https://doi.org/10.29326/2304-196X-2024-13-2-154-163

Gut microbiota and bacterial associations in monkeys with gastrointestinal diseases in the setting of helminth infestation

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ABSTRACT

One of the topical issues of current primatology is spontaneous pathology in monkeys, primarily gastrointestinal infections, which are the leading ones in the morbidity and mortality patterns of the animals raised in captivity. Gastrointestinal pathology in monkeys involves complicated infectious processes, most often of associative type, with the formation of various bacterial and parasitic associations. The study demonstrates the results of gastrointestinal disease and helminth infestation monitoring as well as of the microbial flora spectrum analysis in monkeys in 2017–2022. Mortality of monkeys due to gastrointestinal diseases in the specified period amounted to 60.5%. The postmortem study demonstrated that the leading position in this pathology pattern in monkeys was taken by gastroenterocolitis (62.5%), with dominated chronic atrophic gastroenterocolitis in the acute phase (53.9%). The analysis of the six-year trend in animal mortality showed that the percentage of gastrointestinal diseases remained approximately at the same level every year. Helminth infestations were detected in 22.0% of the diseased animals and in 30.2% of the dead ones. *Trichocephalus trichiurus* was found in 93.3% of the diseased and in 99.7% of the dead monkeys, *Strongyloides* sp. – in 12.2% of the diseased and in 3.3% of the dead animals. Helminths were detected as mono- and less often as mixed infestations. In the isolated microflora, the top position was taken by the representatives of genus *Proteus*. The percentage of pathogenic enterobacteria detections was low, and *Shigella flexneri* was the leader among them. In monkeys that died from gastrointestinal diseases without parasitic infestation, the pathogenic enterobacteria detection rate was 2 times higher than in the infested animals. The microorganisms were isolated as monocultures and in associations. The microorganisms were isolated as monocultures and in associations *Proteus* spp. were detected more often. Gastrointestinal diseases of helminth-bacterial etiology in monkeys require complex therapy of the animals.

Keywords: monkeys, gastrointestinal diseases, pathogenic and opportunistic bacteria, bacterial associations, helminth infestation

Acknowledgements: The work was financed by the state budget, it is included in the research section "Development of models of the most significant human viral infections in laboratory primates in order to study unresolved issues of pathogenesis and immunogenesis, as well as to test the developed means of prevention and treatment, including study of the intestinal microbiome".

For citation: Kalashnikova V. A., Egorova T. P., Demerchyan A. V., Polyakova V. I., Lenshina Ya. I., Ilyazyants D. A., Arshba I. M. Gut microbiota and bacterial associations in monkeys with gastrointestinal diseases in the setting of helminth infestation. *Veterinary Science Today.* 2024; 13 (2): 154–163. https://doi.org/10.29326/2304-196X-2024-13-2-154-163

Conflict of interests: The authors declare no conflict of interests.

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УДК 619:579:616.995.1:616.34:599.8

Кишечная микрофлора и бактериальные ассоциации на фоне гельминтозной инвазии при желудочно-кишечных заболеваниях у обезьян

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РЕЗЮМЕ

Одна из актуальных проблем современной приматологии – спонтанная патология обезьян, в первую очередь желудочно-кишечные инфекции, являющиеся ведущими в структуре заболеваемости и смертности животных, разводимых в условиях неволи. Желудочно-кишечная патология обезьян представляет собой сложные инфекционные процессы, чаще всего протекающие по ассоциативному типу, с формированием разных бактериальных и паразитарных сочетаний. В исследовании представлены результаты мониторинга желудочно-кишечных заболеваний, гельминтозной инвазии и анализа спектра микрофлоры у обезьян, проведенных в течение 2017–2022 гг. Гибель обезьян от заболеваний желудочно-кишечного тракта в указанный период составила 60,5%. При постмортальном исследовании установлено, что в структуре данной патологии у обезьян лидирующая позиция принадлежит гастроэнтероколитам (62,5%), при этом доминировали хронические атрофические гастроэнтероколиты в стадии обострения (53,9%). Анализ динамики гибели животных в течение 6 лет показал, что процент заболеваний желудочно-кишечного тракта из года в год держится примерно на одном уровне. Гельминтозная инвазия выявлена у 22,0% больных и 30,2% погибших животных. Кишечные паразиты *Trichocephalus trichiurus* обнаружены у 93,3% больных и 99,7% погибших обезьян, *Strongyloides* sp. – у 12,2% больных и 3,3% погибших животных. Гельминты выявлены в моноинвазиях, реже – в полиинвазиях. Из выделенной микрофлоры первое место занимают представители рода *Proteus*. Процент выявления патогенных энтеробактерий низкий, но среди них лидирует *Shigella flexneri*. У погибших от желудочно-кишечных заболеваний обезьян без паразитарной инвазии частота обнаружения патогенных энтеробактерий в 2 раза выше, чем у инвазированных животных. Микроорганизмы были выделены в виде монокультур и в ассоциациях. Чаще выявляли сочетания представителей нормофлоры с *Proteus* spp. Желудочно-кишечные заболевания у обезьян гельминто-бактериальной этиологии требуют комплексной терапии животных.

