ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Microbial Community Studying Of The Dogs' Gastrointestinal Tract By The T-Rflp Molecular Genetic Method And Assessing The Natural Resistance Of Animals.

Yury Evgenievitch Kuznetsov[1]*, Sergei Vladimirovitch Engashev[2], Ekaterina Sergeevna Engasheva[3], Ilia Nikolaevitch Nikonov[4], and Nadezhda Victorovna Kuznetsova[1].

[1]St. Petersburg State Academy of Veterinary Medicine, Chernigovskaya str., 5, Saint-Petersburg 196084, Russia.
[2]Moscow State Academy of Veterinary Medicine and Biotechnology – MVA named after K.I. Scriabin (Moscow SAVMB), Akademika Skryabina str, 23, Moscow 109472, Russia.
[3]All-Russian Scientific Research Institute of Veterinary Sanitation, Hygiene and Ecology – branch of All-Russian Scientific Research Institute of Experimental Veterinary Science of the Russian Academy of Sciences, Zvenigorodskoe shosse 5, Moscow, 123022, Russia.
[4]All-Russian Research Veterinary Institute of Poultry Science– Branch of the Federal State Budget Scientific Institution Federal Scientific Center "All-Russian Research and Technological Poultry Institute" of Russian Academy of Sciences, Chernikova str., 48, Saint-Petersburg, Lomonosov 198412, Russia.

ABSTRACT

T-RFLP (Terminal Limiting Fragment) Length polymorphism is a molecular biological method based on the analysis of polymorphism of long-term restriction fragments of microorganism DNA - one of the most promising for today to study the bacterial community and the structure of microbial culture as a whole. Earlier, the study of the microbiota of the gastrointestinal tract of animals was mainly carried out with the help of classical culture methods of microbiology, which do not always allow us to determine the cause of the sharp deterioration in animal health from the fact that many pathogenic microorganisms cannot be cultivated on artificial media, so they are sometimes called uncultivated uncultured). There was a misconception that in carnivorous animals the microbiota of the intestine is meager and few - 10-20 species. In the course of the study, it was found that in 12 experimental dogs, the gastrointestinal tract contains more than 287 species of microorganisms belonging to 13 types, of which 165 species are noncultivated. The second stage of our study was the study of the natural resistance of experimental animals, whose stool samples we took to determine the microbial community of the contents of their gastrointestinal tract. A reliable correlation between biochemical indices of α-, β-, γ-globulins, the level of lysozyme, bactericidal activity, and the presence of dysbiosis was not revealed. A reliable correlation was noted between the disorders in the composition of the present microflora and the activity of phagocytosis, which manifested itself in the enhancement of the phagocytic activity of the cells in the development of dysbiosis. IgG and IgM levels were not significantly different from normal, but there was a tendency for IgM to cling. The amount of IgA turned out to be significantly lower, and there was a correlation between the depth of disturbance in the composition of the intestinal microflora. The level of immune complexes increased depending on the severity of dysbiosis. In dogs with reduced natural resistance against the background of bactericidal and lysozyme activity, the number of immunoglobulins decreases, phagocytic activity decreases. On the basis of the carried out researches, it is possible to assert that dysbiosis is an important pathophysiological compensatory mechanism. Its appearance, apparently, is caused by the need to increase the antigen load. Based on the above, I believe, to conclude that studies on dysbiosis, can indirectly indicate a violation of the immune status of carnivorous, in particular dogs.
Keywords: bacteria, microflora, molecular-genetic method, DNA-sequencing, dog, immunities.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Microflora is a collection of different types of microorganisms besides bacteria, which includes fungi, algae, protozoa, etc., inhabiting any habitat, in our case, it is a living organism. It is established that changing the set of feeds in the diet leads to a reorganization of the ratio of individual microorganisms in the ecosystem of the gastrointestinal tract (GIT) of dogs, and, consequently, to a change in the state of animal health. Therefore, it is of undoubted interest to study the composition of the microbial community of dog feces.

