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Treatment and Determination of the Presence of *Helicobacter* in Shelter dogs by Faecal Antigen Testing and Enzyme–Linked Immunosorbent Assay

Tratamiento y determinación de la presencia de *Helicobacter* en perros de refugio mediante pruebas de antígenos en heces y ensayo inmunoenzimático

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ABSTRACT

In order to determine the presence of *Helicobacter* in shelter Dogs in Yozgat Province of Türkiye by fecal antigen test and ELISA and to treat the infection during the initial period. As the material of the study, faecal antigen test, faecal samples for ELISA, blood samples for haematological and biochemical analyses were collected from 82 dogs in Sorgun animal shelter where food and water bowls were kept in compartments. Infected and control groups were formed according to the results of the fecal antigen test. As a result of fecal antigen test, 6 of 82 animals were antigen positive and 76 were antigen negative. According to ELISA analysis; 53 dogs were antibody positive and 29 dogs were antibody negative. No significance was determined between the groups in terms of age and gender in both tests ($P>0.05$). Dogs in the infected group were treated according to a protocol known as triple therapy, which is used in *Helicobacter* infections. WBC, NEU and MON counts, RDW and % NEU values of the infected group were higher in the hematological examination, while the % LYM values were significantly lower than the control group. In the biochemical examination, the concentrations TNF–α (21.17 pg·mL^{-1} *vs.* 48.21 pg·mL^{-1}), IL–1ß (73.41 pg·mL^{-1} *vs.* 37.60 pg·mL^{-1}) ($P<0.01$) and CRP (644.0 mg·dL^{-1} *vs.* 234.01 mg·dL^{-1}) ($P<0.001$) were found to be higher than those of the control group. As a result, it was determined in the study that the presence of *Helicobacter* was intense in dogs that were in one–to–one contact with each other, including the feeding of dogs in shelter conditions. With the methods used in the study, it is predicted that the presence of *Helicobacter* can be detected and treated in the early stages, thus preventing transmission of *Helicobacter* in dogs.

Key words: Acute phase response; dog; ELISA; *Helicobacter;* faecal antigen test

RESUMEN

Con el objeto de determinar la presencia de *Helicobacter* en perros de refugio de la provincia turca de Yozgat mediante la prueba del antígeno fecal y ELISA y tratar la infección durante el periodo inicial. Como material del estudio, se recogieron pruebas de antígenos fecales, muestras fecales para ELISA, muestras de sangre para análisis hematológicos y bioquímicos de 82 perros en el refugio de animales de Sorgun, donde los cuencos de comida y agua se guardaban en compartimentos. Se formaron grupos infectados y de control según los resultados de la prueba del antígeno fecal. Como resultado de la prueba del antígeno fecal, 6 de 82 animales fueron positivos al antígeno y 76 negativos. Según el análisis ELISA; 53 perros fueron positivos a anticuerpos y 29 perros fueron negativos a anticuerpos. No se determinó ninguna significación entre los grupos en cuanto a edad y sexo en ambas pruebas ($P>0,05$). Los perros del grupo infectado fueron tratados según un protocolo conocido como terapia triple, que se utiliza en las infecciones por *Helicobacter*. Los recuentos de WBC, NEU y MON, los valores de RDW y % NEU del grupo infectado fueron superiores en el examen hematológico, mientras que los valores de % LYM fueron significativamente inferiores a los del grupo de control. En el examen bioquímico, se observó que las concentraciones de TNF–α (21,17 pg·mL^{-1} frente a 48,21 pg·mL^{-1}), IL–1ß (73,41 pg·mL^{-1} frente a 37,60 pg·mL^{-1}) ($P<0,01$) y PCR (644,0 mg·dL^{-1} frente a 234,01 mg·dL^{-1}) ($P<0,001$) eran superiores a las del grupo de control. Como resultado, se determinó en el estudio que la presencia de *Helicobacter* era intensa en perros que estaban en contacto uno a uno entre sí, incluida la alimentación de perros en condiciones de refugio. Con los métodos utilizados en el estudio, se prevé que la presencia de *Helicobacter* pueda detectarse y tratarse en las primeras fases, evitando así la transmisión de *Helicobacter* en perros.

