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Pathomorphological, bacteriological and virological features of pneumonia in captive monkeys

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ABSTRACT

Introduction. Respiratory infections pose a significant challenge in veterinary practice due to their high prevalence across various animal species. Pneumonia and gastrointestinal diseases are leading causes of mortality in captive primates.

Objective. Study of pneumonia incidence in monkeys, the analysis of pulmonary microbiota composition, study of pathomorphological lesions in lung tissue.

Materials and methods. Common methods were used for pathomorphological, microscopic and bacteriological examinations of 1,862 dead monkeys. Lung samples and serum samples from 126 monkeys died of pneumonia in 2021–2024 were tested for acute respiratory viral pathogens as well as for antibodies to them with polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA).

Results. Pneumonia was postmortem diagnosed in 865 monkeys (46.5%). The mortality rate for pneumonia in baby monkeys during their first month of life reached 100%. In baby monkeys under one year of age, the mortality rate was 65.4%. The obtained data showed that the disease incidence in these age groups was high. Deaths of monkeys due to pneumonia were reported throughout the year. Based on postmortem examinations, bilateral polysegmental broncho-pneumonia was the most frequent finding, lobar fibrinous pneumonia affecting the right lung was less common. Microscopic analysis detected purulent exudate and cocci bacteria in the bronchial lumen. The predominant bacteria isolated from lung tissue were enterobacteria (58.5%) and Gram-positive cocci (36.6%). Various microorganisms were isolated but the most frequently enteric bacteria were as follows: *Escherichia coli* (66.1%), *Enterococcus* spp. (27.5%) and *Proteus* spp. (31.5%). The following bacterial pathogens associated with pneumonia were detected: *Staphylococcus aureus* (31.5%), *Klebsiella pneumoniae* (2.2%), *Pseudomonas aeruginosa* (0.8%) and *Streptococcus pneumoniae* (0.6%). Adenoviruses, human parainfluenza viruses of type 1 and type 3 and respiratory syncytial virus (RSV) were also circulated in the monkey colony.

Conclusion. During analysis of microbial etiology of pneumonia in monkeys it shall be considered that pneumonia is frequently arisen as a secondary infection, heavily influenced by underlying gastrointestinal pathologies and immunosuppression.

Keywords: monkeys, pneumonia, pathomorphology, bacteriology, virology

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Патоморфологические, бактериологические и вирусологические особенности пневмоний у обезьян, содержащихся в неволе

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РЕЗЮМЕ

Введение. В ветеринарии проблема возникновения и лечения респираторных инфекций актуальна в связи с высоким уровнем заболеваемости среди разных видов животных. У обезьян, содержащихся в условиях неволи, пневмонии, наряду с желудочно-кишечными заболеваниями, являются одной из главных причин гибели.

Цель исследования. Изучение заболеваемости обезьян пневмонией, анализ спектра легочной микрофлоры, изучение патоморфологических изменений в легочной ткани.

Материалы и методы. При изучении 1862 случаев гибели обезьян патоморфологические, микроскопические и бактериологические исследования проводили по общепринятым методикам. Выявление возбудителей острых респираторных вирусных инфекций и антител к ним осуществляли методами полимеразной цепной реакции и иммуноферментного анализа при исследовании образцов легких и сывороток крови от 126 обезьян, погибших с диагнозом «пневмония» в 2021–2024 гг.