The gastrointestinal tract performs various functions, it is not only digestive but also protective, as well as immune functions, in particular, the intestine participates in the realization of protective reactions of the organism against pathogenic, opportunistic microorganisms and many inorganic substances.

It is known that one of the most important functions of normal microflora is colonization resistance, which determines its protective properties. Under the influence of negative factors, the stability of the ratio of normal native (indigenous) flora, as well as adhesiveness and colonization resistance, is disturbed, which leads to the appearance of pathological processes called dysbiosis [1, 2, 3, 4].

Microflora is a collection of different types of microorganisms of bacteria, which includes fungi, algae, protozoa, etc., inhabiting any habitat, in our case, it is a living organism. It is established that, in the diet, the inroads into the reorganization of the ratio of the individual microorganisms in the ecosystem of the gastrointestinal tract (GIT) of dogs. Therefore, it is of undoubted interest to study the composition of the microbial community of dog feces.

The gastrointestinal tract performs various functions, it is not only digestive but also protective, as well as, in particular, the immune function, in particular, the intestine participates in the realization of the medicinal reactions of the organism against pathogenic, opportunistic microorganisms and many inorganic substances.

It is known that one of the most important functions of normal microflora is colonization resistance. Under the influence of negative factors, stability of the ratio of normal native (indigenous) flora, as well as adhesion and colonization, which leads to the appearance of pathological processes called dysbiosis [5, 6, 7, 8].

The natural resistance of mammals to pathogenic microorganisms and foreign agents is determined by nonspecific cellular and humoral factors. These factors include the protective properties of the skin, mucous membranes, including. the mucous membrane of the intestine, bactericidal activity of blood serum, and other body fluids that contain nonspecific humoral factors - lysozyme, complement, interferon, beta-lysine, properdin, natural antibodies, etc [9, 10].

The indicators of phagocytosis are a sensitive indicator of the protective reactions of the body. Phagocytosis plays an important role in the development of antimicrobial resistance of the organism, in particular, the participation of phagocytes in inflammation is known [11, 12].

The aim of our study was to study the community of the contents of the gastrointestinal tract of dogs using the molecular genetic method of T-RFLP and to identify the correlation between the severity of dysbiotic disorders and the state of resistance of the organism. To achieve the goal, tasks were defined: to conduct an analysis of the bacterial community of feces of 12 dogs and to study the immunological parameters of their blood.

MATERIALS AND METHODS

The studies were carried out in the molecular genetic laboratory of Biotrof LLC, where, between February and May 2016, 12 samples of feces from service dogs aged from 1 to 6 years old were kept in a nursery in St. Petersburg. All samples were taken from clinically healthy animals of the same breed, kept in the same conditions and consuming the same diet.

The object of the study was the microbial community of the studied fecal matter samples, as well as the blood of dogs.

ISSN: 0975-8585

The subject of the study is the use of the molecular genetic method in the study of the bacterial community of the gastrointestinal tract of carnivores, and in particular of dogs, and the identification of correlation links between the severity of dysbiotic disorders and the state of resistance of the organism.

DNA from the contents of the dog feces was isolated by phenol/chloroform extraction and purified by STAB solution.

PCR amplification of the bacterium 16S rRNA genes was performed using primers: 63F (CAGGCCTAACACATGCAAGTC) - labeled at the 5'-end (D4-Well Red); 1492R (TACGGHTACCTTGTTACGACTT).

The amplified fragment was isolated from the agarose gel with a 3M guanidine thiocyanate solution.

Restriction of amplicons was carried out using restriction enzymes HaeIII, HhaI and MspI ("Fermentas"), for 2 hours at 37 ° C. After completion of the restriction, DNA from the reaction mixture was precipitated with ethanol, dissolved in SLS (Beckman Coulter) with the addition of a molecular weight marker of 600 bp. (Beckman Coulter) and separated under capillary electrophoresis with fluorescent detection using an automatic sequencer CEQ8000 (Beckman Coulter).

