbala Province, mid-Iraq. The study agrees with other studies in ranges, but only one epidemiological study represents the endoparasites of fishes, including *L. abu*, with an incidence of 0.8% in Al-Husainia Creek, Karbala province, mid-Iraq. *L. abu* has the most parasite species of the fish studied (six species). The third-stage larval forms of *Contracaecum* spp. were found in the mesenteries and liver of *A. vorax* with a 0.7% incidence, in the intestinal wall of *B. grypus* with a 0.6% incidence, in the body cavity of both *B. sharpeyi* and *B. xanthopterus* with a 0.4% and 0.3%, respectively, and in

the intestinal wall and gonads of *L. abu* with a 0.8% incidence (22). In the north of Iraq, in Sulaimani province, Kurdistan region, Iraq (23), 44 third larval stage *Contraecaecum* were found in 13 infected freshwater fish species from 966 fish species, one species from each of the Bagridae, Heteropneustidae, Mastacembelidae, Mugilidae, Siluridae, and Sisorid. The infection rates ranged from 0.92% to 19.35%, and morphological and genetic analysis (ITS1, ITS2, and COX2) revealed that all the *Contraecaecum* larvae belonged to the same species (*Contraecaecum rudolphii* B). The percentage of *contraecaecum* spp. was 8.74% in 183 samples of freshwater fish *Planiliza abu* collected from the Al-furat river in the-Qar Governorate in southern Iraq (24). Mhaisen et al. 2018 linked the most widely distributed nematode larval forms of the genus *Contraecaecum* in Iraqi fishes and found infection in 44 freshwater and marine fish species. *Contraecaecum rudolphii* Type-B and *Contraecaecum septentrionale* Kreis, 1955 were among the infections. Globally, 42.6% of *Clarias gariepinus* were collected from Chivero Lake, Zimbabwe (18), 6% of European sea basses showed *Anisakis* infection (25), 50% of *Contraecaecum osculatum* in wild Atlantic cod (*Gadus morhua*) in Denmark (26), and 30% of *Contraecaecum* spp. Globally, 42.6% of *Clarias gariepinus* were collected from Chivero Lake, Zimbabwe (18), 6% in European seabasses showed a prevalence of *Anisakis* infection (25), 50% of *Contraecaecum osculatum* in wild Atlantic cod (*Gadus morhua*) in Denmark (26), and 30% of larvae of *Contraecaecum* spp. were detected in fish species from Nasser Lake, Egypt (27). The larval nematode intensity considered low in crustaceans, the first intermediate hosts (7). In these studies, only one nematode (*Contraecaecum* spp.) was found in *L. abu*, which agrees with (28), who researched fish parasites in the Bahr Al-Najaf depression in mid-Iraq for two years, from January 1995 to December 1996. A total of 6992 fishes from 11 different species were infected with only one nematode, *Contraecaecum* spp. The weight of the fish is unaffected by infection or gender. While host length was significantly correlated with infection, (29) found that infection with *Contraecaecum* spp. larvae was positively correlated with fish length as it increased gradually in larger fishes in *Planiliza abu* collected between June 2001 and May 2002 from three locations (Hilla river, Al-Furat fish farm, and Al-Mahaweel drain), Babylon province in central Iraq. According to the present research, the fish length (range in cm) in 2019 was 10-18 cm in November, 5-18 cm in December, 8-15 cm in January and 9-15 cm in February, and 10-15 cm in November, 12-19 cm in December, and 7.5-11 cm in November and December. These ranges are comparable to other studies by (30), who collected 180 fish (*Planiliza abu*) from a fish market in Al-Diwanya city and found a correlation between the length of fish and the infection rate. The infection rate was higher in 180 fish (*Planiliza abu*) with a length of 16 cm than in fish of 6-10 cm and 11-15 cm, respectively. Al-Husainia Creek in Karbala province is a tributary of the Al-Furat river (Euphrates river) that feeds Razzaza lake. The present research differed from another study (29), in which monthly

samples related to season changes of infection with *Contraecaecum* spp. were 11.9% in winter and 47.6% in summer. Because in the present research, no samples were collected in 2020, and none were collected in 2021. However, it was higher in the winter. In November and December 2019, the infection rate was 48.73%, 65.08% in 2020, and 9.6% in 2021, respectively. Human activities, increases and decreases in paratenic or intermediate hosts, birds, and environmental pollution might all be responsible for this study's fluctuation.

In conclusion, the preventive education of stakeholders is necessary to reduce the danger to public health. Consumers should evaluate fish species, origins, and risk factors, such as crustaceans and birds in the same region. If *contraecaecum* larvae are found in the abdominal cavity of a fish, they should be mechanically isolated. To maintain food safety, parasitological surveys for *contraecaecum* larvae in consumed seafood, especially fish hosts, are critical. To limit the danger of human diseases, worms must be killed using heat or cold.

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Ethical Statement

Ethics required are approved by the Ethical Committee of University of Kerbala/ College of Veterinary Medicine.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authors' Contribution

Marwa Jawad: Conceptualization, Methodology, Writing-Original draft preparation, Funding acquisition, Investigation, Data curation, Formal analysis. Zahraa Mohammed Ridha: Conceptualization, Funding acquisition, Investigation, Writing-Reviewing and Edit. Firas Alali: Writing-Reviewing and Edit, Software, Funding acquisition, Project Administration, Supervision, Resources. Saeed El-Ashram: Writing-Reviewing and Edit, Visualization, Funding acquisition, Validation, Supervision. Asaad Sh. M. Alhesnawi: Writing-Reviewing and Edit, Data curation, Formal analysis.

References

- Shamsi S. Parasite loss or parasite gain? Story of *Contracaecum* nematodes in antipodean waters. *Parasite Epidemiology and Control* 2019;4:e00087. doi:10.1016/j.parepi.2019.e00087.
- Castellanos JA, Tangua AR, Salazar L. Anisakidae nematodes isolated from the flathead grey mullet fish (*Mugil cephalus*) of Buenaventura, Colombia. *International Journal for Parasitology: Parasites and Wildlife* 2017; 6:265-270. doi:10.1016/j.ijppaw.2017.08.001.
- Herzog P. Untersuchungen über die Parasiten der subuasse. *rfi. sche des Irak. Archiv für Fischereiwissenschaft* 1969; (2/3):132-141.
- Al-Hadithi IA, Habish AH. Observations on nematode parasite (*Contracaecum* sp.) in some Iraqi fishes. *Bulletin of the Basrah Natural History Museum (Iraq)* 1977;4:71-25.
- Mattiucci S, Palomba M, Nascetti G. Anisakis. First Edition, In: *Encyclopedia of Infection and Immunity*, Walter Oliver. 2021; 774. doi: 10.1016/B978-0-12-818731-9.00075-6.
- Buchmann K, Mehrdana F. Effects of anisakid nematodes *Anisakis simplex* (sl), *Pseudoterranova decipiens* (sl) and *Contracaecum osculatum* (sl) on fish and consumer health. *Food and Waterborne Parasitology* 2016;4:13-22. doi:10.1016/j.fawpar.2016.07.003.
- Aibinu IE, Smooker PM, Lopata AL. Anisakis nematodes in fish and shellfish-from infection to allergies. *International Journal for Parasitology: Parasites and Wildlife* 2019; 9:384-393. doi:10.1016/j.ijppaw.2019.04.007.
- Ángeles-Hernández JC, Gómez-de Anda FR, Reyes-Rodríguez NE, Vega-Sánchez V, García-Reyna PB, et al. Genera and species of the Anisakidae family and their geographical distribution. *Animals* 2020;10:2374. doi: 10.3390/ani10122374.
- Bezerra TN, Decraemer W, Eisendle-Flöckner U, Hodda M, Holovachov O, et al. World database of nematodes. *Contracaecum* Railliet & Henry, 1912. Accessed through: World Register of Marine Species, 2019.
- Gibson D: "*Contracaecum*". WoRMS. World Register of Marine Species, 2017.
- Shamsi S. Recent advances in our knowledge of Australian anisakid nematodes. *International Journal for Parasitology: Parasites and Wildlife* 2014; 3:178-187. doi: 10.1016/j.ijppaw.2014.04.001.
- Mostafa E, Omar M, Hassan S, Samir M. Occurrence and molecular identification of *Anisakis* larval type 1 (Nematoda: Anisakidae) in marketed fish in Egypt. *Journal of Parasitic Diseases* 2020;44 (3):536-345. doi: 10.1007/s12639-020-01222-8.
- Eiras JC, Pavanelli GC, Takemoto RM, Nawa Y. An overview of fish-borne nematodiasis among returned travelers for recent 25 years—unexpected diseases sometimes far away from the origin. *Korean Journal of Parasitology* 2018; 56 (3):215-227. doi: 10.3347/kjp.2018.56.3.215.
- Debenedetti ÁL, Madrid E, Trelis M, Codes FJ, Gil-Gómez F, et al. Prevalence and risk of anisakid larvae in fresh fish frequently consumed in Spain: An Overview. *Fishes* 2019; 4:13. doi:10.3390/fishes4010013.
- Mhaisen FT, Abdul-Ameer KN, Hamdan ZK. Checklists of parasites of fishes of Salah Al-Din province, Iraq. *Biol. Appl. Environ. Res* 2018; 2 (2):180-218.
- Shamsi S, Aghazadeh-Meshgi M. Morphological and genetic characterisation of selected *Contracaecum* (Nematoda: Anisakidae) larvae in Iran. *Iranian Journal of Fisheries Sciences* 2011; 10:356-361.
- Iyaji F, Etim L, Eyo J. Parasite assemblages in fish hosts. *Bio-Research* 2009;7(2):561-570. doi: 10.4314/br.v7i2.56606.
- Barson M. The occurrence of *Contracaecum* sp. larvae (Nematoda: Anisakidae) in the catfish *Clarias gariepinus* (Burchell) from Lake Chivero, Zimbabwe. *Onderstepoort Journal of Veterinary Research* 2004;71 (1):35-39.
- González-Acuña D, Llanos-Soto S, Oyarzún-Ruiz P, Kinsella JM, Barrientos C, et al. Parasites of the neotropic cormorant nannopterum (*Phalacrocorax*) *brasiliensis* (aves, phalacrocoracidae) in Chile. *Revista Brasileira de Parasitologia Veterinária* 2020; 29 (3): e003920. doi:10.1590/S1984-29612020049.
- Zhang Q, Meng M, Huang C, Feng S, Liu J, et al. Identification and genetic characterization of *Contracaecum* sp. (Nematoda: Anisakidae) from China. *Integrative Zoology* 2021;16:929-938. doi:10.1111/1749-4877.12465.
- Al-Awadi HM, Mhaisen FT, Al-Joborae FF. Helminth parasitic fauna of aquatic birds in Bahr Al-Najaf depression, mid Iraq. *Bull. Iraq Nat. Hist. Mus* 2010;11:7-15.
- Al-Saadi AJ, T Mhaisen F, R Hasan H. Endoparasites of seven fish species from Al-Hussainia creek, Karbala province, mid Iraq. *Basrah Journal of Agricultural Sciences* 2010;23:139-146. doi: 10.33762/bagsr.2010.118385.
- Abdullah YS, Abdullah SM, Hussein RH. Morphology and molecular studies of *Contracaecum* larvae (Nematoda: Anisakidae) in some fish species from Sulaimani Province, Kurdistan Region, Iraq. *Basrah Journal of Agricultural Sciences* 2021;34:93-110. doi: 10.37077/25200860.2021.34.1.09.
- Hadi NA, Hussein ZA. Seasonal changes and their impact on the incidence internal parasites of fish *Liza abu* in the-Qar governorate. *Al-Qadisiyah Journal of Pure Science* 2017;22:41-47.
- Díez G, Briaudeau T, Santurtún M, Aboitiz X, Mendibil I, et al. Infection Rate in Seabasses Fed with Viscera Parasitised by Anisakid Larvae. *Acta Parasitologica* 2022; 67: 835-841. doi:10.1007/s11686-022-00525-5.
- Karami AM, Marnis H, Korbut R, Zuo S, Jaafar R, et al. Absence of zoonotic parasites in salmonid aquaculture in Denmark: Causes and consequences. *Aquaculture* 2022; 549:737793. doi:10.1016/j.aquaculture.2021.737793.
- Hamouda AH, Younis AE. Molecular characterization of zoonotic anisakid *Contracaecum* spp. larvae in some fish species from Lake Nasser, Egypt. *Aquaculture Research* 2022;53:2548-2561. doi:10.1111/are.15774.
- Al-Joborae FF, Mhaisen FT, Al-Awadi HM. Parasitic fauna of fishes in Bahr Al-Najaf depression, mid Iraq. *Bulletin of the Iraq Natural History Museum* 2010;11:1-9.
- Al-Zubaidy AB. Prevalence and Densities of *Contracaecum* sp. Larvae in *Liza abu* from Different Iraqi Water Bodies. *JKAU: Mar Sci* 2009; 20: 3-17. doi : 10.4197/Mar. 20-1.1.



Mycobacterium avium subsp. paratuberculosis ile Enfekte Sığırlarda Bazı Akut Faz Protein ve Biyokimyasal Parametre Düzeylerinin Belirlenmesi

Kadir BOZUKLUHAN¹, Oğuz MERHAN², Fatih BÜYÜK³, Enes AKYÜZ⁴, Tahir GEZER⁴,
 Hale ERGİN EĞRİTAĞ⁵, Gürbüz GÖKÇE⁴

¹Kafkas Üniversitesi, Kars Meslek Yüksekokulu, Kars/TÜRKİYE

²Kafkas Üniversitesi, Veteriner Fakültesi, Biyokimya Anabilim Dalı, Kars/TÜRKİYE

³Kafkas Üniversitesi, Veteriner Fakültesi, Mikrobiyoloji Anabilim Dalı, Kars/TÜRKİYE

⁴Kafkas Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, Kars/TÜRKİYE

⁵Mehmet Akif Ersoy Üniversitesi, Veteriner Fakültesi, Biyokimya Anabilim Dalı, Burdur/TÜRKİYE

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Özet: Paratüberkülozis, kronik diyare ve granümatöz enteritis ile karakterize evcil ve vahşi ruminantlarda görülen bulaşıcı bir hastalıktır. Çalışmanın amacı paratüberküloz ile enfekte sığırlarda bazı akut faz protein ve biyokimyasal parametre düzeylerinin belirlenmesidir. Çalışmada ELISA test sonuçlarına göre 15 paratüberküloz ile enfekte ve 15 antikor negatif sağlıklı sığır serumu kullanıldı. Serumlar haptoglobin, seruloplazmin, serum amiloid A (SAA), alkalen fosfataz (ALP), gama-glutamil transferaz (GGT), total protein, üre, kreatinin ve demir ölçümünde kullanıldı. Paratüberkülozisli sığırlar ile kontrol grubundaki hayvanlar karşılaştırıldığında haptoglobin, SAA (P<0.001), üre (P<0.05), seruloplazmin, kreatinin düzeylerinin (P<0.01) ve bunun yanı sıra ALP ve GGT aktivitesinin (P<0.01) arttığı, albümin ve total protein düzeyinin (P<0.01) ise azaldığı belirlendi. Demir düzeyi kontrol grubuna göre azalmakla beraber istatistiksel olarak anlamsız tespit edildi. Sonuç olarak, elde edilen bulguların paratüberkülozisin teşhisine katkı sağlayacağı, haptoglobin ve SAA'nın hastalığın teşhisinde yardımcı parametre olarak kullanılabilecekleri ve bu konuda daha detaylı çalışmaların yapılması gerektiği düşünülmektedir.