Ключевые слова: обезьяны, заболевания желудочно-кишечного тракта, патогенные и условно-патогенные бактерии, бактериальные ассоциации, гельминтозная инвазия

Благодарности: Работа выполнена за счет средств госбюджета, входит в раздел научных исследований «Разработка моделей наиболее значимых вирусных инфекций человека на лабораторных приматах с целью изучения нерешенных вопросов патогенеза и иммуногенеза, а также испытания разрабатываемых средств профилактики и лечения, включая изучение микробиома кишечника».

Для цитирования: Калашникова В. А., Егорова Т. П., Демерчян А. В., Полякова В. И., Леншина Я. И., Ильязянц Д. А., Аршба И. М. Кишечная микрофлора и бактериальные ассоциации на фоне гельминтозной инвазии при желудочно-кишечных заболеваниях у обезьян. *Ветеринария сегодня*. 2024; 13 (2): 154–163. https://doi.org/10.29326/2304-196X-2024-13-2-154-163

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

Gut microbiota condition is one of the important factors of animal and human health. Microorganisms enter the digestive tract immediately after birth and play an important role in the animal's life. The basis of the normal gut flora is known to consist of bifidobacteria and lactobacilli, *Escherichia coli* with normal fermentation, enterococci. Studies show that changes in the quantitative and qualitative composition of the gut microbiota lead to impaired intestinal function and gastrointestinal (GI) diseases [1, 2, 3, 4]. Etiological agents of intestinal infections can be bacteria, viruses, protozoa, helminths, fungi. However, not only microbial concentration plays a significant role in the development of the intestinal diseases, but also associations of different types of microorganisms in which they enter into symbiosis or antagonism and express pathogenicity factors [5, 6]. Helminths also change the quantitative and qualitative composition of the gut microbiota, forming microparasite community [7, 8, 9].

Monkeys are anatomically and physiologically similar to humans, and show natural susceptibility to many infectious diseases [10, 11, 12]. Observation results demonstrate that monkeys, both in their natural habitat and in captivity, suffer from various diseases typical for other animals and humans. In the Adler apery of the Research Institute of Medical Primatology (now Kurchatov Complex of Medical Primatology, National Research Centre "Kurchatov Institute") monkeys of different species are kept. According to our annual data, mortality of monkeys in more than half the cases is due to gastrointestinal diseases [13, 14]. Intestinal diseases of monkeys are complex infectious processes, most often of an associative type, with the formation of various bacteria and parasite combinations [15, 16]. Intestinal parasites infect various species of monkeys both in the wild and in captivity, causing serious diseases of the digestive tract and in some cases leading to animal death [17, 18, 19, 20]. According to foreign publications, nematodes are the most common helminths in monkeys kept in zoos, and they can be transmitted to humans [21, 22]. In the foreign research publications, there is data about intestinal parasites isolated from primates kept in zoos and reserves or from free-living ones, but these data refer to particular species and small groups of monkeys [23, 24, 25]. Moreover, these papers describe only the results of parasitology research.

The relevance of the presented work is determined by the fact that GI diseases are the major cause of the monkeys' mortality in the apery. At the same time, intestinal parasitic infections and bacterial associations that form intestinal diseases in monkeys are still an understudied problem. Our previous studies demonstrated the circulation of protozoa and helminths in the monkeys in the apery and provided some data on the concomitant microbiota [15, 16]. In this paper, we would like to focus on the gut microbiota of nematode-infested monkeys and on the features of bacterial associations during its formation.

The novelty of the work lies in the fact that our study was the first to report on the microbial landscape and bacterial associations in the setting of the invasion by intestinal parasites *Trichocephalus trichiurus* and *Strongyloides* sp. in non-human primates kept in captivity.

The aim of the study was to examine the structure of the gut microbiota of monkeys with intestinal diseases who died due to GI pathology in the setting of helminth infestation.

Table 1
Characteristics of tested monkeys (2017–2022)

Monkey species	Diseased	Dead	Total
Rhesus macaque (*Macaca mulatta*)	172	731	903
Crab-eating macaque (*Macaca fascicularis*)	152	514	666
Southern pig-tailed macaque (*Macaca nemestrina*)	41	45	86
Green monkey (*Chlorocebus sabaeus*)	14	79	93
Anubis baboon (*Papio anubis*)	12	170	182
Hamadryas baboon (*Papio hamadryas*)	18	438	456
Total	409	1,977	2,386

Table 2
Number of tested dead monkeys (2017–2022)