Calculation of the sizes of peaks and their areas was carried out using the Fragment Analysis software module (Beckman Coulter). To identify the peaks, the T-RFLP gram for the three endonucleases (HaeIII, HhaI, and MspI) was processed using the Fragment Sorter program (http://www.oardc.ohio-state.edu/trflpfragsort/index.php).

Studies included the study of some hematological and biochemical indicators. The natural resistance of the body was determined by lysozyme, bactericidal and phagocytic activity. The determination of lysozyme activity was carried out according to the method of Dorofeychik V.G. (1976) with the establishment of circulating immune complexes (CIC), as well as proteinograms (total protein, albumins, globulins (α-, β-, γ-), albumin / globulin ratio (A / G) .The bactericidal activity was determined by the method of Emelianenko P. (1980) using the test culture of Escherichia coli To determine the phagocytic activity of neutrophils, we used the method of Berigan V.M. and Slavskaya E.M. (1986) in our modification. The phagocytic function of blood cells of dogs was investigated in relation to the test of the microbe - Staphylococcus aureus.

RESULTS AND DISCUSSION

For each dog, T-RFLP-grams reflecting the structure of the bacterial community of feces were obtained. With the help of the Frag Sort program, the taxonomic affiliation of the microbial peaks of the dog feces was established (Figure 1).

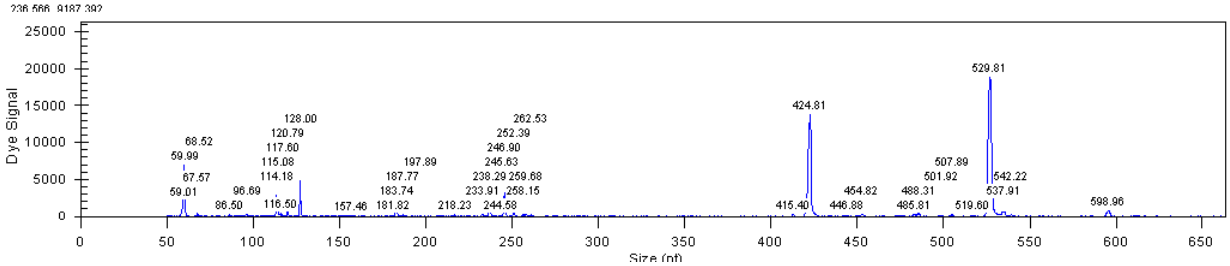

Figure 1: T-RFLP-gram of bacterial community of dog' feces sample No. 1

Among the huge number of microorganisms inhabiting the gastrointestinal tract, lactobacilli and bifidobacteria play an important role in the normalization of the indigenous microflora in animals and humans with enterocolitis and dysbiosis of various etiologies [13, 14, 15].

It was found that the content in feces of dogs of the family Lactobacillaceae, which usually have high antagonistic activity against pathogenic microorganisms, was low. The greatest content of lactobacilli was found in the feces of dogs of samples No. 1, 2, 3 and 6. The proportion of bifidobacteria of the family

ISSN: 0975-8585

Bifidobacteriaceae was also low in the feces of all dogs. The exception was sample No. 4, the content of bifidobacteria in which was 3.42%, (Table 1).

Table 1: Composition of the bacterial community of dog' feces (n = 12)