Результаты. У 865 обезьян postmortem поставлен диагноз «пневмония» (46,5%). Гибель от пневмоний малышей первого месяца жизни приближается к 100%. У детенышей возрастом до одного года показатель смертности составляет 65,4%. Полученные данные свидетельствуют о высокой частоте заболевания в этих возрастных группах. Гибель обезьян от пневмоний регистрируется на протяжении всего года. На основании патолого-анатомических исследований установлено, что чаще возникает двусторонняя полисегментарная бронхопневмония, реже – долевая фибринозная пневмония с развитием воспалительного процесса в правом легком. При микроскопическом исследовании в просвете бронхов выявляются гнойные экссудаты и кокковая микрофлора. Среди бактерий, выделенных из ткани легких, наибольший процент составляют энтеробактерии (58,5%) и грамположительные кокки (36,6%). Спектр выделенных видов микроорганизмов разнообразный, но в большинстве случаев представлен кишечными бактериями: *Escherichia coli* (66,1%), *Enterococcus* spp. (27,5%), *Proteus* spp. (31,5%). Из возможных бактериальных возбудителей пневмоний обнаружены *Staphylococcus aureus* (31,5%), *Klebsiella pneumoniae* (2,2%), *Pseudomonas aeruginosa* (0,8%), *Streptococcus pneumoniae* (0,6%). В стаде обезьян также циркулируют аденовирусы, вирусы парагриппа человека типа 1 и 3 и респираторно-синцитиальный вирус.

Заключение. При анализе возможной роли микроорганизмов в развитии пневмоний у обезьян необходимо учитывать, что у большинства животных заболевание развивалось на фоне патологий желудочно-кишечного тракта и при сниженном иммунитете.

Ключевые слова: обезьяны, пневмония, патоморфология, бактериология, вирусология

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INTRODUCTION

In veterinary practice, pneumonia occurrence and treatment is of current importance due to the its high incidence rate among wild, domestic, farm animals (cats, dogs, cattle, pigs, horses, etc.) and birds. Pneumonia mainly affects baby and young animals, while the disease frequency and severity depend on the keeping conditions, stress, density of animals, their immune status, diet, climate and production practice, as well as coinfection with various pathogens [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11]. In domestic animals, adults and elderly animals are often susceptible to the disease [4, 5, 6].

Low primates are also susceptible to most human pathogens, so it is easy to reproduce some human infectious diseases in them in a similar form [12]. In recent decades, due to the sharp decline in monkey populations in natural habitats and the ban on trapping, primate breeding centres have become the main source of laboratory primates. Animals kept in captivity are in most cases susceptible to intestinal and respiratory diseases. Pneumonia is one of the leading causes of morbidity and often leads to the death of animals [13, 14, 15]. Pneumonia may act either as an independent pathological process or as a concomitant (secondary) disease arising from gastrointestinal disorders, including acute or chronic gastritis and gastroenterocolitis [12, 16]. In addition, pneumonia often occurs in animals recently imported from their natural habitats and undergoing acclimatization [17, 18].

The lungs are a complex ecosystem with a large number of diverse microorganisms interacting both with each other and with the host organism [18, 19]. A functional relationship exists between the microbiomes of the lungs, oropharynx, and intestines – an interconnection that significantly influences the development of both pulmonary and intestinal diseases through the modulation of metabolic, immune, and other physiological processes. Because of the ongoing microbial exchange between the oropharynx and the upper and lower respiratory tracts, the microbiome of the lungs is never static. The dominant microbial population in the microbiome and its size differ significantly between healthy and pathologically altered organs. According to this concept, infectious lung lesions are viewed as a disruption of the existing microbial balance, with the course and outcome of pneumonia being largely determined by interactions among the microorganisms themselves [19].

A critical step in pneumonia diagnosis is the isolation and identification of the involved infectious agents – including bacteria, viruses, and fungi – from the respiratory tract [2, 20]. According to literature data, less than 10% of pneumonia cases are described as polymicrobial, and in more than 50% of cases, the etiological agents of pneumonia in monkeys remain unidentified [19]. According to the literature data, the most common causes of pneumonia in captive monkeys of various species are *Streptococcus* spp., less commonly *Klebsiella pneumoniae*, *Staphylococcus aureus* and other bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus* spp., *Morganella morganii*) [21, 22, 23, 24, 25]. Earlier, staff-members of the Kurchatov Complex of Medical Primatology of National Research Centre "Kurchatov Institute" demonstrated the involvement

of human parainfluenza virus type 3 in the respiratory tract pathology in *Papio anubis* [26].

The importance of this study is highlighted by the fact that respiratory diseases are predominant in the etiological structure of diseases in monkeys and constitute one of the primary causes of mortality for animals in captivity.

