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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Comparative Assessment of Fecal Microbiota in Healthy and Epileptic Dogs

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Abstract: Idiopathic epilepsy is a neurological condition affecting dogs and cats characterized by repeated seizures for which no cause other than a hypothesized genetic predisposition can be identified. The aim of this study was to comparatively evaluate the content of mesophilic aerobic bacteria, coliform bacteria, *Escherichia coli*, *Enterococcus* spp., *Staphylococcus aureus* and *Lactobacillus* spp. in the fecal flora of healthy dogs and dogs diagnosed with epilepsy. Feces of 10 healthy dogs and 10 dogs diagnosed with epilepsy were used as study material. Selective isolation and counts were performed with inoculating the fecal samples were on Tryptic Soy Agar for mesophilic aerobic bacteria, MacConkey Agar for coliform bacteria, Eosin Methylene Blue Agar for *E. coli*, Bile Esculin Agar for *Enterococcus* spp., Mannitol Salt Agar for *S. aureus* and De Man, Rogosa and Sharpe Agar for *Lactobacillus* spp. The numbers of mesophilic aerobic bacteria, coliform bacteria, *E. coli*, *Enterococcus* spp., *S. aureus* and *Lactobacillus* spp. were $6.6x10^6$, $2.6x10^5$, $1.4x10^5$, $1.6x10^5$, $3.2x10^4$ and 4.1×10^4 cfu/mg in the fecal flora of healthy dogs; and $9.0x10^6$, $5.0x10^5$, $2.8x10^5$, $6.4x10^5$, $6.3x10^4$, and $1.2x10^4$ cfu/mg in dogs diagnosed with epilepsy, respectively. As a result of the study, there was a decrease in the number of *Lactobacillus* spp. in the fecal flora of dogs with epilepsy compared to healthy dogs, while the number of other bacteria increased. It was concluded that adding probiotic bacteria to the diet of epileptic dogs could help regulate the gut microbiota and reduce the severity and frequency of seizures.

Keywords: Dog, fecal flora, idiopathic epilepsy.

Sağlıklı ve Epileptik Köpeklerde Fekal Mikrobiyotanın Karşılaştırmalı Değerlendirmesi

Öz: İdiyopatik epilepsi, hipotez edilen genetik yatkınlıktan başka bir nedenin tanımlanamadığı, tekrarlayan nöbetlerle karakterize, köpekleri ve kedileri etkileyen nörolojik bir durumdur. Bu çalışma sağlıklı köpeklerin ve epilepsi tanısı almış köpeklerin fekal florasındaki mezofilik aerobik bakteri, koliform bakteri, *Escherichia coli*, *Enterococcus* spp., *Staphylococcus aureus* ve *Lactobacillus* spp. içeriğini karşılaştırmalı olarak değerlendirmek amacıyla gerçekleştirildi. Çalışma materyali olarak 10 sağlıklı ve 10 epilepsi tanısı almış köpeğin dışkısı kullanıldı. Seçici izolasyon ve sayımlar, dışkı örneklerinin mezofilik aerobik bakteriler için Tryptic Soy Agar, koliform bakteriler için MacConkey Agar, *E. coli* için Eosin Metilen Mavisi Agar, *Enterococcus* spp. için Bile Eskülin Agar, *S. aureus* için Mannitol Salt Agar ve *Lactobacillus* spp. için De Man, Rogosa ve Sharpe Agar'a ekilmesiyle gerçekleştirildi. Sağlıklı köpeklerin dışkı florasında mezofilik aerobik bakteri, koliform bakteri, *E. coli*, *Enterococcus* spp., *S. aureus* ve *Lactobacillus* spp. sayıları sırasıyla $6,6x10^6$, $2,6x10^5$, $1,4x10^5$, $1,6x10^5$, $3,2x10^4$ ve $4,1 \times 10^4$ kob/mg ve epilepsi tanısı konulan köpeklerde sırasıyla $9,0x10^6$, $5,0x10^5$, $2,8x10^5$, $6,4x10^5$, $6,3x10^4$ ve $1,2x10^4$ kob/mg olarak bulundu. Sonuç olarak, epilepsili köpeklerin dışkı florasında *Lactobacillus* spp. sayısında sağlıklı köpeklere kıyasla bir azalma görülürken, diğer bakterilerin sayısında artış görüldü. Epileptik köpeklerin diyetine probiyotik bakteri eklenmesinin bağırsak mikrobiyotasını düzenlemeye ve nöbetlerin şiddetini ve sıklığını azaltmaya yardımcı olabileceği kanısına varıldı.