Palabras clave: Respuesta de fase aguda; Prueba de antígeno en heces; Perro; ELISA; *Helicobacter*

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INTRODUCTION

Helicobacter is a genus of gram–negative, microaerophilic, spiral bacteria containing more than 40 species. Non–*Helicobacter pylori helicobacter*ia (NHPHs) such as *Helicobacter heilmannii sensu stricto* (s.s.), *Helicobacter bizzozeronii* and *Helicobacter felis* have been frequently detected in the stomach of dogs (*Canis lupus familiaris*) and cats (*Felis catus*) [1]. The main types of NHPH found in dogs are *H. heilmannii* s.s., *H. bizzozeronii*, *H. salomonis*, *H. felis* and *H. canis* [2]. Most of the NHPHS may also cause illness in humans. Attention should be paid to the zoonotic importance of NHPHS due to the increased risk of occurrence in people who are in close and intense contact with animals [3]. Since *Helicobacter* pathogens can be excreted through defecation, expectorating, and vomiting, they present faecal–oral, oral–oral and gastro–oral transmission routes [4]. Dogs are the natural hosts of NHPH and harbour these bacteria in their gastric mucosa, intestines and oral cavities; therefore, gastric fluid, saliva and faeces are possible sources of transmission of these bacteria to humans [2].

Invasive and non–invasive methods are used in the diagnosis of *Helicobacter* infection. Among invasive methods with endoscopic biopsy, histological examination, culture, rapid urease test (RUT), and polymerase chain reaction (PCR) are shown; while among non–invasive methods, faecal antigen test, urea breath test (UBT), and serological tests are named [5]. Although gastroscopy, which is within the invasive diagnostic methods, has higher sensitivity and specificity than other methods, it should be noted that many veterinarians do not have the opportunity and ability to perform gastroscopy, in addition to the risks of gastroscopy and associated anaesthesia. For this reason, non–invasive methods such as serology, culture or PCR, which can be made from blood, saliva and faecal samples, are preferred [6]. In addition, it was reported in a study that PCR analyses alone would not be sufficient to associate the presence of bacteria with the disease state in the diagnosis of *Helicobacter*, and this should be supported by histopathological analyses [7].

The faecal antigen test is highly accurate and a rapid test that can be used to confirm the presence of bacteria both at diagnosis and after treatment. The simplicity of the present method does not require prior preparation of the patient, but it is recommended not to apply a proton pump inhibitor (PPI), a 4–week antibiotic treatment, and bismuth compounds two weeks before the test is performed [8].

Erythrocyte synthesis is regulated by many factors such as erythropoietin, B_{12}, folic acid, and vitamin C. *Helicobacter* infections have been associated with vitamin B_{12} deficiency, iron deficiency and iron deficiency anaemia. These infections trigger acute or chronic gastritis, causing the persistence of vitamin B_{12} deficiency [9]. In addition, it is known that high levels of TNF–alpha and interleukin levels are present in *Helicobacter*–induced inflammations and that the local increase may cause structural changes in the gastric epithelial cells [10].

It has been reported that all *Helicobacter* species are highly sensitive to ampicillin, clarithromycin, tetracycline, tylosin, enrofloxacin, gentamicin and neomycin, and over 90% of dogs with *Helicobacter*–induced gastritis are relieved by using a combination of metronidazole, amoxicillin and famotidine. This treatment procedure, known as triple therapy, lasts at least 21 days, increasing the chance of eradication [11]. The aim of the study was to determine the presence of *Helicobacter* in dogs by fecal antigen test and ELISA methods and to provide early treatment, to have information about the prognosis of inflammation by looking at TNF–α, IL–1ß and C–reactive protein concentrations and to measure the effects of the presence of the agent on folic acid (FA) and cobalamin (COB) concentrations.