This study presents novel data by consolidating the results of pneumonia mortality monitoring in captive monkeys in the primate breeding centre, representing the first such investigation carried out in Russia. Accordingly, the present study was aimed at not only bacterial microflora detection in the lungs of dead animals and assessment of serological and molecular-genetic markers of acute viral respiratory infections (AVRIs), but also at description of pathologically altered lung features.

The purpose of the study was to study the pneumonia incidence, to analyse lung microbiota landscape, and to examine lung lesions.

MATERIALS AND METHODS

Animals. A total of 1,862 deaths of monkeys of various species from spontaneous diseases were studied in the period from January 2019 to December 2024. All animals were kept in the primate breeding centre of the Kurchatov Complex of Medical Primatology of National Research Centre "Kurchatov Institute". Dead male (n = 726) and female (n = 1,136) monkeys ranged in age from new-born to 38 years (Table 1).

Postmortem and microscopic examinations. Dead animals were necropsied in the necropsy room of the Laboratory for Pathological Anatomy of the Kurchatov Complex of Medical Primatology. Internal organs and tissues from dead animals were examined to detect pathological lesions. When gross lung inflammation signs were detected, tissue samples were collected for histological analysis. The samples were fixed in a 10% neutral (pH 7.4) formalin solution, then subjected to standard histological processing, followed by mounting into Histomix paraffin medium (BioVitrum, Russia). Histological sections, 4 µm thick, were prepared from the paraffin-embedded tissues and stained with Hansen's hematoxylin and eosin, as well as with Van Gieson's picrofuchsin [27].

Morphological analysis (microscopic examination) was carried out with Axiolab.A1 laboratory microscope (Carl Zeiss Microscope GmbH, Germany). Axiocam 105 colour digital camera (Carl Zeiss Microscopy GmbH, Germany) was used for microphotography.

Bacteriological examination was carried out according to a common technique: a lung smear was made on a slide, Gram-stained, and inoculated in diagnostic nutrient media, followed by biochemical identification of the grown colonies of microorganisms, as described earlier [28].

Virological examination. Serum and lung samples collected in 2021–2024 from 126 dead monkeys with diagnosed pneumonia including 48 *Macaca mulatta*, 22 *Macaca fascicularis*, 7 *Chlorocebus aethiops* ssp., 38 *Papio hamadryas* and 11 *Papio anubis* were used for the virological examination. The lung suspension was prepared using Minilys homogenizer (Bertin Technologies, France) at a ratio of 5–6 g of the material per 1 mL of 0.1M sodium phosphate buffer, pH 7.4; centrifuged with Allegra cold centrifuge (Beckman Coulter, USA) at 3,000 rpm for 30 min for clarification. The resulting 10% supernatant was used for further tests.

Test kits manufactured by ECOlab (Russia) were used for detection of IgG, IgM and IgA antibodies to parainfluenza virus types 1 and 3, respiratory syncytial virus (RSV) and adenovirus. The results of enzyme-linked immunosorbent assay (ELISA) were read using Immunochem-2100 laboratory spectrophotometer (High Technology Inc., USA) at wavelength of 450 nm. The sera reactivity to respiratory viruses was assessed based on OD_{450} values (optical density of ELISA-tested serum samples at a wavelength of 450 nm). The test results were interpreted according to the test-kit manufacturer's instructions.

Table 1
Characterization of dead monkeys by age groups

Monkey species	Age groups						Total
	under 1 month of age	under 11 months of age	1–3 years of age	4–10 years of age	11–15 years of age	16 years of age and older	
Macaca mulatta	52	45	116	176	86	129	604
Macaca fascicularis	73	32	78	148	76	94	501
Macaca nemestrina	15	5	2	8	6	17	53
Chlorocebus aethiops ssp.	11	7	7	18	14	18	75
Papio anubis	30	13	27	40	21	20	151
Papio hamadryas	109	49	47	110	58	71	444
Other species	5	2	7	9	3	8	34
Total	295	153	284	509	264	357	1,862