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Anahtar kelimeler: Dışkı florası, idiyopatik epilepsi, köpek.

INTRODUCTION

Epilepsy was defined as a chronic neurological system disorder that impairs the quality of life of both the sufferer and their family members, can last a lifetime, and is most frequent in dogs (Wessmann et al., 2016). Epilepsy results from congenital or acquired disorders and is characterized by a permanent tendency to recurrent epileptic seizures. Epilepsy appears quickly as a result of aberrant activity in neurons, and presents with motor, autonomic, and behavioral symptoms. Epileptic seizures occur in episodes and are short-lived (usually less than 2-3 minutes). Epilepsy can develop for many reasons. Some cases are genetic and develop a progressive picture produced by genetic and epigenetic factors, while in some cases, inflammatory causes (inflammatory, infectious, trauma, vascular, neoplastic) play a role in the disease development. The cause of most cases of epilepsy is unknown (Hülsmeyer et al., 2015). The true prevalence of epilepsy in dogs is not known exactly, and is estimated to be 0.6-0.75% (Kearsley-Fleet et al., 2013). It has been noted that this rate is comparable to the prevalence of human epilepsy (Löscher, 2022). As a result of epidemiological studies conducted in specific breeds with idiopathic epilepsy, there is evidence for the presence of hereditary epilepsy 3.1% in Labrador retriever, 9.4% in Belgian shepherd, 8.9% in Basset Griffon vendeen and other breeds (Hülsmeyer et al., 2015, Ekenstedt and Oberbauer, 2013).

The microbiota of intestine composed of approximately 1000 species and 10^8-10^{11} cells that provide immunological, metabolic, and protective functions through cytokines, neurotransmitters, and short-chain fatty acids. The gut microbiome of dog is composed of bacteria, archaea, fungi, protozoa, yeasts, viruses, and parasites, though its exact makeup is unknown. Although it is consistent throughout different dog groups, it also has its own distinct characteristics. The most frequent bacteria in dogs are *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, which are rather stable in a healthy intestine. The relative number and composition of gut microbiota are critical for its effective defense against infections and numerous metabolic processes (Tuniyazi et al., 2022). The gut microbiota evolves dynamically in response to both internal (genetic) and external (diet, environment, illnesses, etc.) variables (Wang et al., 2023). Studies have discovered that the intestinal flora and the brain interact. The term Gut-Microbiota-Brain Axis (GMB) describes to the simultaneous communication between the enteric nervous and central systems, as well as the significance of the intestinal microbiota. It is now thought that symbiotic microbiota, in addition to influencing digestive system function, may also have a bidirectional and reversible impact on extraintestinal pathogenic systems such as the neurological system and behavior (Wang and Wang, 2016). Understanding the intestinal microbiota may help us understand the progression and severity of epilepsy, and so predict its prognosis (García-Belenguer et al., 2021).

It was aimed to comparatively evaluate the contents of mesophilic aerobic bacteria (MAB), coliform bacteria (CB), *Escherichia coli*, *Enterococcus* spp., *Staphylococcus aureus* and *Lactobacillus* spp. in the fecal flora of healthy and epileptic dogs in the study.

MATERIAL AND METHOD

Animal Material: The animal material of the study consisted of a total of 20 dogs of various breeds and ages, 10 healthy and 10 epileptics, brought to the Animal Hospital of Ondokuz Mayis University for medical examination between January and July 2023. Breed, age and gender information's of the animals included in the study were presented in Table 1.

Table 1. Informations of dogs constituting the animal material of the study.