Microorganism	Fecal samples											
	1	2	3	4	5	6	7	8	9	10	11	12
Lactobacillaceae	4,52	1,26	11,78	0,58	0,42	5,64	0,26	0,12	0,04	0	0,62	0,3
Bacillaceae	7,93	32,71	13,86	16,21	16,4	13,64	21,4	25,68	26,18	23,16	12,36	14,37
Bifidobacteriaceae	0	0	0	3,42	0,64	0,14	1,6	0	0,68	1,26	0,14	1,23
Ruminococcaceae	0,4	3,87	1,32	2,79	2,1	2,12	2,48	1,84	1,6	2,56	1,32	0,78
Eubacteriaceae	0,63	0,99	0,21	1,21	0,39	1,12	0,82	0	0,2	0,73	0	0
Lachnospiraceae	0	0,44	0,64	0,99	0,96	0,8	0,24	0,34	0,27	1,24	0	0,31
Clostridiaceae	5,49	3,07	2,66	1,58	3,64	6,6	9,98	12,6	2,02	4,54	5,46	1,25
Pseudomonas	0,88	4,33	0	0	7,92	16,06	0,83	0,18	13,83	2,91	2,48	0
Actinobacteria	13,57	11,36	7,09	16,2	18,92	11,3	29,5	33,32	17,73	18,68	6,81	9,56
Bacteroidetes	17	21,75	33,81	14,18	14,54	25,32	14,48	8,62	21,5	26,54	20,77	33,65
Camphylobacteriaceae	0,79	2,32	0,14	0,56	0,88	0,34	1,82	1,24	1,13	1,26	0,48	0
Enterobacteriaceae	0	0	0	0	1,63	0,44	0,09	0	0,52	0	0	0
Unculturedbacteria	48,79	17,9	28,49	42,28	31,56	16,48	16,5	16,06	14,3	17,12	49,56	38,55

It was shown that a considerable number of bacilli of the Bacillaceae family were recorded in the feces of dogs. It should be noted that these microorganisms, as a rule, have high antagonistic activity against pathogenic microorganisms and other useful properties (digestion of carbohydrates of feeds, etc.).

In all samples of feces, useful cellulolytic bacteria possessing the ability to digest carbohydrates of feeds have been fixed. The highest content of bacteria of the family Ruminococcaceae was from 0.4 to 3.87%, the bacteria of the family Lachnospiraceae from 0 to 1.24%, bacteria of the Eubacteriaceae family from 0 to 1.21%. The greatest content of cellulolytic bacteria was found in samples No. 2, 4, 6, 7, 10, the smallest - No. 1, 3, 8, 11, 12, (Figure 2).

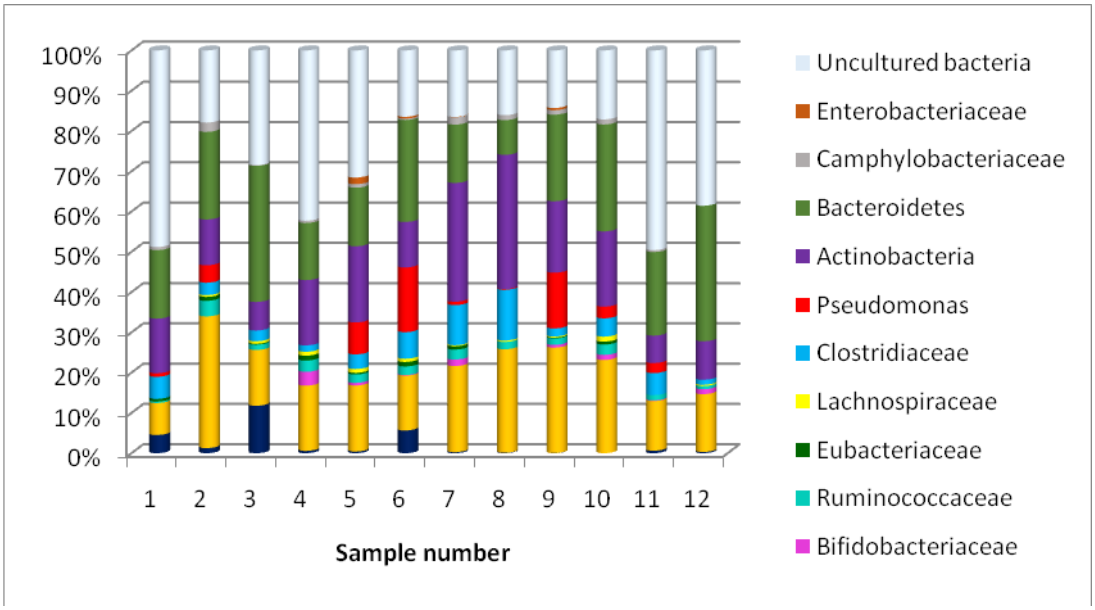

Figure 2: Composition of the bacterial community of dog' feces

The results of the studies showed that the highest Clostridiaceae clostridium content was in samples No. 1, 6, 7, 8, 10, 11 and 12.