MATERIALS AND METHODS

Study population and experimental design

The population consisted of 82 dogs in the Temporary Animal Shelter of the Municipality of Sorgun. Priority was given to those sampled dogs which showed signs of vomiting and gastritis. Sampling was done from 82 dogs in the form of scanning. The samples were collected from different groups of breed, gender and age. Sampling was done by screening from 82 dogs. Samples were collected from different breeds, sex and age groups. Samples were composed of 47 dogs from the 0 > 2 age group, 35 dogs from the 2 > 4 age group, 45 dogs from the female animal group and 37 dogs from the male animal group. According to faecal antigen test results, 2 (4,25%) dogs from 0 > 2 age group and 4 (11,4%) dogs from 2 > 4 age group were positive for *Helicobacter* antigen. These dogs were formed the infected group (n=6) and the antigen negative dogs (n=6) were formed the control group. The study counted with the approval of the Erciyes University Animal Experiments Local Ethics Committee (10.09.2020, 20/128).

Clinical examination

The clinical examination of the 82 dogs was performed. The animals were examined for signs such as loss of appetite, vomiting, abdominal pain, and increased urge to drink water with dehydration.

Blood and faecal collection

Clinical symptoms frequently encountered in infections originating from *Helicobacter* were considered while sampling. 82 dogs with anorexia, hypersalivation or vomiting–like symptoms from the first day of sampling were included in the study. For faecal antigen testing, faecal samples were collected from the rectal region. Blood samples were taken via vena jugularis into EDTA tubes (Vacusel, Konya, Türkiye) for haematological analysis; into vacuum tubes (BD Vacutainer, Plymouth, United Kingdom) for biochemical and serological analysis. The blood samples obtained were centrifuged in a cooled centrifuge (Hettich Universal 320R, Germany) at 4.000 g·5 min^{-1}. After centrifugation, the serum was stored at -80°C (ESCO, ESC–UUS–480A, Singapore) until biochemical and serological analysis.

Treatment protocol

It was paid attention that no antimicrobial treatment was administered to the dogs for the previous week and that the dogs sampled were at least one year old. In the dogs to be sampled, those showing symptoms of vomiting and gastritis were prioritized, and scanning was performed on 82 dogs that formed the material of the study. Faecal antigen tests were performed on the faecal samples. According to the results of faecal antigen tests, positivity (n=6) and negativity (n=6) were determined. Those found positive were accepted as infected group and treatment was applied. The infected group was treated according to the protocol known as triple therapy used in *Helicobacter* infections. Amoxicillin–Clavulanate (Croxilex–BID 625 mg tablet, Turkey) 20 mg·kg^{-1} PO once a day, Metronidazole (Flagyl 500 mg tablet, France) 15 mg·kg^{-1} PO once a day, Ranitidine (Ranitab 150 mg tablet, Turkey) 4 mg·kg^{-1} PO once a day. After treatment, stool samples were collected with sterile test apparatus for 3 weeks and stool antigen test was repeated.

Serological analyses

- **Faecal antigen test**

Faecal samples were taken with rectal swabs from 82 dogs. The *Helicobacter* Antigen Test (Dia Pro Diagnostic Bioprobes, San Giovanni, Italy), which enables the qualitative detection of *Helicobacter* antigen in faecal on an immunochromatographic basis, was applied. The plastic bottle was opened from the screwed part, and an amount of faeces sample of approximately the size of lentils was introduced. The bottle was then shaken to mix the extraction buffer with the sample and waited for about 30 s. 4 drops of the mixture containing faeces and buffer solution were dripped onto the test card. Results were read within 5 min. In the presence of *Helicobacter* antigen in faecal samples, by reacting with the antigen-specific antibodies in the test card, double lines were observed in the positive samples, while a single line was in the negative samples.

- **Enzyme-linked immunosorbent assay (ELISA)**

In the sera from dogs (n=82), animals positive for *Helicobacter* antibody were detected using the Canine *Helicobacter* IgG Elisa kit (Sunlong Biotech, Zhejiang, China) according to the instructions of the manufacturer. Biotek ELX800 (Wermont, USA) device was used for ELISA measurement. ELISA plates were read at 450 nm.