No.	State of health	Race	Gender	Age
1	Healthy	German Shepherd	female	9 months
2	Healthy	Crossbreed	male	2 years
3	Healthy	Crossbreed	female	4 months
4	Healthy	Crossbreed	female	5 months
5	Healthy	Crossbreed	male	3 years
6	Healthy	Crossbreed	male	3 years
7	Healthy	Crossbreed	female	6 months
8	Healthy	Crossbreed	male	3 years
9	Healthy	Crossbreed	male	5 years
10	Healthy	Coil	female	6 months
11	Idiopathic epilepsy	Golden Retriever	female	14 years
12	Idiopathic epilepsy	Border Collie	female	3.5 years
13	Idiopathic epilepsy	Terrier	male	2 years
14	Idiopathic epilepsy	Golden Retriever	female	11 years
15	Idiopathic epilepsy	Cocker Spaniel	male	3 years
16	Idiopathic epilepsy	Crossbreed	male	9 years
17	Idiopathic epilepsy	Golden	female	6 years
18	Idiopathic epilepsy	French Bulldog	female	2.5 years
19	Idiopathic epilepsy	Ibiza Podenco	female	4.5 years
20	Idiopathic epilepsy	Crossbreed	male	7.5 years

The Level I confidence level guideline for the diagnosis of idiopathic epilepsy determined by the International Veterinary Epilepsy Task Force was used as the criteria for the inclusion of epileptic animals in the study group (De Risio et al., 2015). According to this guideline, the presence of two or more unprovoked epileptic seizures in the last 24 h, no significant findings in the physical and neurological examination during the interictal period, and the absence of any abnormalities in complete blood count, urine analysis, serum biochemistry tests, cardiac and abdominal ultrasonography findings, and electrocardiography were used for the diagnosis of idiopathic epilepsy.

The inclusion criteria for the healthy dogs constituting the control group were determined as not having a primary disease, not having a neurological disease diagnosed during physical examination, and not having received any antiepileptic treatment before. None of the animals had any probiotic use in their diet.

For the study, fecal samples were collected directly from the rectum of the animal and placed in sterile fecal containers as at least 10 g. After the fecal samples were collected, they were brought to the Microbiology Laboratory for bacteriological examinations.

Bacteriological Isolation: Stool samples were weighed as 10 mg with a precision scale. They were homogenized with sterile physiological saline (SPS) at a ratio of 1/10 (w/v). Dilutions were prepared with sterile SPS up to 10^{-8}. The prepared dilutions were used for the selective isolation and count of MAB, CB, *E. coli*, *S. aureus*, *Lactobacillus* spp. and *Enterococcus* spp (Sarica et al., 2005).

For the isolation and enumeration of MAB, CB, and *Enterococcus* spp., 0.1 ml of 10^{-2}, 10^{-3} and 10^{-4} dilutions were taken and spread to the three Tryptic Soy Agar (TSA), MacConkey agar (MAC), and Bile Esculin Agar (BEA), respectively. The cultured media were incubated at 37º C for 24 h under aerobic conditions. At the end of the incubation period, media containing 1-20 bacterial colonies were evaluated (all colonies for MAB, pink bacterial colonies for CB and black-esculin-positive bacterial colonies for *Enterococcus* spp.) and the bacterial colonies were counted for calculating the number of these bacteria (Sarica et al., 2005; Gülhan et al., 2015; Özşavlı et al., 2018; Strompfová et al., 2004).

For the isolation and enumeration of *Lactobacillus* spp., 0.1 ml of 10^{-2}, 10^{-3} and 10^{-4} dilutions were taken and spread to the three De Man, Rogosa and Sharpe (MRS) agars. They were incubated in anaerobic conditions at 37ºC for 48 h in a jar with an anaerobic gas pack (Turhan et al., 2016). At the end of the incubation period, the media containing bacterial colonies were counted. After the counting, the number of *Lactobacillus* spp. in the fecal content was calculated.

For the isolation and enumeration of *S. aureus* and *E. coli,* 0.1 ml of 10^{-3}, 10^{-4} and 10^{-5} dilutions were taken and spread onto the three Mannitol Salt Agar (MSA) and Eosin Methylene Blue agar (EMB), respectively. The inoculated media were incubated at 37º C for 24 h under aerobic conditions (Nsofor and Christian, 2012; Patır and Güran, 2018). At the end of the incubation period, colonies that were mannitol positive and formed yellow bright zones were evaluated as *S. aureus;* and metalic highlights for *E. coli*. After the counting of these colonies, the number of *S. aureus* and *E. coli* in the fecal content was calculated.