The largest proportion of bacteroid bacteria Bacteroidetes (genera Bacteroides, Flavobacterium, Flexibacter, Cellulophaga, Prevotella) was found in feces of dogs No. 3, 6, 9, 10, 11 and 12.

In feces of all dogs, a number of pathogenic bacteria were detected - actinobacteria-fils of Actinobacteria, enterobacteria of the Enterobacteriaceae family and campylobacteria (genus Helicobacter).

Now it is established that these pathogens are permanent inhabitants of the gastrointestinal tract of animals and can exist for a long time in the body without causing diseases, and can also be expelled from the body by useful symbiotic representatives of the "normal" microflora, for example, useful lactobacilli and bacilli. However, with unsatisfactory conditions for keeping animals, poor quality of feed, unbalanced feeding, a sharp change in diet, there is a violation of the microflora of the gastrointestinal tract: the active reproduction of pathogenic bacteria and the displacement of representatives of "normal" microflora with all the ensuing negative consequences.

The greatest content of actinobacteria fila Actinobacteria, among which the pathogens of actinomycosis are often found, was in the feces of dogs 4, 5, 7, 8, 9 and 10.

The number of enterobacteria of the Enterobacteriaceae family, among which pathogens (Salmonella sp., Enterobacter sp., Escherichiacoli, Serratia sp., Citrobacter sp., Klebsiella sp., Kluyvera sp., Pantoea sp.) Are often found in feces of all dogs.

The largest content of campylobacteria was found in feces of 2, 7, 8, 9, 10 dogs.

In addition, a significant number of uncultivated bacteria (microorganisms that cannot be detected by classical microbiological methods), as well as pseudomonads, the greatest content of which was found in samples Nos. 6 and 9, was found in the feces of all the animals studied.

The second stage of our study was the study of the natural resistance of experimental animals, whose stool samples we took to determine the microbial community of the contents of their gastrointestinal tract.

To do this, we conducted a hematological study of the blood of experimental animals, the results are given in Tables 2.

Table 2: Hematologic parameters of blood of dogs during the experiment (n = 12) (P ≤ 0,05)

Hemoglobin, g/l	Erythrocytes, xl0^{12}/l	Leukocytes, x10^{9}/l	Platelets, x10^{9}/l
122 ± 4,6	7,56± 0,58	7,83 ± 0,65	368,5+21,8

The results of the conducted studies showed that the number of erythrocytes, leukocytes, as well as the hemoglobin level in all animals during the experiment, were within the limits of the fluctuations of the reference values.

Table 3: Biochemical parameters of blood serum of dogs (n = 12) (P≤0,05)

Total protein, g/l	Albumins,%	α-globulins,%	β-globulins,%	γ-globulins,%
66,4±1,79	38,9±1,08	2,2±0,6	8,7±0,35	15,3±1,58

Table 4: Indices of natural resistance of dogs (n = 12) (P≤0,05)

Bactericidal activity of blood serum,% of E. coli lysis	Lysozyme activity of blood serum,%	CEC, op. units	Ig A	Ig M	Ig G_1	Ig G_2
62,4±8,97	13,94±3,18	0,13±0,03	2,2±0,66	0,97±0,05	5,1±0,6	2,5±0,96

ISSN: 0975-8585

The indicators of phagocytosis are determined by counting the number of phagocytic cells with neutrophils and subsequent counting: phagocytic index, phagocytic activity, and phagocytic intensity. According to the experimental data, the following indicators of the phagocytic activity of neutrophils were calculated on the blood of dogs.