Monkey species	Number of monkeys						Total
	2017	2018	2019	2020	2021	2022	
Rhesus macaque	117	204	115	105	124	66	731
Crab-eating macaque	82	92	89	89	90	72	514
Southern pig-tailed macaque	6	5	6	13	8	7	45
Green monkey	11	20	17	17	3	11	79
Anubis baboon	19	32	43	17	45	14	170
Hamadryas baboon	57	55	75	66	105	80	438
Total	292	408	345	307	375	250	1,977

Table 3
Characteristics of tested dead monkeys (2017–2022)

Monkey species	Dead		Total
	with GI lesions	without GI lesions	
Rhesus macaque	491	240	731
Crab-eating macaque	289	225	514
Southern pig-tailed macaque	21	24	45
Green monkey	52	27	79
Anubis baboon	101	69	170
Hamadryas baboon	242	196	438
Total	1,196	781	1,977

MATERIALS AND METHODS

The study object included 2,386 monkeys of six species of both sexes aged from 10 days to 35 years, which were kept in the apery. This number included 409 monkeys affected with intestinal diseases and 1,977 dead monkeys (Tables 1, 2).

The test samples included feces collected by a rectal smear from live monkeys, in case of dead animals they included the contents of three parts of the intestine (small intestine, caecum, rectum).

Bacteriological, biochemical and microscopic tests were carried out using routine practical methods[1]. The test material was aseptically collected and delivered to the laboratory, where initial inoculation on the diagnostic nutrient media was performed: Endo agar, *Salmonella-Shigella* agar, 5% blood agar, salt egg yolk agar, *Yersinia* selective agar, chromogenic *Candida* agar. The inoculates were cultivated in the thermostat at 37 °C for 24 hours, dishes with *Yersinia* selective agar – at 28 °C for 48 hours, dishes with *Candida* agar – at 24 °C for 48 hours. Isolation of pure cultures and their further identification were carried out according to generally accepted standards: examination of morphological and tinctorial properties (Gram staining of smears), hemolytic and lecithinase activity, and examination of the biochemical properties. The VITEK®2 Compact system (bioMérieux, France) was also used to determine the enterobacteria species. Slide agglutination method with specific sera was used for determination of the serovars of the isolated *Shigella*, *Salmonella*, *Yersinia* strains.

Parasite tests. Conventional microscopy procedure of native feces preparations was used to detect parasite infestation[2]. To identify helminth eggs, a small amount of feces from different places of the test portion was ground on a slide in a drop of 50% glycerol solution until a uniform transparent smear formation, covered with a coverslip and subjected to microscopy at 10 × 10 and 10 × 40 magnification. The extensity of helminth infestation was determined by the number of infected animals to the total number of the tested ones.

In the study of postmortem material, both microscopy of native preparations and macroscopic examination of the contents of the large intestine were used, as a result of which adult helminths were detected.

Statistical data processing and calculations were carried out using GraphPad Prism 8 software. To assess the significance of the differences in the frequency of helminth and bacteria detections in different monkey species in individual test groups the χ^2 goodness-of-fit test was used. All differences were interpreted as significant at $p < 0.05$. The χ^2 test-for-trend was used to determine changes in frequency indicators depending on the test year. Fisher's exact test was used to determine the statistical significance between helminth infestation extensity and monkey species.

RESULTS AND DISCUSSION

From January 2017 to December 2022, 1,977 dead monkeys were tested; 1,196 (60.5%) of them demonstrated GI lesions during the necropsy (Table 3).

Gastrointestinal diseases were often accompanied with pneumonia, signs of cachexia, exicosis, and dystrophy of internal organs. Analysis of the animal mortality trend over 6 years demonstrated that the percentage of GI diseases remained approximately at the same level every year. As can be seen in Table 4, there was a tendency for a slight decrease in the number of monkeys died of GI diseases in 2022.

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[1] Methodological guidelines for the microbiological diagnosis of the enterobacteria-induced diseases: approved by the Ministry of Health of the USSR on 17.12.1984 No. 04-723/3. https://base.garant.ru/71310616/?ysclid=lvdnbim4fh245607194

[2] MUC 4.2.3145-13 Laboratory diagnostics of helminth and protozoa infestations: guidelines (approved by Chief State Medical Officer of the Russian Federation on 26 November 2013). https://docs.cntd.ru/document/1200110752?ysclid=lvdnu57iyo743363677