Statistical Analysis: Statistical evaluation of the obtained data was carried out using descriptive analysis, Pearson correlation analysis and independent samples t test using the SPSS software.

RESULTS

Isolation and Enumeration of MAB: The number of MAB in the fecal flora was determined as in average of 6.6 x 10^6 cfu/mg in healthy dogs and 9.0 x 10^6 cfu/mg in dogs diagnosed with epilepsy (Table 2). When evaluated according to gender, the number of MAB in the fecal flora of healthy female dogs (n=5) was calculated as 6.6 x 10^6 cfu/mg and in female dogs diagnosed with epilepsy (n=6) was 8.3 x 10^6 cfu/mg. The number of MAB in the fecal flora of healthy male dogs (n=5) was determined as 6.5 x 10^6 cfu/mg and in male dogs diagnosed with epilepsy (n=4) was 1.0 x 10^7 cfu/mg (Table 3).

Table 2. Total number of bacteria determined in fecal flora and statistical significance.

	n	Epilepsy	Healthy	P
MAB	10	9.0 x 10^6 ± 5.1 x 10^5	6.6 x 10^6 ± 6.4 x 10^5	0.001
E. coli	10	2.8 x 10^5 ± 2.6 x 10^4	1.4 x 10^5 ± 1.5 x 10^4	0.524
Coliform bacteria	10	5.0 x 10^5 ± 6.4 x 10^4	2.6 x 10^5 ± 7.1 x 10^3	0.00
***Enterococcus* spp.**	10	6.4 x 10^5 ± 7.0 x 10^4	1.6 x 10^5 ± 8.0 x 10^3	0.00
S. aureus	10	6.3 x 10^5 ± 4.3 x 10^3	3.2 x 10^4 ± 2.9 x 10^3	0.00
***Lactobacillus* spp.**	10	1.2 x 10^4 ± 5.82 x 10^5	4.1 x 10^4 ± 3.3 x 10^3	0.00

Table 3. Number of bacteria determined in fecal flora and statistical significance according to the gender.

		Epilepsy	Healthy	P
MAB	**Female**	8.3 x 10^6 ± 9.2 x 10^5	6.6 x 10^6 ± 1 x 10^6	0.035
	Male	1.0 x 107 ± 5.0 x 10^5	6.5 x 10^6 ± 4.9 x 10^5	0.015
CB	**Female**	4.3 x 10^5 ±8.3 x 10^4	2.6 x 10^5 ± 1.1 x 10^4	0.035
	Male	4.8 x 10^5 ± 8.3 x 10^4	2.5 x 10^5 ± 6.2 x 10^3	0.022
E. coli	**Female**	2.7 x 10^5 ± 2.3 x 10^4	1.6 x 10^5 ± 2.2 x 10^4	0.471
	Male	2.9 x 10^5 ± 4.4 x 10^4	1.3 x 10^5 ± 2.1 x 10^4	0.237
ENT	**Female**	7.0 x 10^5 ± 1.2 x 10^5	1.6 x 10^5 ± 1.1 x 10^4	0.022
	Male	5.5 x 10^5 ± 8 x 10^4	1.5 x 10^5 ± 1.4 x 10^4	0.002
LB	**Female**	1.0 x 10^4 ± 8.1 x 10^2	4.7 x 10^4 ± 4.9 x 10^3	0.0
	Male	1.3 x 10^4 ± 6 x 10^2	3.4 x 10^4 ± 4.8 x 10^3	0.009
SA	**Female**	6.4 x 10^4 ± 5 x 10^3	3.3 x 10^4 ± 3.4 x 10^3	0.003
	Male	6.4 x 10^4 ± 7.3 x 10^3	3.1 x 10^4 ± 4.7 x 10^3	0.004

MAB: Mesophilic aerobic bacteria; CB: Coliform bacteria, ENT: *Enterococcus* spp.; SA: *S. aureus*; LB: *Lactobacillus* spp.