- **Haematological parameters**

Hematological parameters (Red blood cell, White blood cell, Platelet indices) were determined with a blood count device (Mindray BC-2800, China) in the blood samples of the infected and healthy groups.

Biochemical analyses

- **Inflammatory parameters**

TNF-α, IL-1β and CRP concentrations in the serums were determined according to the manufacturer's instructions, commercial ELISA test kit (Sunlong Biotech, Zhejiang, China).

- **Vitamins**

Cobalamin (B_{12}) and Folic acid (B_9) concentrations were determined in serums of the infected and control groups according to the manufacturer's instructions using a commercial ELISA assay kit (Sunlong Biotech, Zhejiang, China).

Statistical analysis

IBM SPSS Statistics 25,0 (IBM Corp. Armonk, New York, USA) package program was used for the statistical analysis. The suitability of the data to the normal distribution was evaluated by the histogram, Q-Q plots, and the Shapiro-Wilk tests and was shown as mean±standard deviation or median (min-max). The data presenting a normal distribution were evaluated with the independent two-sample T-test, and the data lacking a normal distribution were evaluated with the Mann-Whitney U test. The relationship between the parameters of the groups was investigated with the help of the Spearman Correlation test. The significance level was considered as $P<0.05$.

RESULTS AND DISCUSSION

Serological findings

- **Faecal antigen test findings**

In the faecal antigen test results, 6 positive (7%) and 76 negative (93%) animals were determined in the dogs (n=82) whose faecal samples were collected. According to the faecal antigen test results, infected (n=6) and healthy groups (n=6) were determined. Faecal antigen tests were repeated for the presence of *Helicobacter* 3 weeks after the treatment was applied to the dogs in the infected group. Negativity was detected in 5 of the infected dogs. According to faecal antigen tests and haematological and clinical findings after the treatment, complete recovery was observed in 5 (85%) of the infected dogs. Antigen positivity was detected in 5 of 45 female dogs and in one of 37 male dogs. Antigen positivity was detected in 2 of 47 dogs in the 0 > 2 age group and in 4 of 35 dogs in the 2 > 4 age group. When evaluated in terms of age and gender, it was determined that antigen positivity rates in faecal antigen tests were higher in the 2 > 4 age group compared to the 0 > 2 group; and in female dogs compared to male dogs. However, these results were not statistically significant ($P>0.05$). Distribution of these rates according to age and gender are given in TABLE I.

TABLE I
Distribution of antigen positivity by age and gender according to the Feacal antigen test (n=82)

Parameters		Antigen positive (n=6)	Antigen negative (n=76)
Age	0 > 2 (n)	2 (4.25%)	45 (95.75%)
	2 > 4 (n)	4 (11.4%)	31 (88.6%)
Gender	Female (n=45)	5 (11.0%)	40 (89.0%)
	Male (n=37)	1 (2,7%)	36 (97.3%)

- **ELISA test findings**

According to the *Helicobacter* IgG results in the serum of the sampled dogs, significant differences were determined in the concentrations of *Helicobacter* IgG in control (n=6) and infected dogs (n=6). According to the normal range of *Helicobacter* IgG (6.93-8.80), results with a value below 6.93 mg·dL^{-1} were considered seronegative, and results with a value of 8.80 mg·dL^{-1} and above were considered seropositive. The mean IgG concentration was 5.93 mg·dL^{-1} in the control group and 10.83 mg·dL^{-1} in the infected group. Seropositivity was detected in 28 of 45 female dogs (62.5%) and 25 of 37 male dogs (67.5%). 53 dogs were positive by ELISA. Antibody positivity was detected in 30 (63.5%) of 47 dogs aged 0 > 2 years and 23 (65.5%) of 35 dogs aged 2 > 4 years. When evaluated in terms of age and gender, it was determined that the seropositivity rates in the ELISA test were higher in the 0 > 2 age group compared to the 2 > 4 age group and in male dogs compared to female dogs. However, these results were not statistically significant ($P>0.05$). The distribution of seropositivity according to *Helicobacter* IgG ELISA test according to age and gender is shown in TABLE II.