Anahtar Kelimeler: Sığır, Paratüberküloz, Akut faz protein, Biyokimyasal parametreler

Determination of Some Acute Phase Protein and Biochemical Parameter Levels in Cattle Infected with *Mycobacterium avium subsp. paratuberculosis*

Abstract: Paratuberculosis is an infectious disease in domestic and wild ruminants characterized by chronic diarrhea and granulomatous enteritis. The aim of the study is to determine some acute phase protein and biochemical parameter levels in cattle infected with paratuberculosis. In the study, 15 paratuberculosis-infected and 15 antibody-negative healthy bovine serums were used according to ELISA test results. The sera were used for the measurement of haptoglobin, ceruloplasmin, serum amyloid A (SAA), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein, urea, creatinine and iron. When the cattle with paratuberculosis and the animals in the control group were compared, it was determined that haptoglobin, SAA (P<0.001), urea (P<0.05), ceruloplasmin, creatinine levels (P<0.01) as well as ALP and GGT activities (P<0.01) increased, while albumin and total protein levels (P<0.01) decreased. Although the iron level decreased compared to the control group, it was found to be statistically insignificant. As a result, it is thought that the obtained findings will contribute to the diagnosis of paratuberculosis, haptoglobin and SAA can be used as auxiliary parameters in the diagnosis of the disease, and more detailed studies should be done on this subject.

Keywords: Cattle, Paratuberculosis, Acute phase protein, Biochemical parameters

1.Giriş

Mycobacterium avium subsp. paratuberculosis tarafından oluşturulan paratüberkülozis, kronik diyare ve granümatöz enteritis ile karakterize, sığır, koyun ve keçilerin yanı sıra antilop ve geyik gibi vahşi ruminantlarda da görülen kontagiyöz bir hastalıktır (1,2). Hastalık canlı ağırlık kaybı, süt veriminde azalma, döl veriminde ve yemden yararlanmada düşme, tedavi ve kontrol program masrafında

artış vb. ekonomik kayıplara neden olmaktadır (3). Zoonoz olması nedeniyle veteriner hekimler, çobanlar ve yetiştiriciler risk altında olup halk sağlığı açısından da hastalık önem arz etmektedir. Hastalığın bulaşması sindirim yoluyla ve intrauterin yolla olmaktadır (4). Paratüberküloz kronik seyirli olduğu için semptomlar çok geç ortaya çıkmaktadır. Hayvanlarda halsizlik, yorgunluk, tüylerin karışık bir hal almasının yanı sıra kronik ishal ve iştahın

normal olmasına karşın aşırı zayıflama görülmektedir (2,5). Hastalığın teşhisinde anamnez ve semptomlar hastalıktan şüphelendirse de kesin teşhis nekropsi ile olmakta ve subklinik olgularda ise serolojik testlerden (PCR, ELISA vb.) faydalanılmaktadır (4,6).

Organizmada, yangı ve doku hasarı gibi yangı doğurucu uyaranlara karşı akut faz yanıt (AFY) oluşmakta ve bunun sonucunda karaciğerden akut faz proteinleri (AFP) sentezlenmektedir (7,8). AFP'ler, interlökin (IL)-1, IL-6 ve tümör nekrozis faktör (TNF)- α gibi yangı doğurucu sitokinlerin hepatositleri uyarılmasıyla sentezlenip kan dolaşımına salınırlar. AFY sırasında üretilen ve kan düzeyi artan (pozitif) veya azalan (negatif) AFP'lerin paraziter hastalıklarda, bakteriyel ve viral enfeksiyonların ayırımında, klinik ve subklinik hastalıkların ayırıcı tanısında, prognozun ve uygulanan tedavi etkinliğinin belirlenmesinde kullanılabileceği bildirilmektedir (9,10). Hastalığın tanısı hem halk sağlığı hem de ekonomik kayıpların azaltılması açısından önemli olmakla beraber, hastalığın subklinik seyir izlemesi ve tanıda kesin bir metodun olmaması nedeniyle tanıya yardımcı parametrelerin kullanılmasına ihtiyaç duyulmaktadır. Bu nedenle çalışmada amacımız paratüberküloz ile enfekte sığırlarda bazı akut faz protein ve biyokimyasal parametre düzeylerinin belirlenmesidir.

2. Materyal ve Metot

Çalışma, Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu (KAÜ-HADYEK/2021-185) onayı ile yapıldı. Çalışmanın hayvan materyalini, 2021-2022 tarihleri arasında Kars ve çevre ilçelerinde aile tipi işletmelerde yetiştirilen paratüberkülozise karşı aşılammış sığırlar (3-5 yaşlı) oluşturdu. Sığırların Vena jugularis'inden antikoagülsüz tüplere kan örnekleri alındı. Elde edilen serumlar analiz yapıncaya kadar -20 °C'de saklandı. Kronik ishal, progresif kilo kaybı, süt veriminde azalma, çene altı ödem vb. semptom gösteren sığırlarda paratüberkülozisin tanısı, %64 sensitivite ve %99 spesifiteye sahip, ELISA kiti (*Mycobacterium paratuberculosis* Antibody Test Kit, 06-07130-26, IDEXX) ile konuldu. Test, üretici firmanın belirtmiş olduğu prosedüre göre yapıldı ve sonuçlar spektrofotometrede 450 nm'de dalga boyunda okunarak kaydedildi. Biyokimyasal parametreleri araştırmak için ELISA test sonuçlarına göre 15 paratüberküloz ile enfekte ve kontrol grubu olarak 15 antikör negatif sağlıklı sığır serumu kullanıldı. Haptoglobin ve seruloplazmin analizi, sırasıyla Skinner ve ark. (11) ve Colombo ve Ricterich (12) tarafından bildirilen yöntemlere göre spektrofotometrik olarak yapıldı. Serum amiloid A (SAA) seviyeleri bir ELISA kiti (Tridelta®, İrlanda) ile ölçüldü. Alkalen fosfataz (ALP), gama-glutamil transferaz (GGT), total protein, üre, kreatinin ve demir düzeyleri ticari test kiti (Biolabo®, Fransa) kullanılarak kolorimetrik olarak (Epoch®, Biotek, ABD) ölçüldü.

2.1. İstatistiksel Analiz

Verilerin analizinde SPSS yazılım programı (Version 20, Chicago, Illinois, ABD) kullanıldı. Yapılan Kolmogorov-Smirnov normalite testi sonucunda gruplar normal dağıldığı için grupların karşılaştırılmasında Student-T testi kullanıldı. İstatistiksel değerlendirmede P<0.05 değeri istatistiksel olarak anlamlı kabul edildi.

3. Bulgular

Paratüberkülozlu sığırlar ile kontrol grubundaki hayvanlar karşılaştırıldığında haptoglobin, SAA (P<0.001), üre (P<0.05), seruloplazmin, kreatinin düzeylerinin (P<0.01) ve bunun yanı sıra ALP ve GGT aktivitesinin (P<0.01) arttığı, albümin ve total protein düzeyinin (P<0.01) ise azaldığı belirlendi. Demir düzeyi kontrol grubuna göre azalmakla beraber istatistiksel olarak anlamsız tespit edildi (Tablo 1).

Tablo 1: Paratüberkülozli ve klinik olarak sağlıklı sığırlarda biyokimyasal parametre düzeyleri

Parametreler	Kontrol (n=15)	Enfekte Grup (n=15)	P
Haptoglobin (g/L)	0.066±0.007	0.152±0.006	P<0.001
Serum Amiloid A (µg/mL)	21.31±1.31	34.78±2.57	P<0.001
Seruloplazmin (mg/dL)	16.54±1.03	24.92±1.32	P<0.01
Albümin (g/dL)	3.48±0.07	2.85±0.06	P<0.01
Total Protein (g/dL)	6.89±0.18	4.91±0.19	P<0.01
ALP (U/L)	32.74±1.19	51.68±2.03	P<0.01
GGT (U/L)	30.28±1.66	40.36±1.62	P<0.01
Üre (mmol/L)	7.21±0.27	8.35±0.30	P<0.05
Kreatinin (µmol/L)	99.22±3.38	119.41±3.60	P<0.01
Demir (µg/dL)	101.94±2.68	96.73±2.80	NS

ALP: Alkalen fosfataz, GGT: Gama-glutamil transferaz, NS: Önemsiz

4. Tartışma ve Sonuç

Doku hasarının nonspesifik belirteci olan AFP'ler, hastalık şiddetini belirlemenin yanı sıra tanı, ayırıcı tanı ve prognozu belirlemede kullanılan önemli belirteçlerdir (13,14). AFP'lerin bazılarının kan düzeyi artarken, bazılarının ise azalmaktadır (7,9). Çalışmada da haptoglobin, SAA ve

seruloplazmin konsantrasyonunun arttığı, albümin düzeyinin ise azaldığı belirlendi.

Ruminantlarda önemli bir AFP olan haptoglobin sağlıklı hayvanlarda çok düşük konsantrasyonda bulunurken, birçok bakteriyel, viral, paraziter ve yangısal hastalıkta düzeyinin arttığı yapılan çalışmalarla ortaya konulmuştur (15-21). Sığırlarda önemli olan diğer bir AFP SAA olup uyarımı takiben 4 saat içerisinde artmaya başlar ve 24-48 saat içerisinde pik seviyeye ulaşır (22). Yapılan çalışmalarda SAA'nın bakteriyel enfeksiyonlarda (23-25), viral enfeksiyonlarda (26,27), paraziter enfeksiyonlarda (15,28), operasyonlardan sonra (29) arttığı ortaya konulmuştur. Sığırlarda orta derecede önemli olan ve enfeksiyon varlığının bir indikatörü olarak kabul edilen seruloplazmin, polimorfonükleer lökosit hücrelerine etki ederek, hücrelerin fagositik ve antimikrobiyal gücünün artmasının yanı sıra oluşan oksijen radikallerinin etkisini azaltma ile hücreleri oksidatif hasara karşı korumaktadır (14,30). Hayvan sağlığının bir belirteci olarak değerlendirilen seruloplazminin diagnostik kullanımının olabileceği de bildirilmiştir (7,31). Doğal enfekte paratüberkülozisli Saanen keçilerinde yapılan bir çalışmada SAA, haptoglobin, fibrinojen ve hepsidin düzeyinde artış olduğu ve paratüberkülozun teşhisini desteklemede belirtilen parametrelerin kullanılabilmesi bildirilmektedir (25). İlave olarak paratüberkülozisli sığırlarda yapılan başka bir çalışmada ise SAA, haptoglobin, C-reaktif protein ve seruloplazmin düzeyinin arttığı, albümin ve total protein düzeyinin ise azaldığını rapor etmişlerdir (31). Yine fokal, multifokal ve yaygın lezyonu olan paratüberküloz ile enfekte sığırlarda yapılan başka bir çalışmada AFP düzeyinin arttığı ve AFP'lerin hastalığın teşhisinde potansiyel biyobelirteç olarak kullanılabilmesi bildirilmektedir (32). Çalışmada da paratüberkülozlu sığırlar ile kontrol grubundaki hayvanlar karşılaştırıldığında haptoglobin, SAA, seruloplazmin düzeyinin arttığı belirlendi. Bu artışın muhtemelen fagositik hücrelerdeki aktivite artışı ve oluşan doku hasarına bağlı olarak ortaya çıktığı düşünülmektedir.

Karaciğerde sentezlenen; taşıma, onkotik basınç sağlama vb. birçok fonksiyonu bulunan albümin; anoreksi, karaciğer, böbrek bozuklukları ve bağırsak malabsorpsiyonu durumunda azalmaktadır (8,33). Ayrıca albümin katabolizmasının doku hasarı ve yangısal durumlarda arttığı ve sonuçta serum düzeyinin de azaldığı bildirilmiştir (9,34). Paratüberküloz ile enfekte sığırlarda yapılan bir çalışmada total protein ve albümin miktarının azaldığı (31), paratüberkülozisli develerde yapılan başka bir çalışmada da albümin düzeyinin azaldığı bildirilmiştir (35). İlave olarak paratüberkülozlu koyunlarda yapılan başka bir çalışmada ise total protein, albümin, kalsiyum ve magnezyum seviyelerinin klinik ve subklinik hastalıklı gruplarda kontrol grubuna göre önemli ölçüde azaldığı bildirilmiştir (36). Yine

Kızıl ve Çeribaşı (37) tarafından paratüberkülozisli koyunda yapılan başka bir çalışmada serum total protein, albümin, kalsiyum ve magnezyum düzeyleri düşük olarak bildirilmiştir. Bu çalışmada da albümin ve total protein konsantrasyonlarının azalması muhtemelen enfeksiyona bağlı olarak gelişen doku hasarı, yangı sonucu artan albümin katabolizması veya bağırsaklardaki şiddetli doku hasarı sonucu oluşan malabsorpsiyon ile ilişkili olabileceği düşünülmektedir.