Table 2
Characterization of fatal pneumonia cases in monkeys

Monkey species	Quantity/%		
	total	females	males
Macaca mulatta	249/41.2	156/40.7	93/42.1
Macaca fascicularis	221/44.1	129/39.5	92/52.9
Macaca nemestrina	33/62.3	21/70.0	12/52.2
Chlorocebus aethiops ssp.	32/42.7	22/44.0	10/40.0
Papio anubis	74/49.0	37/40.2	37/62.7
Papio hamadryas	236/53.2	109/45.8	127/61.7
Other species	20/58.8	8/50.0	12/66.7
Total	865/46.5	482/42.4	383/52.8

Table 3
Characterization of monkeys died of pneumonia by age groups

Monkey species	Age groups												p, χ^2 for trend*
	under 1 month of age		under 11 month of age		1–3 years of age		4–10 years of age		11–15 years of age		16 years of age and older		
	number of animals	%	number of animals	%	number of animals	%	number of animals	%	number of animals	%	number of animals	%	
Macaca mulatta	47	90.4	25	55.6	46	39.7	62	35.2	24	27.9	45	34.9	**0.0005**↑↓
Macaca fascicularis	72	**98.6**	22	68.8	22	28.2	41	27.7	23	30.3	41	43.6	0.7675
Macaca nemestrina	14	93.3	1	20.0	1	50.0	6	75.0	4	66.7	7	41.2	0.9643
Chlorocebus aethiops ssp.	9	81.8	1	14.3	1	14.3	5	27.8	6	42.9	10	55.6	**0.0258**↓↑
Papio anubis	27	90.0	9	69.2	9	33.3	13	32.5	8	38.1	8	40.0	0.1357
Papio hamadryas	100	91.7	40	**81.6**	29	**61.7**	25	22.7	17	29.3	25	35.2	< **0.0001**↓
Other species	5	100	2	100	5	71.4	4	44.4	1	33.3	3	37.5	0.9648
Total	274	92.9	100	65.4	113	39.8	156	30.6	83	31.4	139	38.9	< **0.0001**↑↓

* $p < 0.05$ (χ^2 test – statistical difference in pneumonia diagnosis rates between monkey species). Arrows indicate the trend in detection frequency by age, providing that the test result was statistically significant.

Table 4
Number of monkeys died of pneumonia by year

Monkey species	Year of testing						p, χ^2 for trend*
	2019	2020	2021	2022	2023	2024	
Macaca mulatta	42	32	43	36	44	52	**0.0075**↑↓
Macaca fascicularis	42	44	39	27	32	37	0.4535
Macaca nemestrina	6	9	3	3	8	4	0.7173
Chlorocebus aethiops ssp.	5	5	2	6	10	4	0.3061
Papio anubis	20	8	15	7	14	10	0.3494
Papio hamadryas	43	42	40	45	38	28	0.3828
Other species	8	4	3	2	1	2	**0.0177**↓
Total	166/46.6%	144/45.4%	145/38.2%	126/49.2%	147/51.9%	137/50.8%	0.0910

* $p < 0.05$ (χ^2 test – statistical difference in pneumonia diagnosis rates between monkey species). Arrows indicate the trend in detection frequency by year, providing that the test result was statistically significant.

Nucleic acids were extracted from the prepared 10% lung supernatant using RIBO-prep kit (Central Research Institute of Epidemiology of Rospotrebnadzor, Russia) according to the manufacturer's instructions. Complementary DNA was synthesized on a total RNA matrix using Reverta-L reagent kit (Central Research Institute of Epidemiology of Rospotrebnadzor, Russia) in accordance with the manufacturer's instructions. Resulting cDNAs were amplified using "AmpliSens® AVRI-screen-FL" real-time PCR kit to identify acute respiratory viral infection pathogens (RSV; metapneumovirus; parainfluenza virus types 1, 2, 3 and 4; coronavirus; rhinovirus; adenovirus groups B, C and E; bocavirus) according to the manufacturer's instructions. Amplification and analysis of the results were performed using Rotor-Gene Q device (QIAGEN GmbH, Germany).