Coliform Bacteria Isolation and Enumeration: The number of CB in the fecal flora of healthy dogs was determined as in average of 2.6 x 10^5 cfu/mg and in dogs diagnosed with epilepsy as 5.0 x 10^5 cfu/mg (Table 2). When evaluated according to gender, the number of CB in the fecal flora of healthy female dogs (n=5) was calculated as 2.6 x 10^5 cfu/mg and 4.3 x 10^5 cfu/mg in female dogs diagnosed with epilepsy (n=6). The number of CB in the fecal flora of healthy male dogs (n=5) was determined as 2.5 x 10^5 cfu/mg and in male dogs diagnosed with epilepsy (n=4) as 4.8 x 10^5 cfu/mg (Table 3).

E. coli Isolation and Enumeration: The number of *E. coli* in the fecal flora of healthy dogs and in dogs diagnosed with epilepsy was in average of 1.4 x 10^5 cfu/mg and 2.8 x 10^5 cfu/mg, respectively (Table 2). *E. coli* in the fecal flora of healthy female dogs (n=5) was calculated as 1.6 x 10^5 cfu/mg and in female dogs diagnosed with epilepsy (n=6) as 2.7 x 10^5 cfu /mg. The number of *E. coli* in the fecal flora of healthy male dogs (n=5) was calculated as 1.3 x 10^5 cfu/mg and in male dogs diagnosed with epilepsy (n=4) as 2.9 x 10^5 cfu/mg (Table 3).

Isolation and Enumeration of Enterococcus spp.: *Enterococcus* spp. of the fecal content was calculated as in average of 1.6 x 10^5 cfu/mg in the fecal flora of healthy dogs and 6.4 x 10^5 cfu/mg in dogs diagnosed with epilepsy (Table 2). When evaluated according to gender, the number of *Enterococcus* spp. in the fecal flora of healthy female dogs (n=5) was determined as 1.6 x 10^5 cfu/mg and in female dogs diagnosed with epilepsy (n=6) was 7.0 x 10^5 cfu/mg. In the fecal flora of healthy male dogs (n=5) and in male dogs diagnosed with epilepsy (n=4) 1.5 x 10^5 cfu/mg and 5.5 x 10^5 cfu/mg of *Enterococcus* spp. determined, respectively (Table 3).

Isolation and Enumeration of Lactobacillus spp.: After inoculation on MRS agar for selective isolation of *Lactobacillus* spp., the bacterial colonies evaluated were counted as in average of 4.1 x 10^4 cfu/mg and in the fecal flora of healthy dogs and 1.2 x 10^4 cfu/mg in dogs diagnosed with epilepsy (Table 2). When evaluated according to gender, the number of *Lactobacillus* spp. in the fecal flora of healthy female dogs (n=5) was calculated as 4.7 x 10^4 cfu/mg and in female dogs diagnosed with epilepsy (n=6) was 1.0 x 10^4 cfu/mg. The number of MAB in the fecal flora of healthy male dogs (n=5) was determined as 3.4 x 10^4 cfu/mg cfu/mg and in male dogs diagnosed with epilepsy (n=4) was 1.3 x 10^4 cfu/mg (Table 3).

Isolation and Enumeration of S. aureus: *S. aureus* of the fecal content was calculated as in average of 3.2 x 10^4 cfu/mg in the fecal flora of healthy dogs and 6.3 x 10^4 cfu/mg in dogs diagnosed with epilepsy (Table 2). When evaluated according to gender, the number of *Enterococcus* spp. in the fecal flora of healthy female dogs (n=5) was determined as 3.3 x 10^4 cfu/mg and in female dogs diagnosed with epilepsy (n=6) was 6.4 x 10^4 cfu/mg. In the fecal flora of healthy male dogs (n=5) and in male dogs diagnosed with epilepsy (n=4) 3.1 x 10^4 cfu/mg and 6.4 x 10^4 cfu/mg of *Enterococcus* spp. determined, respectively (Table 3).

Statistical Evaluation of Fecal Flora: The numbers and statistical significance of changes of MAB, CB, *E. coli, S. aureus, Lactobacillus* spp. and *Enterococcus* spp. in fecal samples were evaluated within the scope of the study and shown in Tables 2 and 3.