Kolestasis, akut hepatik nekroz ve böbrek yetersizliklerinde GGT aktivitesinin, kolestasis, kemik oluşumu ve onarımı, hepatobiliyer dolaşımın bozulduğu olgular, endojen/ekzojen glukokortikoidler ve stres durumunda ise ALP aktivitesinin arttığı ifade edilmektedir (33,38). Paratüberküloz ile enfekte develerde yapılan bir çalışmada toplam protein, albümin ve glukoz değerlerinde önemli bir azalma, aspartat transaminaz (AST), alanin aminotransaminaz (ALT), ALP, GGT ve glutamik asit dehidrojenaz aktivitelerinde ise artış olduğu bildirilmiştir (35). Kızıl ve Çeribaşı (37) tarafından paratüberkülozisli koyunda yapılan başka bir çalışmada ise laktat dehidrojenaz, AST, ALT ve ALP aktivitesi, glukoz, total bilirubin ve kolesterol düzeylerinin normal referans aralıklar içerisinde olduğu bildirildi. Çalışmada da ALP ve GGT aktivitesi artmış olup bu artış muhtemelen hastalığın karaciğer fonksiyonlarını etkilemesine ve/veya hastalıktan dolayı oluşan strese bağlı olabilir. Serum üre ve kreatinin renal fonksiyonların değerlendirilmesi için kullanılan parametrelerdir (38). Enfeksiyon ve iştahsızlık durumlarında protein katabolizmasında bir artış oluşmakta ve bu durum serum üre ve kreatinin konsantrasyonlarında bir artışa neden olmaktadır (39). Ayrıca enfeksiyonlarda gelişen sistemik yangıya bağlı olarak da böbreklerde perfüzyon azalmakta, böbreklerin beslenmesi ve fonksiyonları bozulmaktadır (40). Çalışmada da serum üre ve kreatinin düzeylerinde artış yaşanmış olup bu durum protein katabolizmasındaki artıştan veya hastalığa bağlı sistemik yangı sonucu böbrek fonksiyonlarının etkilenmesinden kaynaklanabilir.

Sonuç olarak, elde edilen bulguların paratüberkülozisin teşhisine katkı sağlayacağı, haptoglobin ve SAA'nın hastalığın teşhisinde yardımcı parametre olarak kullanılabilirlikleri ve bu konuda daha detaylı çalışmaların yapılması gerektiği düşünülmektedir.

Kaynaklar

1. Baumgartner W and Khol JL. Paratuberculosis (Johne's disease) in ruminants- an ongoing story. Slovenian Veterinary Research 2016; 43: 5-10.
2. Constable PD, Hinckley KW, Done SH, Grunberg W. Veterinary Medicine. Eleventh Edition. London: W.B. Saunders Company, 2017; p.1591-1593.
3. Hasonova L and Pavlik I. Economic impact of paratuberculosis in dairy cattle herds: a review. Veterinarni Medicina 2006; 51: 193-211.
4. Civelek T. Süt sığırlarında paratüberküloz. Kocatepe Veterinary Journal 2017; 10: 29-32. doi: 10.5578/kvj.35300.

5. Osterstock JB, Sinha S, Seabury CM, Cohen ND. Effect of classifying disease states in genetic association studies for paratuberculosis. *Preventive Veterinary Medicine* 2010; 95: 41-49. doi: 10.1016/j.prevetmed.2010.03.005.
6. Mecitoğlu Z ve Demir G. Sığırlarda paratüberkülozun tanısına ilişkin problemler. *Journal of Research in Veterinary Medicine* 2012; 1: 19-23.
7. Tothova C, Nagy O, Kovac G. Acute phase proteins and their use in the diagnosis of diseases in ruminants: A review. *Veterinari Medicina* 2014; 59: 163-180. doi: 10.17221/7478-VETMED.
8. Merhan O, Bozukluhan K. Acute phase response and some acute phase proteins in animals. Yıldız G, Baran MS, Kaplan O, Durna Aydın Ö. eds. In: *Current Multidisciplinary Studies in Veterinary Medicine I*. Ankara: Iksad Publishing House; 2022; pp. 3-33.
9. Petersen HH, Nielsen JP, Heegaard PMH. Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research* 2004; 35: 163-187. doi: 10.1051/vetres:2004002.
10. Bozukluhan K, Merhan O. Clinical significance of some acute phase proteins in cattle. Kükürt A, Gelen V. eds. In: *Cattle Diseases-Molecular and Biochemical Approach*. London: IntechOpen, 2022; pp.1-13.
11. Skinner JG, Brown RA, Roberts L. Bovine haptoglobin response in clinically defined field conditions. *Veterinary Record* 1991; 128: 147-149. doi: 10.1136/vr.128.7.147.
12. Colombo JP and Richterich R. Zur bestimmung des caeruloplasmin im plasma [on the determination of ceruloplasmin in plasma]. *Schweizerische Medizinische Wochenschrift* 1964; 94: 715-720.
13. Murata H, Shimada N, Yoshioka M. Current research on acute phase proteins in veterinary diagnosis: an overview. *Veterinary Journal* 2004; 168: 28-40. doi: 10.1016/S1090-0233(03)00119-9.
14. Ceciliani F, Ceron JJ, Eckersall PD, Sauerwein H. Acute phase proteins in ruminants. *Journal of Proteomics* 2012; 75: 4207-4231. doi: 10.1016/j.jprot.2012.04.004.
15. Merhan O, Bozukluhan K, Gokce HI. Acute phase proteins and biochemical and oxidative stress parameters in Hypoderma spp. infested cattle. *Journal of the Hellenic Veterinary Medical Society* 2017; 68: 535-540. doi: 10.12681/jhvms.16049.
16. Merhan O, Yılmaz V, Bozukluhan K, Maraşlı Ş, Ögün M. Investigation of some acute phase proteins and antioxidant/oxidant system in infected sheep with bluetongue virus disease. *Journal of Advances in VetBio Science and Techniques* 2022; 7: 259-264. doi: 10.31797/vetbio.1063406.
17. Merhan O, Bozukluhan K, Çelebi Ö, Ögün M, Atakişi E, et al. Levels of acute phase protein and some biochemical parameter in cattle infected with *Mycobacterium bovis*. *Journal of the Faculty of Veterinary Medicine Erciyes University* 2017; 14: 101-105.
18. Kaya S, Merhan O, Kacar C, Colak A, Bozukluhan K. Determination of ceruloplasmin, some other acute phase proteins, and biochemical parameters in cows with endometritis. *Veterinary World* 2016; 9: 1056-1062. doi: 10.14202/vetworld.2016.1056-1062.
19. Bozukluhan K, Merhan O, Özcan A, Gökçe Hİ, Gökçe G: *Toxocara vitulorum* ile doğal enfekte buzağularda serum haptoglobin düzeyi, oksidatif belirteçler ve bazı biyokimyasal parametrelerin düzeyinin belirlenmesi. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 2017; 64: 75-79. doi: 10.1501/Vetfak_0000002778.
20. Bozukluhan K, Merhan O, Kiziltepe S, Ergin Egritag H, Akyuz E, et al. Determination of haptoglobin, some biochemical and oxidative stress parameters in calves with pneumonia. *Fresenius Environmental Bulletin* 2021; 30: 9485-9489.
21. Kırbaş A, Kaman R, Merhan O, Bozukluhan K, Değirmençay S, et al. Evaluation of acute phase response and oxidative damage in sheep naturally infected with *Streptococcus pluranimalium* using haptoglobin, nitric oxide, and malondialdehyde levels. *Fırat University Veterinary Journal of Health Sciences* 2021; 35: 145-150.
22. Trela M, Domanska D, Witkowska-Piłaszewicz O. Diagnostic use of serum amyloid A in dairy cattle. *Agriculture* 2022; 12: 459. doi: 10.3390/agriculture1204045.
23. Bozukluhan K, Merhan O, Büyük F, Çelebi Ö, Gökçe G. Brusellozisli sığırlarda bazı akut faz proteinlerin düzeyinin saptanması. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 2016; 63: 13-16. doi: 10.1501/Vetfak_0000002703.
24. Bozukluhan K, Merhan O, Ogun M, Kurt B, Cihan M, et al. Investigation of haptoglobin, serum amyloid A, and some biochemical parameters in calves with omphalitis. *Veterinary World* 2018; 11: 1055-1058. doi: 10.14202/vetworld.2018.1055-1058.
25. Sevgisunar NM and Şahinduran Ş. Evaluation of some acute phase proteins, cytokines and hepcidin levels in naturally infected Saanen goats with paratuberculosis. *MAKU Journal of Health Science Institute* 2021; 9: 29-37. doi: 10.24998/maeusabed.996891.
26. Merhan O, Bozukluhan K, Kiziltepe S, Gokce HI. Investigation of levels of haptoglobin, serum amyloid A, ceruloplasmin and albumin in cattle with foot-and-mouth disease. *Israel Journal of Veterinary Medicine* 2017; 72: 14-17.
27. Bozukluhan K, Merhan, O, Gökçe Hİ, Ögün M, Atakişi E, et al. Determination of some acute phase proteins, biochemical parameters and oxidative stress in sheep with naturally infected sheepox virus. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 2018; 24: 437-441. doi: 10.9775/kvfd.2017.19167.
28. Kırmızıgül AH, Erkiş EE, Merhan O, Ögün M, Ölmez N, ve ark. *Babesia canis* ile enfekte köpeklerde serum amiloid A, haptoglobin, seruloplazmin ve albumin seviyeleri. *Kocatepe Veteriner Dergisi* 2020; 13: 219-223. doi: 10.30607/kvj.670744.
29. Bozukluhan K ve Gökçe Hİ. Retikuloeritonitis travmatika ve retikuloerikarditis travmatika'lı sığırlarda bazı akut faz proteinlerin araştırılması. *Erciyes Üniversitesi Veteriner Fakültesi Dergisi* 2007; 4: 107-113.
30. Cerone SI, Sansinanea AS, Streitenberger SA, Garcia MC, Auza NJ. Cytochrome c oxidase, Cu, Zn-superoxide dismutase, and ceruloplasmin activities in copper-deficient bovines. *Biological Trace Element Research* 2000; 73: 269-278. doi: 10.1385/BTER:73:3:269.
31. Duran U and Cenesiz S. Determination of acute phase protein levels in cattle with paratuberculosis. *International Journal of Advances in Science, Engineering and Technology* 2019; 46-49.
32. Espinosa J, de la Morena R, Benavides J, Garcia-Pariente C, Fernandez M, et al. Assessment of acute-phase protein response associated with the different pathological forms of bovine paratuberculosis. *Animals (Basel)* 2020 10:1925. doi: 10.3390/ani10101925.
33. Russell KE, Roussel AJ. Evaluation of the ruminant serum chemistry profile. *The Veterinary Clinics of North America Food Animal Practice* 2007; 23: 403-426. doi: 10.1016/j.cvfa.2007.07.003.
34. Gruys E, Toussaint MJM, Niewold TA, Koopmans SJ. Acute phase reactant and acute phase proteins. *Journal of Zhejiang University Science B*. 2005; 6: 1045-1056. doi: 10.1631/jzus.2005.B1045.

35. El-Deeb WM, Fouda TA, El-Bahr SM. Clinico-biochemical investigation of paratuberculosis of dromedary camels in Saudi Arabia: proinflammatory cytokines, acute phase proteins and oxidative stress biomarkers. *Pakistan Veterinary Journal* 2014; 34: 484-488.
36. Balikci E, Gurdogan F. Some biochemical parameters and oxidative stress biomarkers in sheep with paratuberculosis. *Medycyna Weterynaryjna* 2015; 71: 679-682.
37. Kızıl Ö ve Çeribaşı S. Paratüberkülozisl bir koyunda serum biyokimyası ve patolojik bulgular. *Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi* 2010; 24: 163-166.
38. Tennant BC, Center SA. Hepatic function. Kaneko JJ, Harvey JW, Bruss ML. eds. In: *Clinical Biochemistry of Domestic Animals*. New York: Academic Press, 2008; pp.379-412.
39. Gokce HI and Woldehiwet Z. The effects of Ehrlichia (Cytoecetes) phagoctytophila on the clinical chemistry of sheep and goats. *Journal of Veterinary Medicine. Series B* 1999; 46: 93-103. doi: 10.1111/j.0931-1793.1999.00210.x.
40. Aral H. Laboratory in diagnosis and monitoring of systemic inflammatory response syndrome or sepsis. *European Journal of Basic Medical Sciences* 2015; 5: 10-20. doi: 10.15197/sabad.2.5.02.



Heterologue Skin Microbiota Transplantation for Treatment of Sarcoptic Manges in Two Dogs with Zoonotic Transmission

Kerem URAL¹, Hasan ERDOĞAN¹, Songül ERDOĞAN¹

¹Department of Internal Medicine, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Aydın/TURKEY

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Abstract: In the present case report, the authors inspected the feasibility of transferring unenriched (however moistured and pre-biotic treated) skin microbiota communities between two heterologous hosts, namely heterologue skin microbiota transplantation (hSmT), [from apparently healthy donor to other relevant and diseased dogs, with scabies, separately]. Two cross-bred, client-owned dogs (belonging to the same owners) with a diagnosis of scabies and intense pruritus were enrolled. Nivea Clear Up Strips were attached in a total of 3 various apparently healthy integumentary tissue of another donor dog and allowed to dry for 10-12 minutes. This was followed by peeling off, all 3 strips were then transferred to diseased skin tissue (laterolateral area in 2 dogs with scabies, separately) which were also irrigated with isotonic and allowed to attach for at least 12-15 minutes. Finally, all 3 strips were removed. Both in case I and II, Vas pruritus scores were decreased significantly beginning on day 0 (initial hSmT day) to days 21. Vas pruritus score ranged between 8-10 and 7-9, in case I and II, respectively, prior to hSmT [days -14 to day 0]. Prior to hSmT all skin scrape positivity were deemed available on days -14 to 0, whereas after day 2 of hSmT, all 2 dogs gave negative skin scraping results till the end of the study. It should not be unwise to draw preliminary conclusion that transfer of unenriched skin microbiota from a healthy donor to the diseased dogs with scabies, should have helped hastening clinical and parasitological recovery by manipulation of cutaneous microenvironment.

Keywords: Dog, Microbiome manipulation, Microbiome modulation, Skin microbiota transplantation

Zoonotik Bulaşı olan İki Sarkoptik Uyuzlu Köpeğin Sağaltımında Heterojen Deri Mikrobiyota Transplantasyonu

Özet: Bu vaka raporunda yazarlar, iki heterolog konakçı arasında (görünüşe göre sağlıklı donörden diğer uyuzlu hasta köpeklere ayrı ayrı) zenginleştirilmemiş (ancak nemlendirilen ve probiyotik uygulanan) deri mikrobiyota topluluklarının transferinin (hDmT) uygulanabilirliğini inceledi. Uyuz tanısı konulan ve yoğun kaşıntısı bulunan sahipli (aynı hasta sahibine ait olan), melez ırkı 2 köpek çalışmaya dahil edildi. Nivea t bölgesi burun bantları, başka donör bir köpeğin görünüşte sağlıklı toplamda 3 farklı integümenter dokusuna yapıştırıldı ve 10-12 dakika kurumaya bırakıldı. Akabinde bantlar alınarak her 3 bant izotonik ile irriga edilen hasta deri dokusuna (her iki uyuzlu köpekte ayrı ayrı laterolateral bölgelere) yapıştırıldı en az 12-15 dakika yapışmasına izin verildi. Sonunda 3 bantta uzaklaştırıldı. Vas pruritus skorları, hem vaka I hem de II'de, 0. günden (hDmT başlangıç günü) 21. güne kadar önemli ölçüde azaldı. hDmT'den önce [-14. -(0.) günler] Vas kaşıntı skoru vaka I' de 8-10, vaka II' de 7-9 arasında değişmekteydi. hDmT'den önce, deri kazıntıları -14 ila 0. günlerde pozitif iken, hDmT'nin 2. gününden sonra, her iki köpekte çalışmanın sonuna kadar negatif elde edildi. Zenginleştirilmemiş deri mikrobiyotasının sağlıklı bir donörden hasta uyuzlu köpeklere aktarılmasının, deri mikroekolojisinin manipülasyonu ile klinik ve parazitolojik iyileşmeyi hızlandırmış olması gerektiği gibi bir ön yargıya varmak yanlış olmayacaktır.