TABLE II
Distribution of seropositivity by age and gender according to *Helicobacter* IgG ELISA test (n=82)

Parameters n=82		Seronegative	Seropositive	Average value (6,93–8,80) mg·dL^{-1}
Age	0 > 2 (n=47)	17 (36,5%)	30 (63,5%)	7,75
	2 > 4 (n=35)	12 (34,5%)	23 (65,5%)	8,01
Gender	Female (n=45)	17 (37,5%)	28 (62,5%)	8,11
	Male (n=37)	12 (32,5%)	25 (67,5%)	7,75

Haemotological parameters

Haematological parameters of the healthy and infected groups are presented in TABLE III. WBC, NEU, NEU % (P<0.001), MON and RDW–SD (P<0.05) values in the infected group were statistically significantly higher than in the control group, while the LYM % value was found to be statistically significantly higher in the healthy group than in the infected group (P<0.001). There was no statistically significant difference between the groups in terms of LYM, EOS, EOS %, MON %, RBC, HGB, HCT, MCV, MCH, MCHC, RDW–CV, PLT, MPV, PDW and PCT values (P>0.05).

TABLE III
The levels of hematological parameters in infected and control dogs

Parameters	Control (n=6)	Infected (n=6)	*P*
WBC (10^3·µL^{-1})	12.08±4.10	20.93±2.36	0.001
NEU (10^3·µL^{-1})	8.00±2.93	16.92±2.30	<0.001
LYM (10^3·µL^{-1})	2.53±0.91	1.60±1.01	0.130
MON (10^3·µL^{-1})	1.08±0.47	1.76±0.53	<0.05
EOS (10^3·L^{-1})	0.48±0.24	0.64±0.63	0.570
NEU %	66.20±6.03	80.93±7.23	<0.01
LYM %	21.00±4.31	7.63±4.74	<0.001
MON %	8.73±1.24	8.38±2.15	0.737
EOS %	4.07±2.31	3.63±2.77	0.775
RBC (10^6·µL^{-1})	6.06±0.54	5.66±0.82	0.350
HGB (g·dL^{-1})	14.23±0.83	13.75±1.85	0.577
HCT %	41.88±3.74	39.13±6.83	0.407
MCV (fL)	70.00±1.99	69.07±6.59	0.747
MCH (pg)	23.83±1.36	24.38±1.75	0.557
MCHC (g·L^{-1})	340.67±17.58	356.67±48.81	0.477
RDW–CV %	14.08±0.74	16.03±2.22	0.069
RDW–SD (fL)	37.67±2.29	42.63±3.36	<0.05
PLT (10^3·µL^{-1})	260.83±55.48	343.83±102.14	0.111
MPV (fL)	10.03±1.08	9.38±1.44	0.397
PDW (fL)	15.47±0.37	15.46±0.53	1.00
PCT (mL·L^{-1})	2.60±0.53	3.22±0.96	0.193

Data were expressed as mean ± standard deviation

Biochemical findings

- **Inflammatory markers (TNF–α, IL–1β, and CRP)**

The results of the biochemical analysis, inflammation markers, *Helicobacter* IgG ELISA, vitamin B_{12} and folic acid concentrations of the control and infected groups are presented in TABLE II. The concentrations of TNF–α (21.17 pg·mL^{-1} *vs.* 48.21 pg·mL^{-1}), IL–1β (73.41 pg·mL^{-1} *vs.* 37.60 pg·mL^{-1}) (P<0.01) and CRP (644.0 mg·dL^{-1} *vs.* 234.01 mg·dL^{-1}) (P<0.001) were found to be higher than in the control group.