DISCUSSION AND CONCLUSION

The phrase intestinal microbiota is defined as to the population of fungi, bacteria, protozoa, and viruses found in GIT (Wang et al., 2023). Bacterial abundance and richness have been found to increase along the tract. Healthy dogs' small intestines have lower bacterial burdens than their colons with a range from 10^2 to 10^{11} cfu/g of luminal material throughout the GIT (Pilla and Suchodolski, 2020).

Recent research has revealed the intricate relationship between the gut bacteria and the brain. Symbiotic bacteria are hypothesized to alter gastrointestinal function as well as many extra intestinal pathogenic systems such as the nervous system and behavior in a bidirectional and reversible manner. Although the exact mechanism of communication between the gut microbiota and the brain has yet to be fully understood, it appears that the gut microbiota influences the brain not only via neuroanatomical pathways (Wang and Wang, 2016). It has been noted that the diversity of the gut microbiota is required not only for intestinal health but also for the physiological function of other organs, particularly the brain. Epilepsy is more common in certain gastrointestinal conditions. Irritable bowel syndrome (IBS) was more common in epileptic patients than in healthy controls, but it was also related with a higher load of emotional symptoms and insomnia. Another clinical trial found that the prevalence of functional GI problems was substantially higher in epileptic patients than in healthy people. These findings indicate a bidirectional relationship between GI and epilepsy (Wang et al., 2023).

Researchers discovered that transferring microbiota from chronically stressed mice into young mice enhances the development of epilepsy (Medel-Matus et al., 2018). Another study suggested that transplanting microbiota from epileptic mice could cause epileptic seizures by boosting brain excitability in healthy mice. Mice given microbiota to epileptic animals were likely to promote epilepsy than controls, demonstrating that microbiome influences seizures (Mengoni et al., 2021). Citraro et al. (2021) found that FMT from non-transgenic rats reduced seizure frequency and duration in rats with hereditary absence epilepsy.

Studies have found that healthy dogs have varying levels of MAB. In a study of the bacterial ecology in dogs' gastrointestinal tracts, the total MAB count was 6.6 x 10^4 cfu/mg in the ileum and 6.0 x 10^7 cfu/mg in the colon (Davis et al., 1977). In a study on the fecal microbiota of Beagle dogs, total mesophilic aerobic bacteria ranged from 1.9 to 5.0 x 10^7 cfu/mg (Benno et al., 1992). In a study on the immunological state and fecal microbiota of dogs after oral arabinogalactan administration, the total MAB count in the control group was determined to be 3.4 x 10^4 cfu/mg (Grieshop et al., 2002). Fecal bacterial populations in dogs vary with age, with young dogs having 1.7 x 10^7 cfu/mg mesophilic aerobic bacteria and old dogs having 6.6 x 10^7 cfu/mg (Simpson et al., 2002). In a study examining the effect of probiotic supplementation on fecal flora in adult healthy dogs, the total mesophilic aerobic bacteria count in the control group was found to be 6.4 x 10^6 cfu/mg (González-Ortiz et al., 2013). The study found that healthy dogs had significantly lower levels of MAB in their feces compared to epileptic dogs (P<0.05). Healthy dogs, both male and female, had significantly lower MAB than epileptic dogs (P<0.05).

Boothe and Debavalya (2011) showed that fecal coliform levels in healthy dogs ranged from 1.5 x 10^4 to 6.3 x 10^5 cfu/mg. When the small intestine and fecal microbiota of 22 healthy beagle dogs were compared, jejunal coliform content was 3.0 x 10^3 cfu/mg and fecal coliform content was 1.0 x 10^6 cfu/mg (Mentula et al., 2005). In a study looking into the impact of several prospective prebiotics and fiber-rich diets on the composition and activity of the canine intestinal microbiota, coliform bacterial levels in healthy dogs before supplementation were reported to be 1.9 x 10^2 cfu/mg (Biagi et al., 2010). In a study looking at the influence of dietary lactosucrose on the fecal flora of dogs, 5.0 x 10^3 cfu/mg coliform bacteria were found in the fecal flora of healthy dogs before the diet (Terada et al., 1992). In a study examining the effect of probiotic supplementation on the fecal flora of adult healthy dogs, the number of coliform bacteria before treatment was estimated to be 2.3 x 10^3 cfu/mg (González-Ortiz et al., 2013). Healthy dogs had significantly lower levels of coliform bacteria in their feces compared to epileptic dogs (P < 0.05). Healthy dogs, both male and female, have significantly lower CB levels in their flora compared to epileptic dogs (P<0.05).