Statistical data processing. Statistical processing of the data and calculations were carried out using GraphPad-Prism 8 software. To detect changes in frequency metrics across study years or age groups, a χ^2 trend test (Pearson's chi-square) was applied. All differences were interpreted as significant at $p < 0.05$.

Table 5
Number of microorganisms isolated from lungs of the monkeys died of pneumonia (2019–2024)

Microorganism	Year of testing						p, χ^2 for trend	Total
	2019	2020	2021	2022	2023	2024		
E. coli	112	100	106	85	87	82	0.2274	572
Representatives of tribe *Proteeae*	51	50	52	42	62	44	**0.0002**↑↓	301
Klebsiella spp.	18	8	5	4	3	3	**0.0048**↓	41
Enterobacter spp.	7	4	4	1	4	6	0.5613	26
Citrobacter spp.	7	4	0	2	2	0	**0.0344**↑↓	15
Other enterobacteria	4	5	1	4	4	1	0.9126	19
Ps. aeruginosa	1	2	2	0	1	1	0.9926	7
Other non-fermenting bacteria	0	0	1	0	2	6	< **0.0001**↑	9
Bacillus spp.	1	1	6	4	3	7	**0.0024**↓↑	22
Other Gram-positive rods	27	10	0	0	3	2	< **0.0001**↓	42
Staphylococcus spp.	123	64	41	40	62	25	< **0.0001**↓	355
Enterococcus spp.	53	49	40	31	36	29	0.9856	238
Other Gram-positive cocci, including *Streptococcus pneumoniae*	6	3	0	1	2	0	0.0503	12
Candida spp.	0	0	1	0	0	0	0.9320	1
Total	410	300	259	214	271	206	< **0.0001**↓	1,660

* $p < 0.05$ (χ^2 test – statistical difference in detection frequency between microorganism species). Arrows indicate the trend in detection frequency by year, where the test result was statistically significant.

RESULTS AND DISCUSSION

A total of 865 pneumonia cases (46.5% of the total number of dead animals) were detected over a six-year period based on the results of postmortem examinations (Table 2). Pneumonia was more frequently diagnosed in males than in females (52.8 and 42.4%, respectively). Pneumonia mortality rates were generally similar across monkey species, exception for *Papio hamadryas* and *Macaca nemestrina*. In these monkey species, pneumonia caused more than 50% of deaths.

According to the findings, the highest mortality for pneumonia was observed in monkeys during their first month of life (Table 3).

The total number of monkeys that died due to pneumonia averaged between 126 and 166 cases per year, remaining relatively stable in percentage terms over a six-year period (Table 4).

Over the six-year study period, neonatal mortality in monkeys exhibited a steady increase, with pneumonia accounting for 100% of deaths in this age group by 2024.

Analysis of seasonal patterns in monkey mortality due to pneumonia revealed that more than 50% of cases occurred during the autumn-winter period of 2020 and 2024, and during the summer-spring period of 2023. In 2022, mortality due to pneumonia in these animals remained high throughout all seasons.

Pathomorphological features of pneumonia. Polysegmental bronchopneumonia was more frequently observed in monkeys living at the primate breeding centre, whereas lobar fibrinous pneumonia – similar to human croupous pneumonia – was considerably less common. Macroscopically, bilateral pulmonary involvement was the most common finding, with the right lung being affected more extensively or more frequently. In cases of unilateral involvement, pneumonia was observed more frequently in the right lung alone (Fig. 1).

Croupous pneumonia in monkeys was characterized by lobar fibrinous inflammation with pleura lesions. In addition to lobar involvement, large focal pneumonic lesions were detected in the centre of the lobes or extending to the pleura. The affected lung tissue was airless, of dense consistency and greyish-reddish colour. A cloudy, foamy fluid exuded from the cut surface when the affected lung was compressed. The lung lymph nodes were enlarged. Cases of total, bilateral lung damage with alveolar filling by fibrinous-leukocytic exudate and serous fluid buildup were recorded (Fig. 2).