Anahtar Kelimeler: Köpek, Mikrobiyom manipülasyonu, Mikrobiyom modülasyonu, Deri mikrobiyota transferi

1.Introduction

Resident skin microbiota is requisited for archetypal skin functioning, influencing the innate immune respond with prevention of colonization against probable pathogenic microorganisms (1). In several skin conditions, it has to be clearly elucidated whether if changes in the cutaneous microbiome are causative or a result of the cutaneous disorder itself (2).

Prior investigations regarding the influence of sarcoptic mange on the skin microbiome revealed decreased levels of microbial diversity, altered relative abundance along with elevated *Staphylococcus* spp. (3) with similar findings in humans, domestic/wildlife animals with scabies (4-7) and in dogs and humans with allergy (8-11). All aforementioned data confirmed disruption of microbial ecology might participate a major role within the pathogenesis of scabies. Given relevant data, this prompted us to perform this case series of 2 dogs with scabies with zoonotic transmission, in

which both dogs and owners were unresponsive to traditional treatment prior to referral, by veterinary surgeons on the field. Therefore in the present case report, the researchers investigated the feasibility of transferring unenriched skin microbiota communities between two heterologous hosts [from apparently healthy donor to other relevant and diseased dogs, with scabies, separately].

Apart from the purpose of our study, readers should fully inform about hSmT. The skin microbiota could be beneficially altered by several mechanisms. The foremost methodology is hSmT. During hSmT, cutaneous microbiome belonging to a healthy person is transmitted to washed/ disinfected cutaneous location of another person in an attempt to modulate dermatological condition of the latter. This methodology has both advantages and disadvantages [cutaneous microecology is transmitted with its natural environment vs. solely a few bacteria could be collected from an individual skin] (12).

2. Case Presentation

Written owner consent was deemed available for all participant dogs. Day 0 was defined as initial day for heterolog skin microbiota transplantation (hSmT) and it was repeated twice (second application was on day 5). Prior values were analyzed by the help of responsible veterinary surgeon at field conditions (day -14 to day 0 analytes).



Figure 1: Clear up test strips were used as hSmT vehicle at this study.

2.1. Demographic data

Two cross-bred (n=2) client-owned dogs (belonging to the same owners) living at the center of Aydin Municipality) at the age of 2.5 and 4 years old, of both sexes and various sizes with a diagnosis of scabies on referral to Aydin Adnan Menderes University, Faculty of Veterinary, Department of Internal Medicine Clinics. Two dogs, with intense pruritus for several days, beginning from the starting point of housing process, were lived in the same closure. Clinical signs compatible with scabies involved regional/local alopecia, severe erythema, crusts, and scaling localized widely in one of the dogs and mildly involved the other one.

Diagnosis of scabies was mainly based on i) rule out of other relevant skin conditions, ii) cytology, skin scraping, [superficial skin scrapings were withdrawn to those of lesional areas in which exhibited the occurrence of numerous live *S. scabiei* mites in two of the dogs) and iii dermatoscopic analytes. Other relevant supportive analytes involved Vas pruritus score, pruritus triage (field version), skin scrape positivity.

2.2. Skin microbiota transplantation by use of Nivea Clear Up Strips

Nivea Clear Up Strips were unboxed from its original version which was followed by separation of each different strip. In a total of 3 various apparently healthy integumentary tissue with evidence of hair growth [without any skin lesions], the strips were located at moistened skin (Bepanthol Sensiderm Cream, Bayer, Turkey) and allowed to dry for 10-12 minutes. Healthy donor was participated due to its well-recognized history, clinical findings and health status. This was followed by peeling off, all 3 strips were then transferred to diseased skin tissue laterolateral area in 2 dogs with scabies, separately) which were also irrigated with isotonic and allowed to attach for at least 2-15 minutes (Fig 2.). At last step all 3 strips were removed. There were no side effects were noticed in 2 dogs were subjected to hSmT.

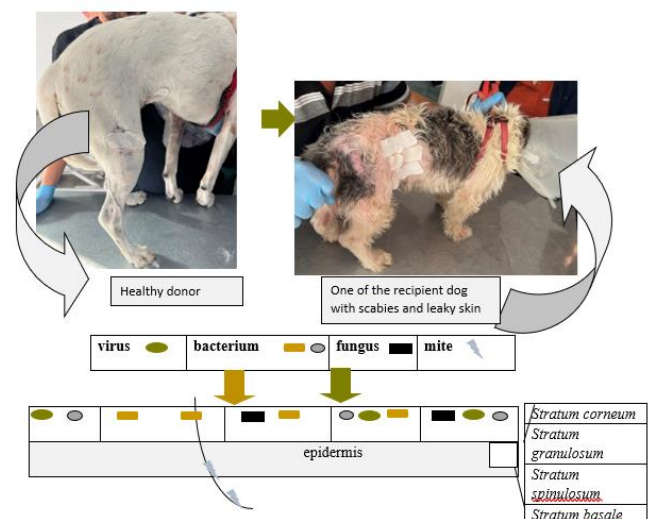


Figure 2: Skin microbiota transplantation (heterolog in origin as defined by apparently healthy donor to diseased one) from the donor to the recipient, as involved at this study. Schematic representation was adopted and transformed from (22). Self-photos belong to the first authors' archive of this study. Days 0 and 5 were the selected timeline for hSmT, which meant the application was available for twice.

2.3. Parasitological and clinical cure

Parasitological and clinical cure were detected by biomarkers of composite analytes involving skin scraping

results, Vas pruritus scores and triage coloring. Interestingly owners were also cured following treatment of both dogs, despite previous therapeutical drug applications (medical doctors for owners, referring veterinary surgeon for dogs) without success.



Figure 3: Case I at referral (prior to hSmT) on day 0 (a), 5 (b) and 10 (c), respectively thereafter hSmT. No more pruritus was evident.



Figure 4: Two dogs enrolled at this study and subjected to hSmT with photographic records obtained on days a) 0 and b) 10. There was no more pruritus, which was one of the criteria for treatment success.

2.4. Composite analytes

Biomarkers as selected, in an attempt to supportive diagnosis, were composed of Vas pruritus scores, skin scrape results and triage levels of pruritus. Both in case I and II, Vas pruritus scores were diminished beginning on day 0 (initial hSmT day) to days 21. Vas pruritus score ranged between 8-10 and 7-9, in case I and II, respectively, prior to hSmT [days -14 to day 0]. Triage levels of pruritus altered from black code to green code in respond to hSmT application. Skin scraping results were deemed negative after day 2 in each case (Fig 5) as shown below. Prior to hSmT all skin scrape positivity were dammed available on days -14 to 0 (Fig 5).

Days	-14	-10	-5	-3	0	2	5	10	21
Case I-Vas pruritus scores	10	10	9	8	9	2	0	0	0
Skin scrape positivity	+	+	+	+	+	+	-	-	-
Triage level of pruritus	Black	Black	Black	Red	Black	Green	Green	Green	Green
Case II-Vas pruritus scores	8	7	9	8	8	1	0	0	0
Skin scrape positivity	+	+	+	+	+	+	-	-	-
Triage level of pruritus	Black	Red	Black	Black	Black	Green	Green	Green	Green

Figure 5: Showing Vas pruritus scores, skin scrape positivity and triage level of pruritus among 2 dogs with zoonotic *S. scabiei* invasion.

3. Discussion and Conclusion

Considering interaction among microbes and mange, diminished microbial variation and elevated pathogenic abundance is compatible with people invased with *S. scabiei* var. *hominis* (13, 14), pigs exploratory infected with *S. scabiei* var. *suis* (3) and dogs/humans presenting allergic integumentary diseases (8, 10, 11, 15). Hence determination of opportunistic pathogens could alter due to host, *Staphylococcus* spp. and *Streptococcus* spp. were frequently encountered. Host existence of mite, ease secondary bacterial invasion via secretion of proteins hindering complement system, that is well recognized as critical participant within the immune respond against mite/bacterial infection (16-18). Mite scrape, in which primary/secondary dermatological signs could thus offer absolute environmental factors for proliferation of opportunistic pathogens. In a prior study analyzing the interaction among mites and microbes to those of 3 different canid species with *S. scabiei* invasion, the principal microbial taxa in relationship with mange exhibited *S. pseudintermedius* and *Corynebacterium* spp., along with *S. agalactiae* [with relevant *Staphylococcus* spp.] significantly altered in abundance. *S. scabiei* invasion in human and pigs demonstrated elevated abundance of *S. aureus* (3, 13). In the present study although cutaneous microbiota was not analyzed (no financial support) hSmT could have helped hastening clinical recovery by probable amelioration of pathogenic bacteria evolved within scabies on to the lesional area.

Previous researches investigating the influence of sarcoptic mange on the skin microbiota, experimental *S. scabiei* var. *suis* invasion of pigs exhibited diminished levels of microbial diversity, changed relative abundance along with elevated *Staphylococcus* spp. (3). Equivalent findings were also detected in humans, domestic/wildlife animals with scabies (4-7) and in dogs and humans with allergic skin conditions [i.e. atopic dermatitis] (8-11). This evidence

suggests that disrupted microbial communities may play a key role in the pathogenesis of sarcoptic mange. In the present study treatment respond with hSmT could be capable of reversing disruption of cutaneous microenvironment by transplantation of beneficial bacteria from healthy donor to the recipient dogs with scabies.

Regarding this context, this case control study asks whether if moving superficial cutaneous microbial communities from a healthy donor to diseased dogs with scabies is feasible or not. This briefly clinical design relied on leaky skin among 2 dogs with scabies and the topographical differences of skin microbiota in each host. We selected sites with a contrasting microbial composition, the hindfeet vs. forefeet, or stifle vs. elbow., as reported previously for human (19). Treatment efficacy was based on negative skin scraping, pruritus triage alterations (color changes as was based on Vas pruritus scores).

In parallel line with therapeutical approach for digestive system disorders with bacterial communities, therapy of integumentary system disorders with microbial transplantation is exhibiting evolution and able to bestow an encouraging approach for therapeutical armamentarium against skin disorders (20, 21). The malodor-causing microbiota was detached via antibacterial compounds and substituted with a cutaneous micro-ecologic niche withdrawn from a non-odorous donor (22). It should be kept in mind that it is unclear that the transplanted bacteria could be capable of stably colonizing the skin. Briefly hSmT could be inadequate for colonization (23) thus, expanded transplantation practices might be warranted (22).

Someone might criticize our study as because of hSmT with an unenriched microbiota substituted lesional areas in 2 dogs with scabies. First of all sometimes the clinician is in a hurry up position due to animal owner attitude and extraordinary behavior. The vast majority of owners lost their time, patient and respect due to economic burden and time wasting treatment trials of prior referrals. For instance, the animal owner involved and participated in each clinical session were presenting sleep disturbance due to itching behavior by themselves. This prompted us to seek for a quick response, in which first author of the study [K.U. (has planned, applied)]. This was due to zoonotic transmission, at the same time, prior to hSmT performed by ourselves (experience of the authors of this manuscript) and several treatment applications were evident (medical doctors for owners and responsible veterinary surgeons for dogs enrolled herein) without evidence of satisfactory results. As was shown in fig. 5. prior to hSmT (days -14 to day 0) triage colors, Vas pruritus scores were remained exactly at the same level, indicating unsatisfactory therapeutical interventions. Hence skin scraping results were all deemed positive from day -14 to day 0. Contrarily with the beginning and application of hSmT parasitological and

clinical cure were both evident. This treatment modality would be a substitute for several old therapeutical interventions. Our competing interest should be warranted with further studies.

References

1. Wanke I, Steffen H, Christ C, Krismer B, Götz F, Peschel A, Schittek B. Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. *Journal of Investigative Dermatology* 2011; 131(2): 382-390. doi:10.1038/jid.2010.328.
2. Zeeuwen PL, Boekhorst J, van den Bogaard EH, de Koning HD, van de Kerkhof P, Saulnier DM, Timmerman HM. Microbiome dynamics of human epidermis following skin barrier disruption. *Genome Biology* 2012; 13(11): 1-18. doi:10.1186/gb-2012-13-11-r101.
3. Swe PM, Zakrzewski M, Kelly A, Krause L, Fischer K. Scabies mites alter the skin microbiome and promote growth of opportunistic pathogens in a porcine model. *PLOS Neglected Tropical Diseases* 2014; 8: e2897. doi:10.1371/journal.pntd.0002897.
4. Walton SF, Holt DC, Currie BJ, Kemp DJ. Scabies: new future for a neglected disease. *Advances in Parasitology* 2004; 57(57): 309-76.
5. Hengge UR, Currie BJ, Jäger G, Lupi O, Schwartz RA. Scabies: a ubiquitous neglected skin disease. *The Lancet Infectious Diseases* 2006; 6(12): 769-779.
6. Almberg ES, Cross PC, Dobson AP, Smith DW, Hudson PJ. Parasite invasion following host reintroduction: a case study of Yellowstone's wolves. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2012; 367(1604): 2840-2851. doi:10.1098/rstb.2011.0369.
7. Fraser TA, Charleston M, Martin A, Polkinghorne A, Carver S. The emergence of sarcoptic mange in Australian wildlife: an unresolved debate. *Parasites & Vectors* 2016; 9(1): 1-11.
8. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, Segre JA. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Research* 2012; 22(5): 850-859. doi: 10.1101/gr.131029.111.
9. Rodrigues Hoffmann A, Patterson AP, Diesel A, Lawhon SD, Ly HJ, Stephenson CE, Suchodolski JS. The skin microbiome in healthy and allergic dogs. *PloS One*, 2014; 9(1): e83197. doi:10.1371/journal.pone.0083197.
10. Williams MR, Gallo RL. The role of the skin microbiome in atopic dermatitis. *Current Allergy AND Asthma Reports* 2015; 15(11): 1-10. doi:10.1007/s11882-015-0567-4.
11. Bradley CW, Morris DO, Rankin SC, Cain CL, Mistic AM, Houser T, Grice EA. Longitudinal evaluation of the skin microbiome and association with microenvironment and treatment in canine atopic dermatitis. *Journal of Investigative Dermatology* 2016; 136(6): 1182-1190. doi: 10.1016/j.jid.2016.01.023.
12. Callewaert C, Knödseder N, Karoglan A, Güell M, Paetzold B. Skin microbiome transplantation and manipulation: Current state of the art. *Computational and Structural Biotechnology Journal* 2021; 19: 624-631. doi:10.1016/j.csbj.2021.01.001.
13. Whitehall J, Kuzulugil D, Sheldrick K, Wood A. Burden of paediatric pyoderma and scabies in North West Queensland. *Journal of Paediatrics and Child Health* 2013; 49(2): 141-143. doi:10.1111/jpc.12095.