For example, animal number 1 during the experiment had a number of phagocytic cells $S_\Sigma = 160$.

Phagocytic index: this is the ratio of the sum of phagocytic staphylococcus cells (S_Σ) to the total number of neutrophils (N_Σ):

$$Phagocytic\ index = \frac{S_\Sigma}{N_\Sigma} = \frac{160}{53} = 3{,}01$$

Phagocytic activity: this ratio of neutrophils participating in phagocytosis to the total number of counted, expressed as a percentage:

$$Phagocytic\ activity = \frac{N_a}{N_\Sigma}100\% = \frac{43}{53}100 = 81{,}1\%$$

Phagocytic intensity: the number of phagocytized staphylococci divided by the number of neutrophils participating in phagocytosis is calculated:

$$Phagocytic\ intensity = \frac{Sum\ St}{Ph.activ} = \frac{S_\Sigma}{N_a} = \frac{160}{43} = 3{,}72$$

After counting the leukocytes and drawing a leukogram, we determined the number of neutrophils involved in phagocytosis, as well as the number of microbial cells phagocytosed by them. Based on the studies, data were obtained that allow one to see, some regularities, between the number of phagocytic cells, the phagocytic index and the phagocytic intensity (Figure 3).

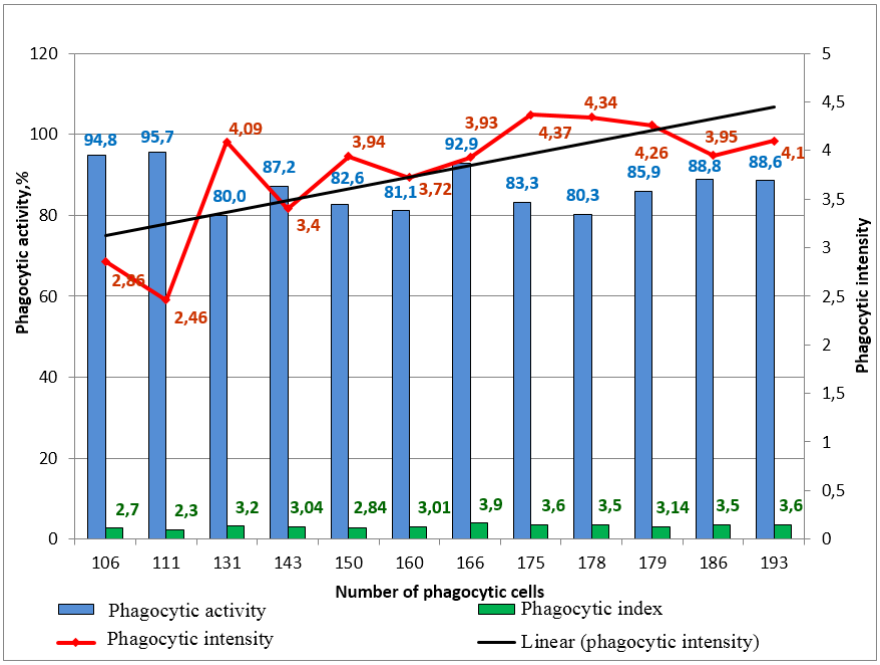

Figure 3: Results of statistical data processing of the study of phagocytic activity of neutrophils

To identify these relationships, it is necessary to statistically process the results of the experiment and construct a regression function. The best estimate of the true regression function is usually due to the use of the least squares method. In this case, the task of determining the parameters of such a function will acquire a

specific meaning: this is the function for which the mean square deviation of the individual values of the effective characteristic from the corresponding values of the function takes the least value.

The multiple coefficients of determination R2, which shows what percentage (out of 100%) of the predicted indicator is determined by the influence of the factors under consideration is of great importance for assessing the quality of the constructed model. If R2 <0.6, then the number of analyzed factors should be clarified and increased.