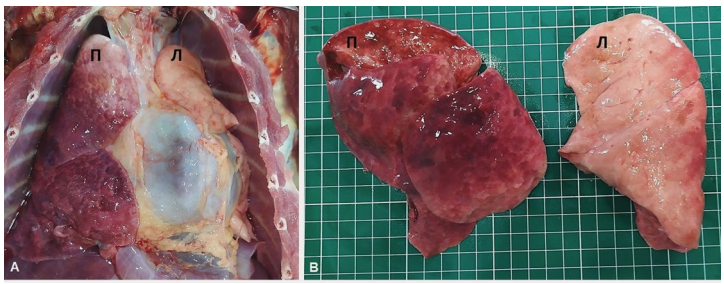

Fig. 1. Macrophotography of heart – lung complex in the monkey with polysegmental bronchopneumonia (Macaca fascicularis, ♀, 3 years old): A – general view; B – all lobes of the right lung are affected; П – right lung; Л – left lung

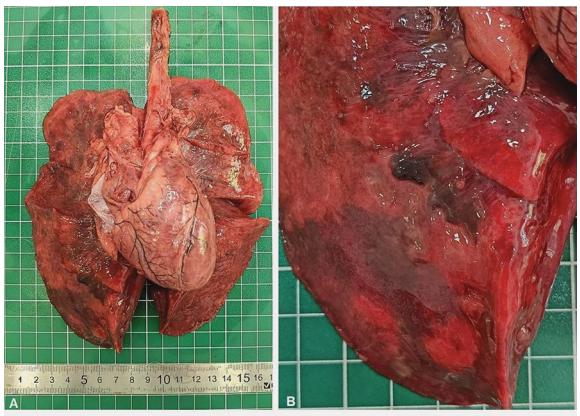

Fig. 2. Macrophotography of heart – lung complex in the monkey with fibrinous pneumonia (Erythrocebus patas, ♂, 10 years old), all lobes of the right and left lungs are completely affected: A – general view; B – fibrin threads on the visceral pleura of the right lung

Fibrin, degenerated leukocytes, and other cell debris were detected within the alveoli in croupous pneumonia cases. There were areas of lung tissue containing only red blood cells in the alveoli. Apparent vascular hyperemia with parietal leukocyte accumulation and capillary stasis within the interalveolar septa were observed (Fig. 3).

Purulent tissue melting foci located generally around the affected bronchi were observed in all croupous pneumonia cases. The bronchial lumen contained purulent exudate and coccoid microflora.

Bronchopneumonia occurred both independently and as a concomitant disease complicating gastrointestinal pathologies. In bronchopneumonia cases, multiple small inflammation foci of grey-red or bluish-purple colour, often merging, that located along the branching bronchi were found in the lungs. The lung tissue around inflammation foci was edematous with hyperemia or severe emphysema, which gave the incision surface a mottled appearance (Fig. 4).

Similar to croupous pneumonia, the microscopic inflammation lesions in these cases were characterized by accumulation of the exudate of various types. Foci of serous fluid mixed with red blood cells as well as polymorphic cellular exudate were found in the alveoli. Leukocytes and mucous with a large amount of bacterial microflora often predominated in the exudate. In all cases, inflammatory lung lesions were combined with the development of focal atelectasis and focal emphysema (Fig. 5).

Thus, croupous pneumonia and bronchopneumonia in monkeys kept in the Kurchatov Complex of Medical Primatology primate breeding centre were characterized by a variety of morphological changes in the lungs. This appears related to the different properties of the disease pathogens.

Asymptomatic pneumonia diagnosed at necropsy only were found in most dead animals. In some cases, differentiation between croupous pneumonia and bronchopneumonia was difficult, as the presenting symptoms were subtle or nonspecific. Intrapulmonary complications including purulent bronchitis, lymphangitis, purulent inflammation foci, extremely rarely – pleural empyema were observed in some animals. The following extrapulmonary lesions were recorded – purulent meningitis, serous-purulent pericarditis.