14. McCarthy JS, Kemp DJ, Walton SF, Currie BJ. Scabies: more than just an irritation. *Postgraduate Medical Journal* 2004; 80(945): 382-387.
15. Wolina U, Hipler UC, Nenoff P. Trichobacteriosis, erythrasma and pitted keratolysis—the spectrum of non-diphtheroid *Corynebacteria*. *Romanian Journal of Clinical and Experimental Dermatology*, 2017; 4(2):64-67.
16. Swe PM, Fischer K. A scabies mite serpin interferes with complement-mediated neutrophil functions and promotes staphylococcal growth. *PLoS Neglected Tropical Diseases* 2014; 8(6): e2928. doi:10.1371/journal.pntd.0002928.
17. Mika A, Reynolds SL, Pickering D, McMillan D, Sriprakash KS, Kemp DJ, Fischer K. Complement inhibitors from scabies mites promote streptococcal growth—a novel mechanism in infected epidermis?. *PLoS neglected Tropical Diseases* 2012; 6(7): e1563. doi:10.1371/journal.pntd.0001563.
18. Bergström FC, Reynolds S, Johnstone M, Pike RN, Buckle AM, Kemp DJ, Blom AM. Scabies mite inactivated serine protease paralogs inhibit the human complement system. *The Journal of Immunology* 2009; 182(12): 7809-7817. doi:10.4049/jimmunol.0804205.
19. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Segre JA. Topographical and temporal diversity of the human skin microbiome. *Science* 2009; 324(5931): 1190-1192. doi: 10.1126/science.117170.
20. Myles IA, Earland NJ, Anderson ED, Moore IN, Kieh MD, Williams KW, Datta SK. First-in-human topical microbiome transplantation with *Roseomonas mucosa* for atopic dermatitis. *JCI Insight* 2018; 3(9). doi: 10.1172/jci.insight.120608.
21. Paetzold B, Willis JR, Pereira de Lima J, Knödlseher N, Brüggemann H, Quist S. R, Güell M. Skin microbiome modulation induced by probiotic solutions. *Microbiome* 2019; 7(1), 1-9. doi:10.1186/s40168-019-0709-3.
22. Ito Y, Amagai M. Controlling skin microbiome as a new bacteriotherapy for inflammatory skin diseases. *Inflammation and Regeneration* 2022; 42(1): 1-13. doi: 10.1186/s41232-022-00212y.
23. Perin B, Addetia A, Qin X. Transfer of skin microbiota between two dissimilar autologous microenvironments: A pilot study. *PLoS One* 2019; 14(12): e0226857. doi:10.1371/journal.pone.0226857.



Medikal Ozonun Deri Lezyonlarının İyileşmesi Üzerine Etkileri

Nevzat Emre ASLAN¹, Hanifi EROL²

¹ Yozgat Bozok Üniversitesi, Veteriner Fakültesi, Cerrahi Anabilim Dalı, Yozgat/TÜRKİYE

² Erciyes Üniversitesi, Veteriner Fakültesi, Cerrahi Anabilim Dalı, Kayseri/TÜRKİYE

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Özet: Fiziksel, kimyasal ve bakteriyel ajanlara karşı bariyer görevi gören deri yaralanmasına bağlı olarak; kaybolan fonksiyonlarını eski haline getirmek ve dokularını onarabilme yeteneğinin yanı sıra yara iyileşmesinde de belirli basamaklardan oluşan bir mekanizmayı içermektedir. Deride yara oluşumunun hemen ardından başlayan hemostazis evresini takiben 2-5 gün boyunca devam eden inflamasyon evresi ve daha sonra proliferasyon evresi başlamaktadır. Bu süreçte anjiyogenezis, granülasyon doku oluşumu, kollajen birikimi ve epitelizasyon birlikte görülmektedir. Yara iyileşmesinin son evresi ise maturasyon ve remodelling evresidir. Ozon mikroorganizmaların yok olmasına ve iyileşme sürecinin uyarılmasını sağlayan güçlü bir oksitleyicidir. Bakteri, mantar ve virüs enfeksiyonlarına karşı immun sistemi aktive etmektedir. Antipatojenik etki gösteren, arteriyollerin vazodilatasyonunu sağlayarak oksijenlenmeyi artıran ozon; basınç altındaki oksijenin dokulara yayılarak anaerobik bakteri üremesi engeller. Epitelizasyon sürecinde matriks birikimi ve hücre çoğalmasını artıran ozon, yara iyileşmesinde daha az inflamatuvar hücre ve daha çok miyofibroblast içeren granülasyon dokusu oluşturmaktadır. Ayrıca toksinleri uzaklaştırarak, besin ve immünolojik faktörler açısından iyileşme sürecine destek sağlamaktadır.

Anahtar Kelimeler: Ozon, Deri, Yara, İyileşme mekanizması

Effects of Medical Ozone on Healing of Skin Lesions

Abstract: Depending on the skin injury, which acts as a barrier against physical, chemical and bacterial agents; It includes a mechanism consisting of certain steps in wound healing, as well as the ability to restore last functions and repair tissues. Following the hemostasis phase, which starts right after the wound formation on the skin, the inflammation phase continues for 2-5 days and then the proliferation phase begins. In this process, angiogenesis, granulation tissue formation, collagen deposition and epithelialization are seen together. The final phase of wound healing is the maturation and remodelling phase. Ozone is a powerful oxidizer that destroys microorganisms and stimulates the healing process. It activates the immun system against bacterial, fungal and viral infections. Ozone, which has an antipathogenic effect and increases oxygenation by providing vasodilation of arterioles; Oxygen under pressure spreads to the tissues and prevents anaerobic bacteria growth. Ozone, which increases matrix accumulation and cell proliferation in the epithelialization process, creates granulation tissue containing less inflammatory cells and more miyofibroblasts in wound healing. It also supports the healing process in terms of nutrients and immunological factors by removing toxins.

Keywords: Ozone, Skin, Wound, Mechanism of healing

1.Giriş

Yüzey alanı bakımından vücudun en büyük organı olan deri; vücudu mekanik hasardan, ultraviyole (UV) ışıklardan, radyasyondan, sıcaklıklardan koruyan bir örtüdür (18). Fiziksel hasar, kimyasal ve bakteriyel ajanlara karşı bariyer görevi gören deri; fonksiyonlarını eski haline getirmek ve dokuları onarabilme yeteneğinin yanı sıra yara iyileşmesinde de belirli basamaklardan oluşan bir mekanizmayı içermektedir (17,23). Yara iyileşmesinin bu basamakları hemostazis, inflamasyon, proliferasyon, maturasyon ve remodelling olarak adlandırılmıştır (7). Yaralanmadan hemen sonra derinin kendini onarma süreci başlamaktadır ve bu sürece mononükleer hücreler, hücre dışı matriks, sitokinler, büyüme faktörleri katılmaktadır (4).

Normalde stratosfer tabakasında bulunduğu bilinen ozon 260-280 nanometre (nm) dalga boyundadır ve yeryüzünü UV ışıklardan ve radyasyondan korumaktadır (12). Ozon tıbbi amaçlı olarak 1840 yılında Schonbein tarafından keşfedilmiştir (28). Ozonun bakteri, mantar, virüs ve birçok protozoonu inaktive ettiği için terapötik olarak kullanıldığı bildirilmiştir (25). Ayrıca ozon iyileşme sürecini hızlandırmasının yanı sıra inflamatuvar yanıtı ve ödemi azaltmak için kullanılmaktadır (14). Dolayısıyla ozon yaranın iyileşme evrelerinde farklı roller üstlenir öncelikle kontaminasyonu önlemeye yardımcı olur, daha sonra aktive olan oksijeni serbest bırakarak granülasyon doku oluşumu ve epitelizasyonu tetiklemektedir (15).

2. Derinin iyileşme mekanizması

Epidermis ve dermis tabakalarından oluşan deri; çevresel faktörler, fiziksel hasarlar, patojenler ve sıvı kaybına karşı vücudu koruyan bir bariyer görevi görür (4).

Epidermis, temel olarak keratinosit, melanosit, Langerhans hücreleri ve Merkel hücrelerinin oluşturduğu derininin en dış katmanıdır (10). Keratinositler yara iyileşmesinde epidermal yanıtta önemli derecede sorumludurlar. Keratinositler çoğaldıkça yaşlı hücreleri yüzeye doğru iterek yerlerini genç hücrelerin almasını sağlayarak 48 günde bir yenilenme döngüsünü devam ettirir (7). Dermis ise intersitisyel ve sellüler yapılardan oluşmaktadır. (10).

Yara iyileşmesi birbirini takip eden 4 fazdan oluşmaktadır; hemostazis, inflamasyon evresi, proliferasyon, maturasyon ve remodelling evreleridir (7).

2.1. Hemostazis

Yara oluşumunun hemen ardından başlayan hemostazis evresi; vazokonstriksiyon, primer ve sekonder hemostazis adımlarından oluşur. Bu süreçte trombositler ve fibrinojenler önemli rol almaktadır (18). Trombositler pıhtı oluşumuna etkilerinin yanı sıra iyileşme sürecini etkileyen büyüme (growth) faktörlerinin ve sitokinlerin üretiminde de rol alır (21). Hepatositlerden salınan ve dolaşıma katılan fibrinojen kan pıhtılaşmasında görev alan bir komponenttir. Yara oluşumunun ardından oluşan ilk cevap damarların vazokonstriksiyonu ile birlikte kanamanın durdurulmasıdır. Primer hemostazis; subendotelial matriks içinde kollajenin açığa çıkmasıyla oluşan trombosit agregasyonu ve bununla birlikte trombosit tıkaç oluşumunu içerir. Sekonder hemostazis; fibrin ağının oluştuğu, çözünmeyen ipliklere dönüştürüldüğü pıhtılaşma kademesini içeren adımdır. Böylece trombosit ve fibrinin oluşturduğu trombus kanamayı durdurur ve ileri süreçlerde görev alacak olan growth faktörler ve diğer komponentler için iskele görevi görür (18).

2.2. İnflamasyon evresi

Nötrofillerin ortaya çıkmasıyla başlayan inflamasyon evresi 2-5 gün boyunca devam eder. Nötrofiller bölgesel patojenlerin yıkılmasını sağlayan enzimler ve fagositoz yeteneği ile bu süreçte önemli rol almaktadır. Ayrıca nötrofiller Tümör Nekroz Faktör- α (TNF- α), İnterlökin-1 β (IL-1 β), ve İnterlökin-6 (IL-6) salınımında görev almaktadır. Yara oluşumundan 3 gün sonra makrofajlar bölgede hem patojenlerin fagositozu hem de growth faktör, kemokinler ve sitokinlerin sekresyon süreçlerini destekler. Ayrıca makrofajlar; apoptotik hücrelerin uzaklaştırılması, yaralanmayı takiben doku yenilenmesi ve hücre proliferasyonu gibi birçok fonksiyonda görev alır (17).

2.3. Proliferasyon

Hemostazın sağlanması ve inflamatuvar yanıtın dengelenmesinin ardından kademeli olarak proliferasyon evresi başlamaktadır. Bu süreçte anjiyogenezis, granülasyon doku oluşumu, kollajen birikimi ve epitelizasyon birlikte görülmektedir (21).

Anjiyogenezis: Yara oluşumundan iyileşmesine kadar olan süreçte neovaskülarizasyonu ifade eden önemli bir süreçtir. Bu damarlaşma geçici bir yara matriksi oluşturarak hemostaza katkıda bulunur. Geçici yara ortamı yeni damar oluşumu ve rejenerasyon için başlangıç noktasıdır, yarının besleyici perfüzyonu ve hücre kalıntılarının ortadan kaldırılmasını sağlamaktadır. Neovaskülarizasyon sürecindeki bozulma; venöz yetmezlik, arteriosklerotik hastalık veya diyabetik ayak yaralarında görülen kronik ülserlere yol açabilir (23).

Granülasyon Doku Oluşumu: Yarının yeniden şekillenmesi esnasında yerini normal bağ doku ile değiştiren granülasyon dokusu temelde aktif fibroblastlar tarafından oluşturulur ve yara kasılmasının yanı sıra yeni ekstra sellüler matriks sentezi yaparlar (18).

Kollajen Birikimi: Yara oluşumunu takiben 5-6 günde zirve yapan kollajen, hemostaz evresinde oluşan geçici fibrin matriksine fibroblastların bağlanmasıyla üretilmeye başlar. Erken evrelerde oluşan tip-3 kollajen zamanla normal dokularda bulunan tip-1 kollajene dönüştürülür (1).

Epitelizasyon: Aktif trombosit ve makrofajların ürettiği Transforming Growth Faktör- α ve Epidermal Growth Faktör (EGF) ile birlikte sitokinlerinde etkisiyle epitelizasyon uyarılır. Keratinosit Growth Faktör sentezi ve IL-6 senteziyle yara kenarlarındaki keratinositlerin yara bölgesine göçüyle epitelizasyonun başladığı belirtilmiştir (3).

2.4. Maturasyon ve remodelling evresi

Yara iyileşme evreleri içinde en uzun süre devam eden maturasyon ve remodelling evresi tip-3 kollajenin tip-1 kollajene dönüşmesiyle başlayan, kan damarları ve granülasyon dokularının gerilemesiyle birlikte tip-1 kollajen oluşumuna bağlı yara geriliminin arttığı evredir. Bu aşamadaki aşırı fibröz oluşumu, tedavisi zor olan hipertrofik skarlaşmaya ya da keloid oluşumuna neden olabilmektedir (18).