In a study of the bacterial flora of the gastrointestinal system in dogs, 4.0 x 10^3 cfu/mg *E. coli* was found in the colon (Davis et al., 1977). In a research on the effect of probiotic use on fecal microbiota, the average number of *E. coli* was 2.0 x 10^4 cfu/mg in the healthy dog group that did not take probiotics (Pascher et al., 2008). The microbiome of healthy dogs with gastrointestinal disorders was studied, and the average *E. coli* concentration was found to be 2.5 × 10^4 cfu/mg (Blake et al., 2019). In a study on the alterations in bacterial fecal ecology caused by antibiotic usage, the average *E. coli* count in dogs not receiving antibiotics was found to be 3.1 x 10^4 cfu/mg (Sørum and Sunde, 2001). Another similar study with the same goal revealed an *E. coli* concentration of 6.7 x 10^3 cfu/mg (Lawrence et al., 2013). The effects of dietary fructooligosaccharide on the *E. coli* population in dogs' feces were investigated, and the average fecal *E. coli* in dogs fed a standard diet was calculated to be 3.8 x 10^4 cfu/mg (Willard et al., 2000). González-Ortiz et al. (2013) found that the *E. coli* count was 1.4 x 10^3 cfu/mg in a study looking at the effects of probiotic supplementation on the fecal flora of adult healthy dogs. When the immunological state and fecal microbiota of dogs were studied after oral arabinogalactan administration, the average *E. coli* count in the control group was reported to be 9.1 x 10^4 cfu/mg (Grieshop et al., 2002). According to the study's findings, the number of *E. coli* in the feces of healthy dogs was lower than that of epileptic dogs, although this difference was not statistically significant (P>0.05). Fecal flora analysis revealed a statistically significant difference (P<0.05) between male and female dogs with epilepsy in terms of *E. coli* presence.

Enterococcus spp. concentration was found to be 5.8 x 10^2 cfu/mg in the flora of healthy dogs (Blake et al., 2019). In a study examining the effects of antibiotic treatment on fecal flora in dogs, it was discovered that the fecal flora included 3.9 x 10^4 cfu/mg enterococci (Sørum and Sunde, 2001). Furthermore, 2.0 x 10^5 cfu/mg enterococci were found in the fecal microbiome of 22 healthy beagle dogs (Mentula et al., 2005). In a study looking at the impact of several possible prebiotics and fiber-rich meals on the composition and activity of the canine intestinal microbiota, the enterococci count was 1.3 x 10^3 cfu/mg in healthy dogs before supplementation (Biagi et al., 2010). The association between fecal enterococci population and age was discovered in dogs, with 9.3 x 10^3 cfu/mg enterococci in young dogs and 7.6 x 10^3 cfu/mg in elderly dogs (Simpson et al., 2002). In a study on the effect of probiotic supplementation on fecal flora in adult healthy dogs, it was discovered that the quantity of enterococci in the fecal flora of dogs who did not receive probiotics was 2.8 x 10^3 cfu/mg (González-Ortiz et al., 2013). Our investigation found that healthy dogs' feces contained an average of 1.6 x 10^5 cfu/mg enterococci. Healthy dogs had significantly lower levels of *Enterococcus* spp. compared to those with epilepsy (P<0.05). Healthy dogs had significantly lower levels of *Enterococcus* spp. in their flora compared to epileptic dogs (P<0.05).