- **Vitamins B_{12} and B_9 (folic acid)**

Serum cobalamin (9.54 pg·dL^{-1}, *vs.* 17.18 pg·dL^{-1}) (P<0.05) and folic acid (2004.40 ng·mL^{-1}, *vs.* 870.85 ng·mL^{-1}) (P<0.01) concentrations of the infected group were found to be significantly lower than in the control group. These findings are presented in TABLE IV.

TABLE IV
The concentrations of inflammatory markers and vitamins in infected and control dogs

Parameters	Control (n=6)	Infected (n=6)	*P*
TNF–α (pg·mL^{-1})	21.17	48.21	<0.01
IL–1β (pg·mL^{-1})	37.60±8.40	73.41±20.29	<0.01
CRP (mg·dL^{-1})	234.01±74.99	644.04±203.94	<0.001
Helicobacter IgG (mg·dL^{-1})	5.93	10.83	<0.01
B_{12} (pg·dL^{-1})	17.18±5.14	9.54±2.21	<0.05
Folic acid (ng·mL^{-1})	2004.40±355.92	870.85±324.47	<0.001

Data were expressed as mean ± standard deviation

Correlation findings

Spearman correlation analysis was applied to TNF–α, IL–1β, *Helicobacter* IgG, CRP, B_{12} and folic acid parameters in control and infected dogs. Statistically significant positive and high correlations were determined between *Helicobacter* IgG and TNF–α, IL–1β and CRP. Statistically significant, negative and moderate correlations were found between *Helicobacter* IgG and B_{12} and folic acid. Statistically significant, negative and high correlations were observed between TNF–α and B_{12} and folic acid. There was a statistically significant, positive and very high correlation between TNF–α and CRP. Statistically significant, negative and high correlations were also observed between CRP and B_{12} and folic acid. Statistically significant, positive and high correlations were found, as well, between CRP and IL–1β and between B_{12} and folic acid. Correlation results between parameters in control and infected dogs are given in TABLE V.

In this study, for the first time in Türkiye, the determination of *Helicobacter* agents in shelter dogs by faecal antigen test and the ELISA method, along with the application of the treatment in the early period of the infection, were accomplished. There are many methods for the diagnosis of the presence of *Helicobacter* of gastric origin. However, each of these methods has both advantages and disadvantages. The use of one or more tests, the accessibility of the tests, the equipment needed in the laboratories, and the clinical conditions of the sick animals to be sampled are determinative for the methods chosen in the diagnosis [8]. Most immunoassay methods rely

TABLE V
Spearman correlation results between parameters in control and infected dogs

Parameters	IgG	TNF–α	IL–1β	B_{12}	CRP	Folic acid
IgG	1.000	0.664*	0.727**	-0.594*	0.678*	-0.832**
TNF–α		1.000	0.490	-0.741**	0.811**	-0.790**
IL–1β			1.000	-0.350	0.643*	-0.503
B_{12}				1.000	-0.685*	0.650*
CRP					1.000	-0.741**
Folic acid						1.000

on the detection of *Helicobacter* antibodies in the serum. In the case of active infection, IgM levels can be detected, followed by an increase in IgG and IgA levels, which remain consistently high until the infection clears [5]. In the presented study, faecal antigen test and *Helicobacter* IgG ELISA test in serum, which are among the non–invasive methods, were used to diagnose the presence of *Helicobacter*.