3. Ozon

Güneş ışınlarının oksijenle etkileşimi sonucunda oluşan ve stratosfer tabakasında bulunan ozon üç oksijen atomundan oluşmaktadır (25). Medikal olarak tıbbi ozon jeneratörlerinden elde edilen oksijen-ozon karışımının %3-5 konsantrasyonlarında ozon içerdiği ve bu konsantrasyonların tedavide etkin olduğu belirtilmektedir (15). Ozon, mikroorganizmaların yok olmasını ve iyileşme sürecinin

uyarılmasını sağlayan güçlü bir oksitleyicidir. Bakteri, mantar ve virüs enfeksiyonlarına karşı immun sistemi aktive etmektedir (16). Ozonun periodontal patojenik mikroorganizmaları azalttığı, enfekte travmatik yaraların iyileşmesini kolaylaştırdığı, diyabetik hastalarda oksidatif stresi önlediği, iyileşmeyi hızlandırdığı ve buna bağlı olarak amputasyon oranlarını azalttığı bildirilmiştir (8). Ayrıca ozonun intravenöz kullanımı sonrası miyokardiyal hastalarda kan kolesterol konsantrasyonunu azaltarak enfarktüs oluşumunun engellenmesi üzerine olumlu etkisinin olduğu bildirilmektedir (11). Ancak ozon düşük dozlarda anti-inflamatuar etki göstererek iyileşmeye katkı sağlasa da yüksek konsantrasyonlarda uygulandığında inflamasyonun artmasına sebep olabileceği belirtilmektedir (16). Sistemik ve lokal olarak uygulanabilen ozon, lokal olarak uygulandığında eritrosit ve lökosit gibi çeşitli hücrelerin aktivasyonu sonucu sistemik yan etkilere neden olabileceği bildirilmektedir (28).

Ozon intravenöz olarak; hepatit B ve C, genel immun yetersizlikleri ve romatizmal artrit vakalarında, intramuskuler olarak; allerji, akne ve frunkulozis vakalarında, rektal olarak; ülseratif kolit ve anal fissür vakalarında, intra-artiküler olarak; artropatilerde, intradiskal yolla; lumbal disk hernilerinde, subkutan yolla; ağrı yönetiminde uygulanabilmektedir (9).

Ozonun inhale edilmesinin pulmoner sistem ve diğer organlarda toksik etki gösterebileceği belirtilmiştir. Bilinen yan etkileri; baş ağrısı, bulantı, kusma, öksürük, rhinitis, epifora, üst solunum yollarında irritasyondur (13). Ayrıca ozonun; gebelik, hipertroidizm, anemi, şiddetli myastenia olgularında kontrendike olduğu belirtilmiştir. Ozonun tedavi amaçlı kullanımında fiziksel özelliğinden dolayı yarılanma ömrüne dikkat edilmesi gerekmektedir. Yarılanma ömrünün 20 °C'de 40 dakika ve 0 °C'de 140 dakika olduğu bildirilmiştir (20).

4.Ozonun deri yarası üzerine etkisi

Birinci Dünya Savaşı sırasında ozonun bakterisit özelliklerinden dolayı enfekte yaralarda, yanıklarda ve fistüllerde tedavi amaçlı kullanıldığı belirtilmektedir (24).

Antipatojenik etki gösteren, arteriyollerin vazodilatasyonunu sağlayarak oksijenlenmeyi artıran ozon; basınç altındaki oksijenin dokulara yayılarak anaerobik bakteri üremesini engeller. Toksinlerin uzaklaştırılmasını, besin ve immunolojik faktörler açısından destek sağlar (24).

Antimikrobiyal etkisi nedeniyle yara iyileşmesinde kullanılan ozonun oluşturduğu hücresel mekanizma ile ilgili iki hipotez vardır. Birincisi; *Candida albicans* ve *Staphylococcus aureus* gibi patojenleri direkt öldürerek serbest oksijen radikallerinin salınımıyla güçlü antioksidan etki göstermesi, diğeri ise deri lezyonlarında oksijen gerilimini artırmasıdır. Ozonun, deride yoğun halde

bulunduğu bilinen *Staphylococcus aureus* ve *Staphylococcus epidermidis* gibi bakterileri birkaç dakika içinde inaktive ettiği bildirilmiştir (29). Topikal formda ozon özellikle yara iyileşmesinde adjuvan olarak kullanılmaktadır (2). Epitelizasyon sürecinde matriks birikimi ve hücre çoğalmasını artıran ozon, yara iyileşmesinde daha az inflamatuvar hücre ve daha çok miyofibroblast içeren granülasyon dokusu oluşturmaktadır (22). Yüksek dozda kullanımına bağlı özellikle solunum yollarında toksikasyona neden olabilen ozon deride antioksidan seviyesini azaltarak lipid peroksidasyonunu artırdığı bildirilmektedir. Ozon, toksik konsantrasyonlarda derinin lipid ve protein bileşenlerinin modifikasyonuna ve oksidasyonuna neden olmaktadır. Bunun yanı sıra uygun dozlarda kullanılan ozon hücre döngüsünü hızlandırmakla birlikte nükleer faktör kapp B (NFkB) gibi redoks transkripsiyon faktörlerinin aktivasyonu ile büyüme faktörlerinin sentezini indükleyebilmektedir. İnflamatuar yanıtlar ve yara iyileşmesi için düzenleyici olan NFkB IL-8, TNF- α ve TNF- β için aktivatördür (6). Vasküler Endotelial Growth Faktör, Fibroblast Growth Faktör (FGF), Platelet Derived Growth Faktör gibi yara iyileşmesinde önemli rol oynayan çeşitli endojen büyüme faktörlerinin ekspresyonunun uyarılmasında rol almaktadır (16). Proliferasyonu uyarıcı ve miyofibroblastik anjiyogenezis ile ilgili yolları aktive eden FGF ekspresyonu kollajen üretimi ile sonuçlanan fibroblast aktivasyonunu da uyarılmasını sağlamaktadır (22,26). Ayrıca ozonun, daha yüksek sayıda miyofibroblast ile ilişkili makrofajların sayısında bir artışa ve maturasyon ve remodelling evresinde proliferatif neovaskularizasyon ve vasküler regresyona neden olması sebebiyle ozonun etki mekanizması FGF-2 ekspresyonu ve miyofibroblastik farklılaşma ile ilişkilendirilmiştir (22). Yara iyileşmesinde bildirilen diğer mekanizma, ozonun peroksitlere parçalanmasına yol açan yara eksudatları ile etkileşimine bağlı olarak doku onarımını uyarması ve bölgede oksijenlenmeyi artırmasıdır (16). Oksijene duyarlı türler trombosit agregasyonunu uyarır ve yara iyileşmesinde önemli rol alan büyüme faktörlerinin salınımını tetikler (16).

Pchepiorka ve arkadaşları (14) 2020 yılında yaptıkları deneysel çalışmada 24 adet ratta bukkal mukozada, cerrahi olarak, bilateral yara oluşturmuş ve ratları 6 gruba ayırmışlardır. İlk iki gruptan birincisine ozon tedavisi uygulayıp diğer gruba müdahale edilmemiş ve 1. gün her iki gruptaki ratları sakrifiye ederek histopatolojik olarak incelemişlerdir. Her iki grupta benzer bulgular olduğunu belirtmişlerdir. Üçüncü gruba ise ozon tedavisine devam edilirken dördüncü gruba herhangi bir müdahalede bulunulmamış ve 3. gün sakrifiye etmişlerdir. 3. günde ozon tedavisi uygulanan grupta fibrin ağı ve kollajenizasyonun arttığını, kontrol grubunda ise infiltrasyonun arttığını belirtmişlerdir. Son olarak beşinci gruba ozon tedavisi uygulanırken ve altıncı gruba müdahalede bulunulmamış ve 7. gün sakrifiye ederek yine histopatolojik olarak

incelemişler ve ozon tedavisi gören gruptaki hayvanlarda kollajen liflerinin yaygın bir şekilde biriktiğini buna karşın kontrol grubunda infiltrasyonun daha yoğun olduğunu vurgulamışlardır.

Rosul ve Patskan (19) 2016 yılında yaptıkları çalışmada diyabet görülen 47 hastada oluşan yaralarda, geleneksel tedavi ve ozon tedavisinin etkinliğini karşılaştırmışlardır. Geleneksel tedavi olarak hastalara, kan şekeri düzenleyici müstahzarlar, antibiyoterapi, antikoagulant preparatlar diğer gruba ise 200 ml salin solüsyonu ozonlanarak günde bir kere intravenöz olarak uygulamışlardır. Yüzeysel ve derin ülser görülen hastalarda 12 ile 14 gün arasında değişen sürelerde tedavilere devam etmişler ve lipid peroksidasyonu ile antioksidan düzeylerini değerlendirmişlerdir. Sonuç olarak ozonun yara iyileşme evrelerini hızlandırdığını, lipid peroksidasyonu ve antioksidan düzeylerini geliştirdiğini ve böylece tedavi sürecinin kısaltılmasına katkı sağlayarak hastaların hastanede kalış süresini kısalttığını belirtmişlerdir.

Wen ve arkadaşları (27) 2022 yılında yaptıkları çalışmada; radyoterapi sonrası aktinik ülser, kronik venöz bacak ülseri, sistemik sklerozda digital ülser, periferik arteriyel hastalığa bağlı iskemik ülser, diyabetik ayak ülseleri görülen hastalardan oluşan grupları, alt gruplara bölerek bir gruba antibiyotik diğer gruba ozon tedavisi uygulanan hastalarda ozon tedavisinin etkinliğini araştırmışlardır. Ozon tedavisi uygulanan gruplarda herhangi bir yan etkinin olmadığını, yara iyileşme sürecinin hızlandığını ve amputasyon oranlarının azaldığını belirtmişlerdir. Bu durum ozonun antioksidan olması ve terapötik mekanizmasının yanı sıra hastalardaki yara boyutlarının ve şiddetinin farklılık göstermesi nedeniyle de olabileceğini ve bu yüzden ozon tedavisinin etkinliğini ve güvenliğini doğrulamak için diğer yara tiplerinde de çalışmalar yapılması gerektiğini önermişlerdir.

Coelho ve arkadaşları (5) 2015 yılında yayımladıkları olgu sunumunda; 10 yaşında 320 kilogram ağırlığındaki bir kısırakta sağ-ön ayakta kronik laminitis olgusu tespit etmişler. Radyolojik muayene sonrası distal falanksın 30° yer değiştirdiğini belirtmişlerdir. Kas içi, peritendinöz ve intrarektal ozon tedavisini haftada iki kez 20 seans şeklinde uygulamışlar ve 6 ay sonra iyileşme olduğunu ve radyolojik bulguların normale döndüğünü belirtmişlerdir.

5.Sonuç

Medikal ozonun veteriner hekimlikte klinik kullanımı yaygınlaşmaya başlamıştır. Bu derlemede medikal ozonun yara iyileşmesi üzerine etkileri hakkında bilgi verilmiştir. Geniş kullanım alanına sahip olan, ucuz ve kullanım kolaylığı sağlayan medikal ozonun antimikrobiyal etkisi, büyüme faktörlerinin uyarılmasını sağlaması nedeniyle veteriner hekimlikte klinik kullanımda avantaj sağlayacağı düşünülmektedir.

Kaynaklar

1. Beldon P. Basic science of wound healing. *Surgery* 2010; 28(9): 409-412. doi: 10.1016/j.mpsur.2010.05.007.
2. Borges GA, Elias ST, Silva SMM, Magalhaes PO, Macedo SB, et al. In vitro evaluation of wound healing and antimicrobial potential of ozone therapy. *J Craniomaxillofac Surg* 2017; 45(3): 364-370. doi: 10.1016/j.jcms.2017.01.005.
3. Broughton G, Janis JE, Attinger CE. The basic science of wound healing. *Plast Reconstr Surg* 2006; 117(7): 12-34. doi: 10.1097/01.prs.0000225430.42531.c2.
4. Canedo-Dorantes L, Canedo-Ayala M. Skin acute wound healing: A comprehensive review. *J Inflamm Res* 2019; 19(1): 1-15. doi: 10.1155/2019/3706315.
5. Coelho CS, Bernadi WA, Ginelli AM, Spagnol T, Gardel SL, et al. Use of ozone therapy in chronic laminitis in a horse. *J Ozone Ther* 2015; 1(1): 1-6.
6. Fitzpatrick E, Holland OJ, Vanderlelie JJ. Ozone therapy for the treatment of chronic wounds: A systematic review. *Int Wound J* 2018; 15(4): 633-644. doi: 10.1111/iwj.12907.
7. Gantwerker EA, Hom DB. Skin: Histology and physiology of wound healing. *Clin Plast* 2012; 39(1): 85-97. doi: 10.1016/j.cps.2011.09.005.
8. Gulmen S, Kurtoglu T, Meteoglu I, Kaya S, Okutan H. Ozone therapy as an adjunct to vancomycin enhances bacterial elimination in methicillin resistant staphylococcus aureus mediastinitis. *J Surg Res* 2013; 185(1): 64-69. doi: 10.1016/j.jss.2013.05.085.
9. Kozat S, Okman EN. Has ozone therapy a future in veterinary medicine? *J Anim Sci* 2019; 3(1): 25-34.
10. Lai-Cheong JE, McGrath JA. Structure and function of skin, hair and nails. *Medicine (UK)* 2013; 41(6): 317-320. doi: 10.1016/j.mpm.2013.04.017.
11. Mohammed Al-Dalain S, Martinez G, Candelario-Jalil E, Menendez S, Re L et al. Ozone treatment reduces markers of oxidative and endothelial damage in an experimental diabetes model in rats. *Pharmacol Res* 2001; 44(5): 391-396. doi: 10.1006/phrs.2001.0867.
12. Maslennikov OV, Kontorshchikova CN, Gribkova IA. Ozone therapy in practice Health Manual, Ministry Health Service of the Russian Federation The State Medical Academy of Nizhny Novgorod. Russia, 2008.
13. Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marques J. Ozone therapy in medicine and dentistry. *J Contemp Dent* 2008; 9(4): 1-9. doi: 10.5005/jcdp-9-4-75.
14. Pchepiorka R, Moreira MS, Lascane NA, Catalani LH, Allegrini S, et al. Effect of ozone therapy on wound healing in the buccal mucosa of rats. *Arch Oral Biol* 2020; 119(1): 1-7. doi: 10.1016/j.archoralbio.2020.104889.
15. Peteoca A, Istrate A, Goanta AM, Ionuşcu I, Tarase A. The use of ozone therapy in veterinary medicine: a systematic review. *AgroLife Sci J* 2020; 9(2): 226-239.
16. Pivotto AP, Banhuk FW, Staffen IV, Daga MA, Ayala TS, et al. Clinical uses and molecular aspects of ozone therapy: A review. *Online J Biol Sci* 2020; 20(1): 37-49. doi: 10.3844/ojbsci.2020.37.49.
17. Reinke JM, Sorg H. Wound repair and regeneration. *Eur Surg Res* 2012; 49(1): 35-43. doi: 10.1159/000339613.
18. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. *Physiol Rev* 2019; 99(1): 665-706. doi: 10.1152/physrev.00067.2017.
19. Rosul MV, Patskan MB. Ozone therapy effectiveness in patients with ulcerous lesions due to diabetes mellitus. *Wiadomosci Lekarskie* 2016; 69(1): 7-9.