The processing of statistical data across the group of animals given in Table 5 showed that there is a sufficiently high degree of dependence of phagocytic activity on the number of phagocytic cells. The correlation coefficient, which shows the relationship between the phagocytic intensity and the number of phagocytic cells, is R = 0.77. The coefficient of determination is R2 = 0.5859, i.e. phagocytic intensity is almost 60% dependent on the number of phagocytic cells.

Table 5: Indices of phagocytic activity of dogs during the experiment (n = 12) (P≤0,05)

Phagocytic activity	Phagocytic index	Phagocytic number
86,7±7,85	3,19±0,8	3,76±0,95

The dependence found shows that phagocytic intensity increases with increasing number of phagocytic cells. The overall coefficient of the equation is 2.0231 is constant for a given group of dogs and probably reflects the overall physiological state of the animals. Logarithmic dependence suggests that with increasing number of phagocytic cells, the rate of increment of phagocytic activity slows down and approaches saturation.

Let us consider the possibility of using this dependence to evaluate phagocytic activity, in the presence of data on the number of phagocytic cells on the example of a dog under number 1:

$$y=0,77421gx+2,0231=0,7742x1g160+2,0231=3,9$$

The absolute error is defined as the difference between the experimental data obtained during the experiments and the calculation for the proposed model:

$$\Delta = 3,72-3,9 = -0,18$$

The relative error in the calculations for the proposed model is

$$\delta = \frac{\Delta}{Ph.inten} x100 = \frac{-0,18}{3,72} x100 = 4,8\%$$

The data presented indicate that the error in the calculations performed for this model does not exceed 6.0% on average. Therefore, this model can be used in research.

CONCLUSION

Thus, as a result of the T-RFLP analysis, the composition of the bacterial microflora of dog feces was established. Separately, it should be noted that the microbial profile of each animal is unique.

Despite the fact that all animals participating in the experiment were clinically healthy, which is confirmed by hematological studies, it is nevertheless shown that the composition of the bacterial community has deviations from the norm.

Along with the high content of useful lactobacilli in the feces of the family Lactobacillaceae and bacilli of the family Bacillaceae, practically no bifidobacteria were recorded. The greatest total content of bacilli and lactobacilli was found in the feces of dogs No. 2, 3, 8, 9, 10.

In the feces of all dogs, a number of pathogenic bacteria were identified - Clostridiaceae clostridia, Bacteroidetes bacteroidoid bacteria (genera Bacteroides, Flavobacterium, Flexibacter, Cellulophaga, Prevotella), Actinobacteria filament actinobacteria, Enterobacteriaceae enterobacteria and Campylobacteria (Helicobacter genus).

The results of the studies showed that the Clostridiaceae clostridium contained the highest content in samples No. 6, 7, 8, 10, 11 and 12.

The largest proportion of bacteroid bacteria Bacteroidetes (genera Bacteroides, Flavobacterium, Flexibacter, Cellulophaga, Prevotella) was found in feces of dogs No. 3, 6, 10, 11 and 12.

The greatest content of actinobacteria fila Actinobacteria, among which there are frequent pathogens of actinomycosis, was in the feces of dogs 7, 8, 9 and 10.

The largest content of campylobacteria was found in feces of 2, 7, 8, 9, 10 dogs.

The number of Enterobacteriaceae enterobacteria in feces of all dogs was low.

In total, the greatest total number of pathogens was detected in the feces of dogs No.6, 7, 8, 9 and 10.

Subbotin V.V., Danilevskaya N.V. (2002), studying the indigenous microflora of the intestine of adult dogs by classical methods, came to the following results: in the first place, according to their data, animals in feces contain bifidobacteria (59.15%), lactobacillus - in second place (16.89%), further enterococci (12.39%), enterobacteria (11.55%), other microorganisms (0.02%). We see that the cultural method does not allow us to establish the entire breadth and diversity of microorganism species found in the gastrointestinal tract in dogs.