Table 4
Trend in the monkeys' mortality due to GI diseases, 2017–2022

Monkey species	Number of dead / %						Trend test*	Total / %
	2017 ($n = 292$)	2018 ($n = 408$)	2019 ($n = 345$)	2020 ($n = 307$)	2021 ($n = 375$)	2022 ($n = 250$)		
Rhesus macaque ($n = 731$)	77/65.8	140/68.6	79/68.7	75/71.4	85/68.5	35/53.0	**< 0.0001 (↑↓)**	491/67.2
Crab-eating macaque ($n = 514$)	50/61.0	64/69.6	44/49.4	46/51.7	46/51.1	39/54.2	0.3145	289/56.2
Southern pig-tailed macaque ($n = 45$)	5/83.3	3/60.0	3/50.0	5/38.5	3/37.5	2/28.6	0.5544	21/46.7
Green monkey ($n = 79$)	9/81.8	8/40.0	11/64.7	15/88.2	2/66.7	7/63.6	0.5575	52/65.8
Anubis baboon ($n = 170$)	10/52.6	12/37.5	25/58.1	12/70.6	34/75.6	8/57.1	**0.0429 (↑↓)**	101/59.4
Hamadryas baboon ($n = 438$)	26/45.6	27/49.1	39/52.0	28/42.4	75/71.4	47/58.8	**< 0.0001 (↑↓)**	242/55.3
Total	177/60.6	254/62.3	201/58.3	181/59.0	245/65.3	138/55.2	–	1,196/60.5

* $p < 0.05$ (χ^2 criterion – statistical difference of detections relative to monkey species).
Arrows show the trend of changes in detection frequency over the years upon statistical significance of the test.

Postmortem examination of dead monkeys with GI lesions showed that in 35.3% of cases the GI lesions were in the form of enterocolitis ($n = 422$), in 62.5% – gastroenterocolitis ($n = 748$) and in 0.6% – gastritis ($n = 7$). Furthermore, in 0.6% of cases, the intestinal lesions were associated with infectious pathology, i.e. yersiniosis ($n = 4$), pseudotuberculosis ($n = 3$). Malignant neoplasms were reported in 1.0% of monkeys: gastric adenocarcinoma ($n = 3$), intestinal adenocarcinoma ($n = 9$). According to the data obtained, the GI diseases included dominating chronic atrophic gastroenterocolitis in the acute stage (53.9%), as well as chronic forms of enterocolitis. In case of stomach lesions, only chronic atrophic gastritis was reported in monkeys (Table 5).

As a result of parasite tests, helminth infestation was detected in 22.0% of the diseased monkeys and in 30.2% of the dead ones (Table 6). Two types of intestinal parasites were detected – *Trichocephalus trichiurus* and *Strongyloides* sp. The detection frequency of *Trichocephalus trichiurus* was 93.3% in the diseased monkeys ($n = 84$) and 99.7% in dead ones ($n = 360$). *Strongyloides* sp. were detected in 11 (12.2%) diseased and 12 (3.3%) dead animals. It was established that *Strongyloides* sp. mono-infestations were detected in 6 (6.7%) diseased and 1 (0.3%) dead monkeys, in other cases the helminths were detected as part of mixed infestations.

Table 6 demonstrates that in the crab-eating macaques with intestinal diseases the helminth detection frequency was higher than in the dead ones. As for Anubis baboons, *Trichocephalus trichiurus* was detected somewhat more often in the intestines of the dead animals. The frequency of infection with these parasites in the diseased and dead rhesus monkeys was almost the same. The same situation was reported in hamadryas baboons. Over the 6-year period, a small number of southern pig-tailed macaques and green monkeys were examined, however, the resulted data demonstrated that helminths were more often detected in the diseased animals of these species than in the dead ones. It was noted that *Strongyloides* sp. were found only in 3 species: rhesus macaques, green monkeys and hamadryas baboons. Thus, the results of the work showed that *Trichocephalus trichiurus* often infected

Table 5
GI diseases and lesions in monkeys (2017–2022)

GI diseases	Number of animals / %	Lesions, number / %			
		acute	chronic atrophic	chronic with complications	CAGE (exacerbation)
Enterocolitis	422/35.3	25/5.9	368/87.2	29/6.9	–
Gastroenterocolitis	748/62.5	25/3.3	69/9.2	9/1.2	645/86.2
Gastritis	7/0.6	0	7	0	–
Infectious pathology	7/0.6	–	–	–	–
Malignant neoplasms	12/1.0	–	–	–	–
Total	1,196/100	50/4.2	444/37.1	38/3.2	645/53.9

CAGE – chronic atrophic gastroenterocolitis.

Table 6
Helminth infestation extensity in monkeys (2017–2022)

Monkey species	Diseased, infested / %	Dead monkeys with GI lesions, infested / %	$p < 0.05$
Rhesus macaque	23/13.4	72/14.7	0.2497
Crab-eating macaque	14/9.2	10/3.5	**< 0.0001**
Southern pig-tailed macaque	23/56.1	9/42.9	**< 0.0001**
Green monkey	9/64.3	16/30.8	0.0662
Anubis baboon	8/66.7	73/72.3	**0.0134**
Hamadryas baboon	13/72.2	181/74.8	**< 0.0001**
Total	90/22.0	361/30.2	

non-human primates, and this coincided with the data of the foreign studies [26, 27, 28].