Hong *et al.* [12] applied the *Helicobacter* faecal antigen test [HpSA] and faecal PCR tests to identify *Helicobacter* spp. from faecal samples taken from eight dogs showing signs of gastritis and detected positivity in only two dogs with the faecal antigen test. Faten *et al.* [13] applied the faecal antigen test in 25 dogs without clinical signs of gastritis; likewise, Haggag *et al.* [14] in 50 dogs, and no positivity was detected in both studies. In the present study, the faecal antigen test was applied to a total of 82 dogs showing clinical symptoms, and seropositivity was found in 6 of them. Samples were collected from shelter dogs showing similar clinical symptoms at the same time period and under the same conditions. Fecal antigen test and ELISA were performed on a total of 82 dogs. As a result of faecal antigen test, positivity was found in 6 of them (7.5%). Seropositivity was found in a large number of samples by ELISA test. Seropositivity was found in 30 out of 47 (63.5%) in the 0 > 2 age group and in 23 out of 35 (65.5%) in the 2 > 4 age group by ELISA method. It was determined that ELISA test was able to detect positivity at higher levels compared to faecal antigen test. However, these results were not statistically significant (P>0.05). Waheeb *et al.* [5] found 20,5% seropositivity in 18 of the samples taken from 88 dogs by *Helicobacter* IgG ELISA method. Moussa *et al.* [15] applied *Helicobacter* IgG ELISA to the blood serum of 30 dogs owned by people with gastritis symptoms and found seropositivity in all of the samples. In this study, a seropositivity rate of 64.6% was detected in 53 of the samples taken from 82 dogs living in shelter conditions.The fact that the rate of *Helicobacter* IgG seropositivity in dogs owned by people with gastritis symptoms is higher than that in dogs living in shelter conditions reveals the zoonotic feature of the *Helicobacter* agent. It was determined that faecal antigen positivity was more common in the 0 > 2 age group compared to the 2 > 4 age group and in male dogs compared to female dogs. However, these results were not statistically significant (P>0.05).

In studies related to *Helicobacter spp.* in dogs, some haematological changes have been reported [16,17,18,19,20,21,22]. It has been reported that lymphocytosis due to lymphofollicular hyperpyloplasia occurs in dogs with *Helicobacter* infection [16]. Since *Helicobacter* agents can cause lymphoplastic, eosinophilic or chronic gastritis, neutrophilic leukocytosis is encountered in the blood in such cases [17]. Red blood cell (RBC) synthesis is regulated by many factors, such as erythropoietin, iron, vitamin B_{12}, folic acid, and vitamin C. Decreases in erythropoietin, iron, vitamin B_{12}, folic acid levels and, therefore, a decrease in RBC, can be observed in *Helicobacter* infections [9]. Meral *et al.* [18] reported a decrease in erythrocyte and hemoglobin counts, an increase in platelet counts and a decrease in hematocrit ratios in infected dogs. Testault *et al.* [19] reported an increase in leukocyte, neutrophil and granulocyte counts and a decrease in erythrocyte and hemoblobin counts in infected dogs. Baan *et al.* [20] determined a decrease in hematocrit rates in infected dogs compared to healthy dogs in their study. Meral *et al.* [17] determined an increase in leukocyte and lymphocyte counts and a decrease in hemoglobin count in infected dogs.

Patel *et al.* [21] on the other hand, revealed a significant increase in neutrophil count in infected dogs. Papagiannakis *et al.* [22] reported a decrease in platelet concentrations in *Helicobacter*–induced gastrointestinal diseases. There are similarities between the results of these studies and the results obtained in the present study.

Acute and chronic inflammation caused by *Helicobacter* can damage epithelial cells and cause inflammatory oedema, atrophy, and necrosis/apoptosis. IL–1 and TNF–α are important proinflammatory cytokines in this process [23]. *Helicobacter* colonization causes an inflammatory reaction in the gastric mucosa; this is mediated by neutrophils and mononuclear cells and is characterized by increases in various cytokines such as TNF–α and IL–1ß [24]. TNF–α is involved in the regulation of immunity and inflammation and is widely and constitutively expressed by activated immune cells and by fibroblasts, endothelial and epithelial cells that respond to proinflammatory cytokines [25]. The increase in TNF–α concentration in *Helicobacter*–infected chronic atrophic gastritis (CAG) is associated with the degree of chronic inflammation. Soluble TNF receptors (TNFRs) have been shown to be actively produced in *Helicobacter*–infected gastric mucosa. High levels of IL–1ß secretion by infected cells suggesting cellular damage, whatever the cause, may act synergistically with *Helicobacter* infection to exacerbate the damage to the gastric epithelium [22]. Park *et al.* [26] stated that the TNF–α level in *Helicobacter* infection increased 3 times in the infected group compared to the control group, and the IL–1ß level increased 3.5 times in the infected group compared to the control group. Seim–Wikse *et al.* [27] reported that CRP concentrations in infected dogs were higher than those in healthy dogs.