In a study of the fecal microbiota composition in healthy dogs and dogs with gastrointestinal disorders, *Lactobacillus* spp. was shown to be present at an average of 1.9 x 10^3 cfu/mg (Blake et al., 2019). Sørum and Sunde (2001) found an average of 3.9 × 10^1 cfu/mg *Lactobacillus* spp. in the fecal flora of healthy dogs. Mentula et al. (2005) found that the jejunal *Lactobacillus* spp. content was 3.0 x 10^1 cfu/mg and the fecal content was 7.0 x 10^3 cfu/mg. In a study looking at the impact of several putative prebiotics and fiber-rich diets on the composition and activity of the canine intestinal microbiota, the *Lactobacillus* spp. count in healthy dogs before supplementation was found to be 1.2 x 10^3 cfu/mg (Biagi et al., 2010). *Lactobacillus* spp. were found in the fecal flora of healthy dogs fed a standard diet at 2.5 × 10^3 cfu/mg (Terada et al., 1992). The connection between fecal *Lactobacillus* spp. and age in dogs was found to be 2.1 x 10^3 cfu/mg in young dogs and 2.7 x 10^3 cfu/mg in old dogs. There were no significant differences between them (Simpson et al., 2002). Similarly, the number of *Lactobacillus* spp. found in healthy canines was 1.5 x 10^3 cfu/mg (González-Ortiz et al., 2013). In another investigation to investigate the change in fecal microbiota with the administration of probiotics in dogs, the amount of *Lactobacillus* spp. in healthy dogs that did not get probiotics was found to be 5.2 x 10^2 cfu/mg (Pascher et al., 2008). The number of *Lactobacillus* spp. found in feces of dogs with idiopathic epilepsy was 1.5 × 10^3 cfu/mg (González-Ortiz et al., 2013). In a study to examine the change in fecal microbiota with the use of probiotics in dogs, the amount of *Lactobacillus* spp. in the group of healthy dogs that did not get probiotics was found to be 5.2 x 10^2 cfu/mg (Pascher et al., 2008). In a small population research, feces were collected from an epileptic dog and a healthy dog who did not take medicine, lived in the same residence, and ate the same diet. The study on amplicon sequencing of 16S rRNA gene revealed statistically no significant variation in flora composition between control and epileptic dogs (Muñana et al., 2020). Our study found that healthy dogs had a higher number of *Lactobacillus* spp. compared to epileptic dogs, which was statistically significant (P<0.05). Healthy dogs had significantly larger levels of *Lactobacillus* spp. compared to epileptic dogs, regardless of gender (P < 0.05).

In their investigation on the fecal microbiota of Beagle dogs, Benno and Mitsuoka (Benno and Mitsuoka, 1992) found a fecal staphylococci level of 2.5 x10^2 cfu/mg. Similar findings were achieved in a study of the bacterial ecology of dogs' gastrointestinal systems, which determined that the staphylococci count in the ileum was 2.5 x10^2 cfu/mg. In a study comparing the small intestine and fecal microbiota in healthy Beagle dogs, jejunal staphylococci were calculated to be 7.0 x 10^1 cfu/mg, while 4 × 10^2 cfu/mg staphylococci were found in the feces (Mentula et al., 2005). When the influence of dietary lactosucrose on the fecal flora of dogs was studied, 5.0 x10^1 cfu/mg staphylococci were found in healthy dogs fed a standard diet (Terada et al., 1992). In a study examining the association between fecal staphylococci population and age in dogs, 1.0 x10^1 and 6.0 x10^1 cfu/mg enterococci were found in young and senior dogs, respectively (Simpson et al., 2002). The study found that healthy dogs, both male and female, had significantly lower levels of *S. aureus* in their feces than dogs with epilepsy (P<0.05).

The study examined the contents of MAB, CB, *S. aureus*, *E. coli*, *Lactobacillus* spp., and *Enterococcus* spp. for the statistical significance of changes in fecal samples collected from 10 healthy and 10 epileptic dogs of varied breeds and ages. The study found that healthy dogs had significantly lower levels of MAB, CB, *E. coli*, S. *aureus*, and *Enterococcus* spp. in their feces compared to epileptic dogs (P<0.05). Healthy dogs had significantly larger levels of *Lactobacillus* spp. in their feces compared to epileptic dogs (P<0.05). The lower number of *Lactobacillus* spp., which also has probiotic properties, and the higher number of MAB, CB, *E. coli*, *S. aureus*, and *Enterococcus* spp. in the fecal flora of dogs with epilepsy compared to healthy dogs were evaluated as indicators that the fecal flora of dogs with epilepsy was disrupted to the detriment of probiotic bacteria. This study's comparative data on the bacterial microflora of epileptic dogs suggests that introducing probiotic bacteria to their diets may help regulate the intestinal microbiota and reduce the frequency and severity of epileptic episodes.

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