In 2017–2022, as a result of bacteriological tests of the feces of the diseased monkeys and intestinal contents of the dead ones, 1,468 microorganisms were detected and isolated gut microbiota was characterized by species diversity; 242 microorganisms were isolated from the feces of the diseased monkeys. The proportion of gram-negative gut microbiota was 80.6% ($n = 195$), gram-positive – 18.6% ($n = 45$), yeast-like fungi – 0.8% ($n = 2$). Representatives of the *Enterobacteriaceae* family were found in 43.5% of the diseased animals with pathogenic enterobacteria isolated in 1.9% of monkeys ($n = 8$) and opportunistic ones – in 41.6% ($n = 170$). Coccal microorganisms detected in 9.1% of animals included *Staphylococcus* spp. (6.6%), hemolytic *Enterococcus* spp. (2.2%), gram-positive diplococci (0.3%); 1,226 microorganisms were isolated from the dead monkeys, of which 95.4 % belonged to the gram-negative gut microbiota ($n = 1{,}170$), 2.8% belonged to the gram-positive gut microbiota ($n = 34$); the proportion of yeast-like fungi amounted to 1.8% ($n = 22$). In 96.0% of the dead animals, enterobacteria prevailed in the isolated gut microbiota ($n = 1{,}148$), of which the proportion of pathogenic ones amounted to 7.2% ($n = 83$), and opportunistic – to 92.8% ($n = 1{,}065$). Gram-positive cocci were found in the intestines of 1.5% of the dead monkeys ($n = 18$), while *Staphylococcus* spp. was detected in 0.7% of the animals, hemolytic *Enterococcus* spp. in 0.3%, and gram-positive diplococcus in 0.5%. Pathogenic and opportunistic gut microbiota was not detected in 282 dead and 220 diseased monkeys (23.6 and 53.8%, respectively). No bacterial growth was reported after inoculation of the samples from 3 diseased monkeys onto the nutrient media (0.7%).

The study results demonstrated that representatives of the genus *Proteus* dominated in the gut microbiota, their detection was three times more likely in the dead animals than in the diseased ones (55.0 and 16.4%, respectively).

Klebsiella spp., *Staphylococcus* spp., hemolytic *Enterococcus* spp., *Enterobacter* spp., *Pseudomonas aeruginosa* were more often isolated from the diseased animals. *Providencia* spp., *Enterobacter* spp., *Shigella flexneri*, *Morganella morganii, Klebsiella* spp.*, Citrobacter* spp. were more often detected in the dead animals with GI diseases (Fig. 1).

Analysis of the bacterial gut microbiota composition of monkeys indicated (Fig. 2) that non-helminth-infested animals with the highest frequency demonstrated *Klebsiella* spp. (7.8% – in the diseased animals, 5.5% – in the dead animals) and *Enterobacter* spp. (5.0% – in the diseased animals, 7.6% – in the dead animals). At the same time, *Staphylococcus* spp. (6.9%), hemolytic *Escherichia coli* (6.0%), *Pseudomonas aeruginosa* (4.4%) were more often isolated from the diseased monkeys without parasite infestation, and *Providencia* spp. (5.6%), *Citrobacter* spp. (5.2%), *Morganella morganii* (4.4%) were most frequent in the dead parasite-free animals. As for helminth infested animals, *Staphylococcus* spp. (5.6%), *Klebsiella* spp. (4.5%), *Bacillus* spp. (4.5%) were most often detected in the diseased monkeys, and *Providencia* spp. (16.9%), *Morganella morganii* (6.7%) were found in the dead ones.

It was noted that pathogenic enterobacteria were not detected in the helminth infested diseased animals, while in the helminth-free monkeys *Shigella flexneri* were isolated in 2.5% of cases ($n = 8$). In dead monkeys without helminth infestation, the frequency of the detection of pathogenic enterobacteria (*Shigella flexneri*, rare *Salmonella* serovars, *Yersinia* spp.) was 2 times higher than in the infested animals – 8.3% ($n = 69$) and 3.9% ($n = 14$), respectively. As a result, we assume that presence of the intestinal helminths can reduce the number of bacterial pathogens, occupying their niche in the gut biocenosis.

When analyzing the trend in the frequency of isolation of gut microbiota bacteria over six years, a consistently high annual percentage of *Proteus* spp. detections was established (Table 7).

Monitoring of the isolation of pathogenic and opportunistic bacteria in monkeys during 2017–2022 showed decrease in the frequency of detections of *Klebsiella* spp., *Morganella morganii*, *Shigella* spp., *Pseudomonas* spp., hemolytic *Enterococcus* spp. and increase in the frequency of detections of hemolytic *Escherichia coli*, *Enterobacter* spp., *Providencia* spp., *Serratia* spp., *Yersinia* spp., yeast-like fungi of genus *Candida*. The decrease in the frequency of detections of *Klebsiella* spp. and *Shigella flexneri* might be associated with the use of *Klebsiella* phage and intestinal phage in the treatment of the animals. The highest percentage of detections of *Citrobacter* spp., *Klebsiella* spp., *Morganella morganii*, *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Bacillus* spp., as compared with other years, was recorded in 2019.