Lin *et al.* [28] found a significant increase in TNF–α and IL–1ß in infected dogs and reported that these excessive increases in TNF–α and IL–1ß concentrations may be effective in the inflammatory response to *Helicobacter* infections. Thus, they demonstrated that the pathogenesis of *Helicobacter* infection is associated with high levels of cytokinins such as TNF–α and IL–1ß. In the present study, serum TNF–α, IL–1ß and CRP concentrations were significantly higher in the infected group (P<0.01).

In the stomach, free cobalamin binds to haptocorrin, which in dogs is mostly produced by parietal cells in the gastric mucosa. Haptocorrin is digested by pancreatic proteases in the duodenum, and then free cobalamin is bound to the intrinsic factor. Although intrinsic factor is mainly produced by the exocrine pancreas in dogs, it is also produced in the parietal cells of the stomach. Parietal cells that produce both haptocorrin and intrinsic factor are typically lost in gastropathies. This condition leads to hypocobalaminemia in dogs [29]. In the changes in the microbiota of the gastrointestinal tract, there is a decrease in the absorption of folic acid from the proximal small intestine and of cobalamin by the ileum, depending on the density of the bacterial

presence. However, a diet high in folate may result in increased serum folate concentrations regardless of disease. Unrelated to the source of infection, pathological conditions in the ileum can also damage cobalamin receptors and thus lead to cobalamin malabsorption [30].

It was stated by Andres *et al.* [31] that this condition is caused by food cobalamin malabsorption syndrome, and stomach diseases associated with *Helicobacter* infection are among the most important causes of this syndrome. Yanik *et al.* [32] state that *Helicobacter* infection, which can become chronic, causes vitamin B_{12} and folate absorption disorder, leading to an increase in circulating homocysteine levels. Accordingly, increased homocysteine levels due to *Helicobacter* infection are thought to play a role in endothelial dysfunction. Seim-Wikse *et al.* [27] stated in their study that folic acid and cobalamin concentrations are biomarkers for *Helicobacter* infections that can cause chronic gastritis. Sobczyńska-Malefora *et al.* [33] reported that *Helicobacter*-derived infections could predispose patients to pernicious anaemia by inducing autoantibodies against antigens in the gastric mucosa. It is predicted that this effect may be due to *Helicobacter*-induced gastric atrophy and may be due to the inhibition of B_{12}, an important vitamin that needs to be absorbed by the body, due to disruptions in the intestinal transit of the foods ingested. Pernicious anaemia, a stage of chronic vitamin B_{12} deficiency, has been associated with *Helicobacter* infection. *Helicobacter* infection can cause malabsorption of different micronutrients, including vitamin B_{12}. In patients with vitamin B_{12} deficiency, *Helicobacter* eradication is followed by increased serum vitamin B_{12} levels and decreased serum homocysteine levels [34]. Mwafy *et al.* [35] cobalamin (B_{12}) was found to be 378.2 pg·mL^{-1} in the control group and 262.5 pg·mL^{-1} in the infected group. Similarly, in the presented study, serum cobalamin (B_{12}) levels were found to be lower in the infected group than in the control group (P<0.05)(TABLE III). Augustin *et al.* [36] think that folate concentrations above the reference range may be caused by serious bacterial growth in the small intestine levels, and folate concentrations lower than the reference range may be caused by infections originating from *Helicobacter*. In the present study, it was determined that folic acid concentrations of the infected group decreased significantly compared to the control group (P<0.001)(TABLE III).

CONCLUSIONS

In conclusion, this study demonstrates that the parameters used in this study are effective markers in the prognosis and follow-up of the treatment process of *Helicobacter*-borne infection in dogs, as well as the usefulness of fecal antigen testing and ELISA methods in the early diagnosis of the presence of the causative agent.

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Conflict of interest

The authors declare no conflict of interest.

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