20. Sciorsci RL, Lillo E, Occhiogrosso L, Rizzo A. Ozone therapy in veterinary medicine: A review. *Res Vet Sci* 2020; 130: 240-246. doi: 10.1016/j.rvsc.2020.03.026.
21. Singh S, Young A, McNaught CE. The physiology of wound healing. *Surgery* 2017; 35(9): 473-477. doi: 10.1016/j.mpsur.2017.06.004.
22. Soares CD, Morais TM, Araujo RM, Meyer PF, Oliveira EA, et al. Effects of subcutaneous injection of ozone during wound healing in rats. *Growth Factors* 2019; 37(1-2): 95-103. doi: 10.1080/08977194.2019.1643339.
23. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. Skin wound healing: an update on the current knowledge and concepts. *Eur Surg Res* 2017; 58(1-2): 81-94. doi: 10.1159/000454919.
24. Sunnen GV. Ozone in medicine: overview and future directions. *J Adv Med* 1988; 1(3): 159-174.
25. Sunnen GV. Ozone, nitric oxide and avian influenza; preliminary theoretical considerations and possible directions. *Ozonics International* 2005; 1-10.
26. Svystonyuk DA, Ngu J, Mewhart HE, Lipon BD, Teng G, et al. Fibroblast growth factor-2 regulates human cardiac myofibroblast mediated extracellular matrix remodeling. *J Transl Med* 2015; 13(1): 1-11. doi: 10.1186/s12967-015-0510-4.
27. Wen Q, Liu D, Wang X, Zhang Y, Fang S, et al. A systematic review of ozone therapy for treating chronically refractory wounds and ulcers. *Int Wound J* 2022; 19: 853-870. doi: 10.1111/iwj.13687.
28. Viebahn-Haensler R. Milestones of medical ozone. *Rev Soc Esp Dolor* 2005; 12(2): 3-9.
29. Zeng J, Lu J. Mechanisms of action involved in ozone-therapy in skin diseases. *Int Immunopharmacol* 2018; 56(138): 235-241. doi: 10.1016/j.intimp.2018.01.040.

BOZOK VETERİNER BİLİMLERİ (BOZOK VET BİL) YAZIM KURALLARI

AMAÇ

Bozok Veteriner Bilimleri'nde, Veteriner Klinik Bilimleri, Veteriner Klinik Öncesi Bilimleri, Veteriner Temel Bilimleri, Gıda Hijyeni ve Teknolojisi, Zootekni ve Hayvan Besleme alanlarında hazırlanmış güncel ve özgün değeri olan orijinal araştırma makaleleri, olgu sunumları, derlemeler, kısa bildiriler ve editöre mektuplar yayımlanarak ulusal ve evrensel bilime katkı sağlamak amaçlanmıştır.

KAPSAM

Bozok Veteriner Bilimleri Yozgat Bozok Üniversitesinin bilimsel yayın organı olup Haziran ve Aralık aylarında olmak üzere yılda iki kez yayımlanır. Derginin kısaltılmış ismi 'Bozok Vet Sci'dir. Yayın hayatına 2020 yılından itibaren başlayacak olan Bozok Veteriner Bilimleri hakemli ve bilimsel süreli dergi olarak yayımlanacaktır.

Dergimizde, Türkçe ve İngilizce dillerinden birinde hazırlanmış olan ve daha önce başka bir dergiye eş zamanlı olarak sunulmamış Veteriner Klinik Bilimleri, Veteriner Klinik Öncesi Bilimleri, Veteriner Temel Bilimleri, Gıda Hijyeni ve Teknolojisi, Zootekni ve Hayvan Besleme alanlarında hazırlanmış orijinal araştırma makalesi, olgu sunumu, davetli ve editör onayı alınmış derlemeler, kısa bildiriler ve editöre mektuplar yayımlanır.

YAZIM KURALLARI (MAKALENİN-YAZININ HAZIRLANMASI)

1. Yazıların sorumlulukları yazarlarına aittir. Gönderilen yazının yayımlanabilmesi için, yayın kurulunca tayin edilen danışmanlar tarafından uygun bulunması şarttır. Dergide yayımlanan yazılar için ücret ya da karşılık ödenmez. Kabul edilmeyen yazılar ve ekleri, aksi belirtilmediği takdirde iade edilmez.
2. Derginin yayın dili Türkçe ve İngilizce. Yayının başında, Türkçe "Özet", İngilizce "Abstract" kısımları yer almalıdır. Özet (Abstract) bölümü 200 kelimeyi geçmemelidir.
3. Metinde sade ve anlaşılır bir yazım dili kullanılmalı, bilimsel yazım tarzı benimsenmeli, gereksiz tekrarlardan kaçınılmalı ve kısaltmalar ilk kullanıldığı yerde tanımlanmalıdır.
4. Bozok Veterinary Sciences'nde yayına kabul edildiği takdirde her türlü yayın hakkının devredildiğine dair beyanları kapsayan "Copyright Form - Yayın Hakkı Devir Sözleşmesinin" sorumlu yazar tarafından imzalanarak pdf formatında gönderilmesi gerekmektedir.
5. Dergiye sunulan çalışmaların "etik kurul onayı" sorumluluğu yazarlara aittir. Bununla beraber Editör, gerektiğinde yazarlardan etik kurul belgesi isteme hakkını saklı tutar.
6. Makalede yer alan tüm yazarların bir bilimsel araştırmacı tanımlama sistemi olan ORCID ID (Open Researcher and Contributor Identifier) kayıt numarası bilgisini makale gönderilme aşamasında sisteme yüklemesi gerekmektedir. ORCID ID kaydı, <http://orcid.org> adresinden ücretsiz yapılabilir
7. Yazışma adresinde belirtilen yazar; tüm yazışmalardan, makale üzerindeki değişikliklerden (yazar sayı ve sırası dahil) ve yayına kabul edilen yazıların matbaa provasının düzeltilmesinden sorumludur.
8. Elektronik sunum: Yayın inceleme sürecini hızlandırmak amacıyla yazılar tam olarak elektronik olarak sunulmalıdır.
9. Yayınlanması istenen çalışmalar; Microsoft Word 6.0 veya daha üst versiyonda, *Times Roman* yazı karakterinde 12 punto, çift aralıklı, sayfanın tüm kenarlarında 3 cm boşluk olacak şekilde ve ilk sayfadan başlayacak şekilde satır numaraları ile birlikte yazılmalıdır. Çalışmada yer alan yazarlar ile ilgili bilgiler "Başlık Sayfası-Title Page" ile "Esas Doküman-main document" den ayrı sunulmalıdır. Orijinal araştırma ve derleme makalelerinde 16 sayfa, literatür listesi mümkünse ise 30 adet sınırını, şekil ve tablo sayısı ise 8 adet sınırını aşmaması tercih edilmelidir. Kısa bildiri ve olgu sunumlarında 10 sayfayı aşmamalıdır.
10. Bozok Veteriner Bilimleri'ne gönderilen yazılar, aşağıdaki sıraya göre (Başlık, Özet, Metin, Kaynaklar, Tablolar ve Şekiller) düzenlenmeli, Tablo ve Şekiller ayrı sayfalarda belirtilmelidir.
11. Dergiye gönderilen çalışmalar Abstract, Özet, Giriş, Materyal ve Metot, Bulgular, Tartışma ve Sonuç, Kaynaklar başlıklarından oluşmalıdır. Giriş, Materyal ve Metot, Bulgular, Tartışma ve Sonuç bölümleri numara verilerek belirtilmelidir (1.Giriş, 2.Materyal ve Metot, 3.Bulgular, 4.Tartışma ve Sonuç). Alt başlıklar 1.1., 1.2., şeklinde ardışık olarak numaralandırılmalıdır. Referanslar bölümü numaralandırılmamalıdır.

a. Başlık: Başlık kısa, açık, tüm harfleri büyük ve yazı için uygun olmalıdır. Özellikle elektronik sunumda makalenin sadece başlığı, (yazar ve kurum adresi vermeksizin) yazılmalıdır. Bu yöntem, yazıların uzmanlarca tarafsız bir şekilde değerlendirilmesini sağlamak amacıyla uygulanmaktadır.

b. Özet: Türkçe yazılarda Türkçe ve İngilizce özet olmalıdır. İngilizce yazılarda Türkçe özet de gereklidir. Özet, 250 kelimedenden daha uzun olmamalı; amaç, materyal ve metot, bulgular ile sonucunu içermelidir. Özetlerin

altına 4-6 adet anahtar kelime verilmelidir. Türkçe anahtar kelimeler "Türkiye Bilim Terimleri (TBT)"ne uygun olarak verilmelidir (Bkz. <http://www.bilimterimleri.com>). İngilizce anahtar kelimeler "Medical Subject Headings (MESH)" e uygun olarak verilmelidir (Bkz. <http://www.nlm.nih.gov/mesh/MBrowser.html>).

c. Metin: Araştırma makalelerinde; Giriş, Materyal ve Metot, Bulgular ile Tartışma ve Sonuç bölümleri, olgu sunumlarında ise; Giriş, Olgu Sunumu, Tartışma ve Sonuç bölümleri olmalıdır. Bölüm başlıkları ilk harfi büyük olacak şekilde küçük harfler ile yazılmalıdır. Yazılarda "Systeme International (SI)" birimleri kullanılmalıdır. Derleme makaleler için hazırlanan özet derlemenin konusu hakkında bilgi ve derlemenin amacından oluşmalıdır. Derleme makalesi "Giriş" ile başlamalı, yazar/lar tarafından belirlenecek ara başlıklarla devam etmeli, "Sonuç" ve "Kaynaklar" ile tamamlanmalıdır.

d. Sembol, birim ve kısaltmalar: Dergimiz, *Scientific Style and Format, The CSE Manual for Authors, Editors, and Publishers*, Council of Science Editors, Reston, VA, USA (7th ed.) tarafından belirtilen sistemi kabul etmektedir. ×, μ, η, veya v gibi semboller MS Word sembol listesinden seçilerek kullanılmalıdır. Derece (°) sembolü gösterimi için; "O" harfinin veya "0" rakamının üst simge şeklinde gösterilmesi ile yapılmamalı sembol menüsünden kullanım tercih edilmelidir. Çarpım "x" harfi değil sembol menüsü (×) kullanılmalıdır. Sayı, birim ve matematiksel semboller (+, -, ×, =, <, >), kullanıldıktan sonra bir boşluk bırakılmalı (örneğin., 3 kg), yüzde işaretinden sonra boşluk bırakılmamalıdır (örneğin, %45). Latince et al., in vitro veya in situ terimleri italic olarak gösterilememelidir.

e. Kaynaklar: Kaynaklar metin içinde parantez içinde numara ile belirtilmelidir. Birden fazla kaynağa atıf yapılacaksa aynı parantez içerisinde belirtilmelidir örn, (3,5,7-11). Literatür listesinde yer alan kaynakların her biri için metinde atıf yapılmalıdır.

Beşten fazla yazarı olan kaynaklarda, beşinciden sonrası için "et al." eki kullanılmalı, aşağıda verilen sistematik ile noktalama işaretleri ve yazım kurallarına dikkat edilerek yazılmalıdır.

- Kaynak süreli yayın ise;** Örnek: Durmuş İ, Demirtaş ŞE, Can M, Kalebaşı S. Determining egg consumption habits in Ankara. *Tavukçuluk Araştırma Dergisi* 2007; 7: 42-45 (article in Turkish with an English abstract).
- Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S et al. Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance* 2018; 11: 1645-1658. doi: 10.2147/IDR. S173867.
- Kaynak editörlü kitaptan bir bölüm ise;** Örnek: Gay CC, Besser TE. *Escherichia coli septicaemia in calves*. Gyles CL. eds. In: *Escherichia Coli in Domestic Animals and Humans*. Wallingford: CAB International, 1994; pp.75-90.
- Kaynak kitap ise;** Örnek: Varley H, Gowenlock AH, Bell M. *Practical Clinical Biochemistry*. Fifth Edition. London: William Heinemann Medical Books Ltd, 1984; p. 685.
- Kaynak editörlü kitap ise;** Örnek: Constable PD, Hinckliff KW, Done SH, Grunberg W. *Veterinary Medicine*. Eleventh Edition. London: W.B. Saunders Company, 2017; p.57.
- Kaynak kongre bildirisi ise;** Örnek: Kırbas A, Degirmencay S., Kilinc AA, Eroglu MS. Increased cardiac troponin-I concentration and cardiac enzyme activities in neonatal calves with sepsis. Second International Veterinary Internal Medicine Congress. October, 11-13, 2019; Ankara-Türkiye.
- Kaynak tez ise;** Örnek: Kırbas A. Elâzığ, Samsun, Sivas, Tokat ve Yozgat illerindeki sığır ve koyunlarda Kırım Kongo Kanamalı Ateş virüs enfeksiyonunun seroprevalansının araştırılması, Doktora tezi, Fırat Üniv Sağ Bil Ens, Elâzığ 2009; s.1-2. (thesis in Turkish with an English abstract).

Web tabanlı erişimler kaynak olarak gösterilmemelidir.

f. Tablolar; kaynaklar kısmından sonra, her bir tablo ayrı sayfada olacak şekilde verilmelidir. Tablo başlıklarının yalnızca ilk harfleri büyük olmalıdır. Tablo başlıkları tablonun üzerinde bulunmalı ve **Tablo 1. (Table 1.)** şeklinde numaralandırılmalıdır. Tablolarda iç ve yan kılavuz çizgiler kullanılmamalıdır. Tanımlayıcı bilgi ve açıklamalar tabloların altına yerleştirilmelidir.