The microorganisms were isolated both as mono-infestations and mixed infestations. Combinations of normal flora representatives with *Proteus* spp. were more often detected. Thus, in the diseased helminth-infested monkeys, the association of *Escherichia coli* + *Proteus* spp. was reported in 10.0% of cases, *Escherichia coli* + *Enterococcus* + *Proteus* spp. – in 4.5% of cases, *Escherichia coli* + *Enterobacter* spp. + *Staphylococcus* spp. – in 2.2% of cases. The highest percentage of simultaneous detections of *Escherichia coli* + *Proteus* spp. was also reported in the diseased helminth non-infested monkeys (9.4%). Combinations of *Escherichia coli* + *Enterococcus* spp. + *Proteus* spp. were reported in 4.1% of animals; bacterial associations of normal flora involving *Escherichia coli* + *Enterococcus* spp. + *Klebsiella* spp. and *Escherichia coli* + *Enterococcus* spp. + *Staphylococcus* spp. were detected in 3.5% of monkeys. Combinations of hemolytic *Escherichia coli* with *Enterococcus* spp. were reported in 2.5% of the animals, normal flora with *Enterobacter* spp. – in 2.2% of the cases. Associations of *Escherichia coli* + *Serratia* spp., *Escherichia coli* + *Klebsiella* spp., *Escherichia coli* +

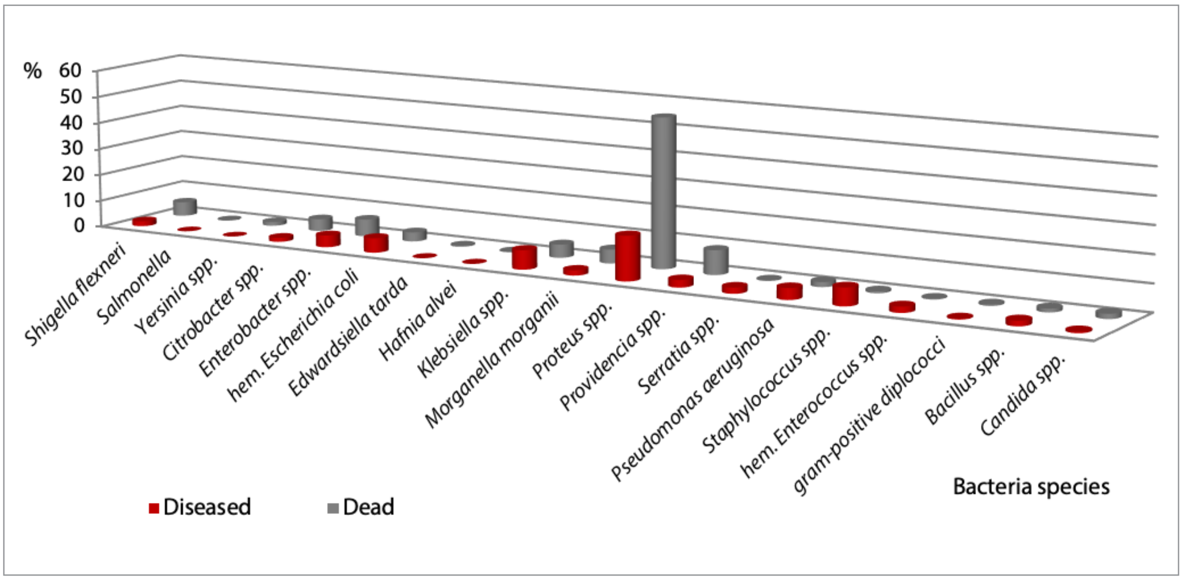

Fig. 1. Gut microbiota composition (or species diversity) in tested monkeys (2017–2022)