Foreign scientists J. Suchodolski, K. Simpson, carrying out a molecular-phylogenetic analysis of the bacterial rRNA of the 16S gene in 5 dogs, determined that about 99% of the whole microbiocenosis of the intestine in dogs is made up of bacterial types of Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria. According to their data in the large intestine, Clostridiales, Bacteroides, Prevotella, Fusobacteria, Bacteroidetes and Actinobacteria (Bifidobacterium species) are considered to be an important source of such metabolites, as essential fatty acids and favorably affect the animal's body. The results obtained by the authors are similar to our data, with a few exceptions.

The purpose of the parallel examination was to identify the correlation between the severity of dysbiotic disorders and the state of resistance of the organism. A reliable connection between biochemical parameters of α-, β-, γ-globulins, the level of lysozyme, bactericidal activity and the presence of dysbiosis was not revealed. There was a significant correlation between disturbances in the composition of normal microflora and activity of phagocytosis, which manifested itself in the enhancement of the phagocytic activity of cells in the development of dysbiosis. The amount of IgG and IgM from the norm was not significantly different, but there was a tendency to increase IgM. The level of IgA was significantly lowered, and there was a correlation between the depth of disturbances in the composition of the intestinal microflora. The level of immune complexes increased depending on the severity of dysbiosis.

In dogs with reduced natural resistance against the background of bactericidal and lysozyme activity, the level of immunoglobulins decreases, and phagocytic activity increases.

On the basis of the studies conducted, it can be argued that dysbiosis is an important pathophysiological compensatory mechanism that reflects temporary or permanent disturbances in the system of general body protection. Its appearance, apparently, is caused by the need to strengthen the antigen load, which supports not only the mobilization readiness of the intestinal epithelium but also the immune cells in a state of constant tension. Based on the above, we consider it possible to conclude that studies on dysbiosis may indirectly indicate violations of the immune status of carnivores, in particular, dogs.

The results obtained by us can be used to analyze the immunity intensity in animals under the conditions of the nursery under the same conditions and can be useful for practicing veterinarians.

ISSN: 0975-8585

REFERENCES

[1] Antonchik T.M. Topical issues of clinical microbiology. M., 1985, pp. 32-36

[2] Vershigora, A.E. Sanitary microbiology / A.E. Vershigora, L.V. Grigorieva, V.A. Yaroshenko // Kiev: Zdorov'ya. - 1967. - 200 P.

[3] Golosova T.V., FainsteinF.E., MartynovaV.A. Infection and natural immunity in leukemia. M.: Medicine, 1980. P. 107.

[4] Deichman G.I. The role of natural resistance of the organism in the response to the emergence, growth and metastasis of the tumor (literature review). VINITI. Series Oncology, No. 13, M., 1984. pp. 46-97.

[5] Lenzner A. Lenzner H.P., Mikelsaar M.E. Lactoflora and colonization resistance. Antibiotics and medical biotechnology. 1987. 3. pp. 173-179.

[6] Liz'ko N.N. Dysbacteriosis of experimental states. Antibiotics and medical biotechnology. 1987. 3. pp. 184-186,

[7] Smolianskaya A.Z. Dysbacteriosis-infectious processes of mixed etiology. Antibiotics and medical biotechnology. 1987. 3. pp. 186-190.

[8] Subbotin V.V., Danilevskaya N.V. Microflora of the intestines of dogs: physiological significance, age dynamics, dysbacteriosis, correction. Journal "Veterinarian", M. 2002, 1.

[9] Benno, Y., Nakao, H., Uchida, K.Impact of the advances of age onthe gastrointestinal microflora of beagle dogs. Journal of Veterinary Medicine and Science 1992, 54. pp. 703-706.

[10] Suchodolski J.S. Intestinal microbiota of dogs and cats: a bigger world than we thought. Vet Clin North Am Small AnimPract2011. 41. pp. 261-272.

[11] Van der Waaij D. Evidence of immunoredulation of the composition of intestinal microflora and its practical consequences. Eur. J. Clin. Microbiol. Infect. Dis., 1988.