Örnek:**Table 1.** Determination of elements in Dogfish Liver certified reference material

Concentration ($\mu\text{g g}^{-1}$)			
	Certified ^a	Found ^b	R(%)
A I ^c	200	215 \pm 10	108
V c	0.6	0.56 \pm 0.01	93
Cr ^c	1.4	1.52 \pm 0.02	109
Co ^c	0.25	0.28 \pm 0.02	112
As	9.66 \pm 0.62	9.55 \pm 0.16	99
Cd	24.3 \pm 0.8	24.2 \pm 0.3	100
Cu	31.2 \pm 1.1	31.7 \pm 0.4	102
Fe	1833 \pm 75	1914 \pm 65	104
Pb	0.16 \pm 0.04	0.16 \pm 0.02	100
Hg	2.58 \pm 0.22	2.31 \pm 0.02	90
Ni	0.97 \pm 0.11	0.94 \pm 0.03	97
Se	8.3 \pm 1.3	8.3 \pm 0.2	100
Ag	0.93 \pm 0.07	0.86 \pm 0.01	92
Zn	116 \pm 6	113 \pm 1	97

^a At 95 % confidence level

^b $\bar{x} \pm SD$, n=3, ^cInformation value

g. Her resim, grafik ve çizim; şekil olarak kabul edilip **Şekil 1. (Figure 1.)** gibi yazılmalı, her biri ayrı sayfada olacak şekilde verilmelidir. Tanımlayıcı bilgi ve açıklamalar şekil ismi ile birlikte şeklin altına yerleştirilmelidir. Resimler 300dpi çözünürlükte olmalıdır.

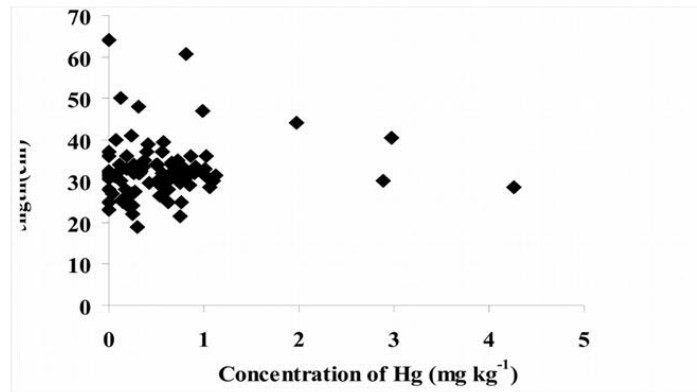
Örnek:

Figure 1. Concentration of Hg (mg kg⁻¹)

Yayının baskı öncesi matbaa provasý yazýþmadan sorumlu yazara gönderilir ve üç gün içerisinde kontrol edilerek dergiye geri gönderilmesi istenir.

Her yayın için Bozok Veteriner Bilimleri'nin ilgili sayısı yazýþmadan sorumlu yazara gönderilir. Makalelerin PDF türü tam metin dosyalarına derginin web sayfasından erişilebilir.

BOZOK VETERINARY SCIENCES (BOZOK VET SCI)

WRITING RULES

Purpose

In Bozok Veterinary Sciences, by publishing original research articles, case reports, reviews, short papers and letters to the editor with current and original value prepared in the fields of Veterinary Clinical Sciences, Veterinary Preclinical Sciences, Veterinary Basic Sciences, Food Hygiene and Technology, Animal Science and Animal Nutrition. It is aimed to contribute to national and universal science.

Scope

Bozok Veterinary Sciences is the scientific publication of Yozgat Bozok University and is published twice a year, in June and December. The abbreviated name of the journal is Bozok Vet Sci. Bozok Veterinary Sciences, which will start its publication life in 2020, will be published as a peer-reviewed and scientific periodical.

In our journal, an original research article, case report, prepared in the fields of Veterinary Clinical Sciences, Veterinary Preclinical Sciences, Veterinary Basic Sciences, Food Hygiene and Technology, Animal Science and Animal Nutrition, which was prepared in one of the Turkish and English languages and was not presented simultaneously to another journal, invited and editor-approved reviews, short papers and letters to the editor are published.

WRITING RULES (PREPARATION OF THE ARTICLE-ARTICLE)

1. Responsibilities of the articles belong to their authors. In order for the submitted manuscript to be published, it must be approved by the advisors appointed by the editorial board. No fee or compensation is paid for the articles published in the journal. Unaccepted manuscripts and their appendices will not be returned unless otherwise stated.
2. The publication languages of the journal are Turkish and English. At the beginning of the publication, the Turkish "Abstract" and the English "Abstract" sections should be included. The abstract section should not exceed 200 words.
3. A plain and understandable writing language should be used in the text, scientific writing style should be adopted, unnecessary repetitions should be avoided and abbreviations should be defined where they are first used.
4. If accepted for publication in Bozok Veterinary Sciences, the "Copyright Form - Copyright Transfer Agreement", which includes the declarations regarding the transfer of all kinds of publication rights, must be signed by the responsible author and sent in pdf format.
5. Responsibility for the "ethics committee approval" of the studies submitted to the journal belongs to the authors. However, the Editor reserves the right to request an ethics committee document from the authors when necessary.
6. All authors in the article are required to upload the ORCID ID (Open Researcher and Contributor Identifier) registration number information, which is a scientific researcher identification system, to the system at the time of submitting the article. ORCID ID registration can be done free of charge at <http://orcid.org>.
7. The author specified in the correspondence address; He is responsible for all correspondence, changes on the article (including the number and order of the author) and correction of the printing proof of the articles accepted for publication.
8. Electronic submission: Manuscripts should be submitted fully electronically in order to speed up the publication review process.
9. Studies to be published; It should be written in Microsoft Word 6.0 or higher, in Times Roman font, 12 points, double-spaced, with 3 cm margins on all sides of the page, and with line numbers starting from the first page. Information about the authors in the study should be presented separately from the

- "Title Page" and "Main Document". It should be preferred that the original research and review articles should not exceed 16 pages, the literature list should not exceed 30 if possible, and the number of figures and tables should not exceed 8. Short papers and case reports should not exceed 10 pages.
10. Manuscripts sent to Bozok Veterinary Sciences should be arranged in the following order (Title, Abstract, Text, References, Tables and Figures), Tables and Figures should be indicated on separate pages.
 11. Studies submitted to the journal should consist of Abstract, Introduction, Material and Method, Results, Discussion and Conclusion, References. Introduction, Material and Method, Results, Discussion and Conclusion sections should be numbered (1. Introduction, 2. Material and Method, 3. Findings, 4. Discussion and Conclusion). Sub-headings should be numbered sequentially as 1.1., 1.2.,... The references section should not be numbered.
 - a) **Title:** The title should be short, clear, all capital letters and appropriate for the text. Especially in the electronic submission, only the title of the article (without giving the author and institution address) should be written. This method is applied to ensure that the articles are evaluated impartially by experts
 - b) **Abstract:** In Turkish articles, there should be an abstract in Turkish and English. Turkish abstracts are also required for English articles. The abstract should not be longer than 250 words; should include the purpose, material and method, findings and conclusion. 4-6 keywords should be given under the abstracts. Turkish keywords should be given in accordance with "Turkish Science Terms (TBT)" (See <http://www.bilimterimleri.com>). English keywords should be given in accordance with "Medical Subject Headings (MESH)" (See <http://www.nlm.nih.gov/mesh/MBrowser.html>).
 - c) **Text:** In research articles; Introduction, Material and Method, Results and Discussion and Conclusion sections, in case reports; There should be Introduction, Case Report, Discussion and Conclusion sections. Chapter titles should be written in lowercase letters with the first letter capitalized. "Systeme International (SI)" units should be used in manuscripts. The summary prepared for the review articles should consist of information about the subject of the review and the purpose of the review. The review article should start with "Introduction", continue with subheadings to be determined by the author/s, and should be completed with "Conclusion" and "References".
 - d) **Symbols, units and abbreviations:** Our journal accepts the system specified by Scientific Style and Format, The CSE Manual for Authors, Editors, and Publishers, Council of Science Editors, Reston, VA, USA (7th ed.). Symbols such as \times , μ , η , or v should be selected from the MS Word symbol list and used. For degree ($^{\circ}$) symbol display; It should not be done by showing the letter "O" or the number "0" as superscript, it should be preferred to use from the symbol menu. Symbol menu (\times) should be used, not the product letter "x". Numbers, units, and mathematical symbols (+, -, \times , =, <, >) should be followed by a space (e.g. 3 kg), not a percent sign (e.g. 45%). Latin et al., in vitro or in situ terms should not be shown in italics.
 - e) **References:** References should be indicated in the text with numbers in parentheses. If more than one source is to be cited, it should be stated in the same parenthesis, eg (3,5,7-11). Reference should be made in the text for each of the sources in the literature list.
 12. In references with more than five authors, "et al." suffix should be used, and it should be written in the following systematic, paying attention to the punctuation marks and spelling rules.
 - a) **If the source is a periodical;** Durmuş İ, Demirtaş ŞE, Can M, Kalebaşı S. Determining egg consumption habits in Ankara. Tavukçuluk Araştırma Dergisi 2007; 7: 42-45 (article in Turkish with an English abstract).
 - b) Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S et al. Antibiotic resistance: a rundown of a global crisis. Infection and Drug Resistance 2018; 11: 1645-1658. doi: 10.2147/IDR.S173867.
 - c) **If the source is a chapter from the edited book;** Gay CC, Besser TE. Escherichia coli septicaemia in calves. Gyles CL. eds. In: Escherichia Coli in Domestic Animals and Humans. Wallingford: CAB International, 1994; pp.75-90.
 - d) **If the source book;** Varley H, Gowenlock AH, Bell M. Practical Clinical Biochemistry. Fifth Edition. London: William Heinemann Medical Books Ltd, 1984; p. 685.
 - e) **If the source is an edited book;** Constable PD, Hinckliff KW, Done SH, Grunberg W. Veterinary Medicine. Eleventh Edition. London: W.B. Saunders Company, 2017; p.57.
 - f) **If the source is the congress notice;** Kirbas A, Degirmencay S., Kilinc AA, Eroglu MS. Increased cardiac troponin-I concentration and cardiac enzyme activities in neonatal calves

with sepsis. Second International Veterinary Internal Medicine Congress. October, 11-13, 2019; Ankara-Türkiye

- g) **If the source is thesis**; : Kırbaş A. Elâzığ, Samsun, Sivas, Tokat ve Yozgat illerindeki sığır ve koyunlarda Kırım Kongo Kanamalı Ateş virüs enfeksiyonunun seroprevalansının araştırılması, Doktora tezi, Fırat Üniv Sađ Bil Ens, Elâzığ 2009; s.1-2. (thesis in Turkish with an English abstract).

Web-based access should not be cited as a source.

- f) **Tables**; After the references part, each table should be given on a separate page. Only the first letters of table titles should be capitalized. Table headings should be above the table and numbered as Table 1. (Table 1.). Inside and side guide lines should not be used in tables. Descriptive information and explanations should be placed below the tables.

Sample :

Table 1. Determination of elements in Dogfish Liver certified reference material

	Concentration ($\mu\text{g g}^{-1}$)		
	Certified ^a	Found ^b	R(%)
Al ^c	200	215 \pm 10	108
V ^c	0.6	0.56 \pm 0.01	93
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Cd	24.3 \pm 0.8	24.2 \pm 0.3	100
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Ni	0.97 \pm 0.11	0.94 \pm 0.03	97
Se	8.3 \pm 1.3	8.3 \pm 0.2	100
Ag	0.93 \pm 0.07	0.86 \pm 0.01	92
Zn	116 \pm 6	113 \pm 1	97

^a At 95 % confidence level

^b $\bar{x} \pm SD$, n=3, ^cInformation value

Every picture, graphic and drawing; should be accepted as figures and written like Figure 1. (Figure 1.), each one should be given on a separate page. Descriptive information and explanations should be placed under the figure along with the figure name. Pictures must be at 300dpi resolution.

Sample:

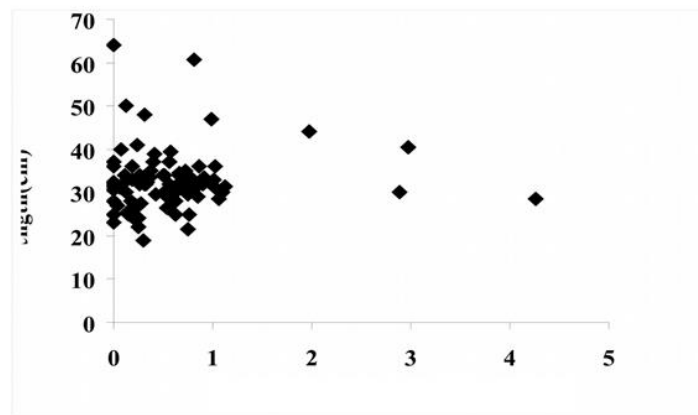


Figure 1. Concentration of Hg (mg kg⁻¹)

The prepress proof of the publication is sent to the corresponding author and it is requested to be checked and returned to the journal within three days.

For each publication, the relevant issue of Bozok Veterinary Sciences is sent to the corresponding author. PDF-type full-text files of the articles can be accessed from the journal's web page.

BOZOK VETERİNER BİLİMLERİ
Yayın Hakları Devri Sözleşmesi

Makale Türü: () Araştırma () Olgu Sunumu () Derleme () Kısa bildiri () Editöre mektup

Makale Başlığı:.....

Biz türü ve başlığı yukarıda belirtilmiş makalenin yazarları olarak; Bozok Veteriner Bilimleri'nin yazım ve yayın şartlarını bilerek ve kabul ederek hazırlayıp yayımlanması dileğiyle Bozok Veteriner Bilimleri Editörlüğüne gönderdiğimiz makalenin orijinal olduğunu, kısmen veya tamamen daha önce yayımlanmadığını veya eşzamanlı olarak başka bir yayın kuruluşuna gönderilmediğini, makale yayımlandıktan sonra ortaya çıkabilecek her türlü bilimsel ve etik sorumluluğun bize ait olduğunu ve Bozok Veteriner Bilimleri'nin hiçbir sorumluluk taşımayacağını, danışman ve dergi editörü tarafından gerekli görülen düzeltmelerle birlikte her türlü yayın hakkını, yazının yayımlandığı tarihten itibaren Bozok Veteriner Bilimleri'ne devrettiğimizi taahhüt ederiz.

Bununla birlikte yazarların telif hakkı dışında kalan patent vb. tescil edilmiş hakları, yazarların kitap ve dersler gibi çalışmalarında makalenin tümü ya da bir bölümünü ücret ödemeksizin kullanım hakkı, ticari amaçla kullanmamak üzere makaleyi çoğaltma hakkı saklıdır.

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