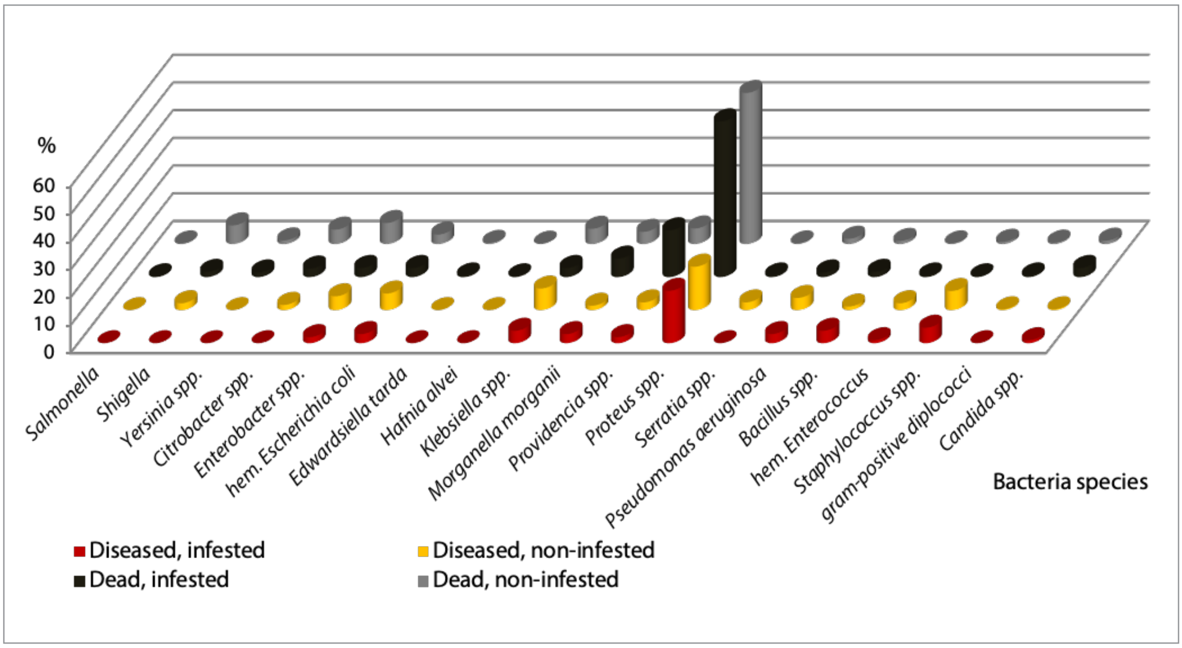

Fig. 2. Effect of helminth infestation on the frequency of detection of the pathogenic and opportunistic bacteria in monkeys (2017–2022)

Enterococcus spp. + *Pseudomonas aeruginosa* were detected in 1.9% of the tested monkeys. The remaining variants of microbial associations were identified in rare cases.

In the dead monkeys the bacterial associations were more diverse. Thus, two-component associations were found in 53.0% of cases, three-component associations in 36.7%, four-component associations in 6.7%, five-component associations in 2.5% and six-component associations in 0.3% of cases. As in the diseased monkeys, *Proteus* spp. were most often detected concurrently with the normal flora in the dead monkeys (39%). Combinations involving normal flora with other opportunistic bacteria were much less common. Thus, co-growth of *Escherichia coli* and *Enterococcus* spp. with the following bacteria were observed: *Providencia* spp. – in 3.5% of cases, *Enterobacter* spp. – in 2.9% of cases, *Shigella flexneri* – in 2.4% of cases, *Citrobacter* spp. – in 2.0% of cases, *Morganella morganii* – in 1.8% of cases, and *Klebsiella* spp. – in 1.3% of cases. In rare cases, *Proteus* spp. were isolated from the intestines of the dead animals together with *Citrobacter* spp., *Enterobacter* spp., *Yersinia* spp. without normal flora. The proportion of concurrent detections of *Proteus* spp. + *Enterococcus* spp. amounted to 1.0%.

Associations of yeast-like fungi of genus *Candida* in the intestines of the dead monkeys with one representative of the opportunistic microbiota were detected more often than with two ones (13 and 4 cases, respectively). Incidence of *Candida* spp. in association with *Proteus* spp. (11 monkeys) was higher than with *Klebsiella* spp. (3 monkeys) and *Pseudomonas aeruginosa* (2 monkeys). Therefore, combinations of pathogenic and opportunistic bacteria with yeast-like fungi in helminth-infested

Table 7
Frequency of microbiota detection in GI diseased and dead monkeys (2017–2022)

Detected microorganisms	Quantity / %						Trend test*	Total / %
	2017 ($n = 177$)	2018 ($n = 254$)	2019 ($n = 201$)	2020 ($n = 181$)	2021 ($n = 245$)	2022 ($n = 138$)		
Citrobacter spp.	8/4.5	13/5.1	17/8.5	13/7.2	3/1.2	6/4.3	0.1953	60/5.0
Enterobacter spp.	7/4.0	15/5.9	14/7.0	17/9.4	20/8.2	20/14.5	**0.0007 (↑)**	93/7.8
Hemolytic *Escherichia coli*	9/5.1	8/3.1	8/4.0	13/7.2	14/5.7	12/8.7	**0.0445 (↑↓)**	64/5.4
Edwardsiella tarda	2/1.1	0	0	1/0.6	2/0.8	0	0.7845	5/0.4
Hafnia alvei	1/0.6	0	1/0.5	0	0	0	0.9010	2/0.2
Klebsiella spp.	11/6.2	13/5.1	27/13.4	24/13.3	4/1.6	8/5.8	0.3492	87/7.3
Morganella morganii	6/3.4	15/5.9	21/10.4	9/5.0	13/5.3	5/3.6	0.7308	69/5.8
Proteus spp.	86/48.6	168/66.1	121/60.2	103/56.9	171/69.8	77/55.8	0.0914	726/60.7
Providencia spp.	7/4.0	17/6.7	24/11.9	19/10.5	28/11.4	22/16.0	**0.0002 (↑↓)**	117/9.8
Salmonella of rare serovars	2/1.1	1/0.4	0	0	0	0	**0.0279 (↓)**	3/0.3
Serratia spp.	1/0.6	1/0.4	1/0.5	0	0	10/7.2	**0.0002 (↓↑)**	13/1.1
Shigella flexneri	0	30/11.8	17/8.5	9/5.0	9/3.7	6/4.3	0.3172	71/5.9
Yersinia spp.	0	3/1.2	2/1.0	6/3.3	0	6/4.3	**0.0330 (↓↑)**	17/1.4
Pseudomonas aeruginosa	3/1.7	11/4.3	18/9.0	5/2.8	2/0.8	0	0.0186 (↑↓)	39/3.3
Bacillus spp.	0	6/2.4	10/5.0	3/1.7	0	5/3.6	0.7586	24/2.0
Hemolytic *Enterococcus*	7/4.0	1/0.4	0	1/0.6	2/0.8	2/1.4	0.1159	13/1.1
Staphylococcus spp.	4/2.3	3/1.2	11/5.5	1/0.6	12/4.9	4/2.9	0.2040	35/2.9
Gr+ diplococcus	1/0.6	5/2.0	0	0	0	0	**0.0183 (↑↓)**	6/0.5
Candida spp.	0	2/0.8	7/3.5	1/0.6	1/0.4	13/9.4	**0.0001 (↑)**	24/2.0

* $p < 0.05$ (χ^2 criterion – statistical difference of detections relative to monkey species).
Arrows show the trend of changes in detection frequency over the years upon statistical significance of the test.

monkeys can aggravate the course of GI diseases due to the simultaneous involvement of pathogenicity factors of various microorganisms and parasites in the development of the infectious process.

As for the species composition of the microbiota, the following *Enterobacteriaceae* species were isolated from monkeys*: Citrobacter freundii*, *C. diversus*, *C. amalonaticus*, *Enterobacter aerogenes*, *E. agglomerans*, *E. cloacae*, *E. gergoviae*, *Klebsiella pneumoniae*, *K. oxytoca*, *K. ozaenae*, *Proteus vulgaris*, *Pr. mirabilis*, *Pr. penneri*, *Providencia stuartii*, *P. rettgeri*, *P. alcalifaciens*, *Serratia marcences*, *S. odorifera*; *Enterococcus*: *Enterococcus faecalis*, *E. faecium*; *Staphylococcus*: *Staphylococcus aureus*, *S. haemolyticus*; as well as yeast-like fungi: *Candida krusei*, *C. glabrata*, *C. tropicalis*.

In conclusion, it can be noted that an important role in the development of GI diseases in monkeys is also played by weakened immunity due to various external factors, including stress, breaches of veterinary and sanitary, zootechnical and animal hygiene rules of feeding and keeping monkeys, which lead to normal gut microbiota disorders and opportunistic microbiota activation. Thus, GI diseases of helminth and bacterial etiology in monkeys require complex therapy. When keeping monkeys in captivity, there is a risk of parasite and pathogenic microorganism transmission to handlers due to human-animal contact. The detected *Trichocephalus trichiurus* and *Strongyloides* sp. are dangerous to humans, therefore it is necessary to comply with safety requirements when working with the diseased monkeys (regular deworming of animals, daily cleaning of cages and enclosures, and strict compliance with the personal hygiene rules). Knowledge about the parasitic and bacterial agents of spontaneous intestinal infections in monkeys is necessary for proper and safe breeding and keeping of these rare animals in captivity and for the practical use of monkeys in biomedical research.

CONCLUSIONS

The following conclusions were made based on the study results.

1. *Trichocephalus trichiurus* are prevalent in the non-humane primates kept in the apery.
2. Etiology of GI diseases in monkeys involves various associations of diverse bacteria with prevailing representatives of the *Enterobacteriaceae* family.
3. The dominant microorganisms were *Proteus* spp., which were isolated from 16.4% of the monkeys with GI diseases, and from 55.0% of the dead animals.
4. The percentage of pathogenic enterobacteria detections was low (diseased monkeys – 1.9%, dead monkeys – 7.2%), but *Shigella flexneri* was the leader among them.
5. In helminth non-infested monkeys, the pathogenic enterobacteria detection frequency was higher than in the infested ones.
6. Associative GI diseases of helminth and bacterial etiology require complex therapy of monkeys.
7. When keeping monkeys in captivity, there is a risk of parasite and pathogenic microorganism transmission to the handlers due to human-animal contact.

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Received 12.03.2024
Revised 09.04.2024
Accepted 07.05.2024

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