Microbial landscape in lungs. Gram-positive cocci were found in all smears during examination of lung

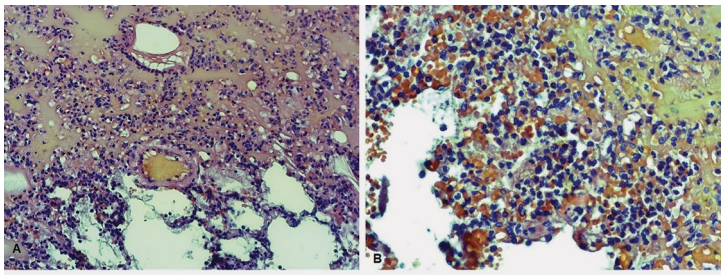

Fig. 3. Microscopic lesions in lungs of the monkey with lobar pneumonia (Macaca mulatta, ♂, 5 years old): A – alveolar edema, fibrin threads in the alveolar lumen, degenerated leukocytes (hematoxylin and eosin staining, magnification 200×); B – alveolar edema, erythrocyte and leukocyte accumulations in alveolar lumen (hematoxylin and eosin staining, magnification 400×)

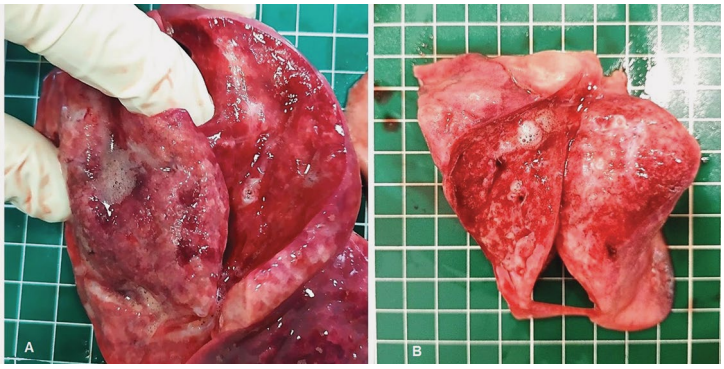

Fig. 4. Macroscopic lesions in lungs of the monkey with bronchopneumonia (Papio anubis, ♂, 5 years old): A, B – alveolar edema (foamy fluid on section), mottled pattern in lung tissue

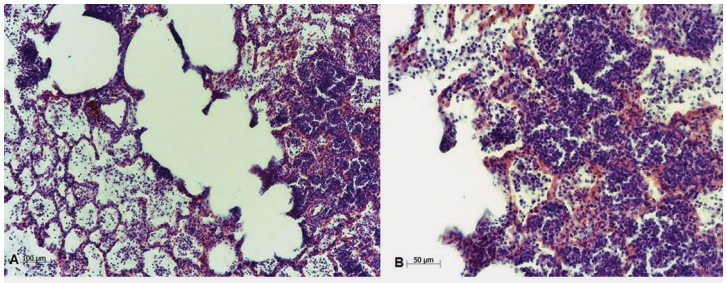

Fig. 5. Microscopic lesions in lungs of the monkey with bronchopneumonia (Papio anubis, ♂, 5 years old): A – focal emphysema (dilated alveoli, thinned and partly disrupted alveolar walls), polymorphic cellular exudate in the alveoli lumen (hematoxylin and eosin staining, magnification 100×); B – polymorphic cellular exudate in the alveoli lumen (hematoxylin and eosin staining, magnification 200×)

Table 6
Indicators of viral infections

Indicators / Monkey species	Parainfluenza virus type 1		Parainfluenza virus type 3		RSV		Adenovirus
	IgG	IgA	IgG	IgA	IgG	IgM	IgG
Monkeys that died in 2021–2022							
Macaca mulatta	10/15* 66.7%	0/15 0%	0/15 0%	0/15 0%	n/d	n/d	2/15 13.3%
Macaca fascicularis	3/4 75.0%	0/4 0%	0/4 0%	0/4 0%	n/d	n/d	0/4 0%
Chlorocebus aethiops ssp.	1/3 33.3%	0/3 0%	0/3 0%	0/3 0%	n/d	n/d	0/3 0%
Papio anubis	3/15 20.0%	0/15 0%	0/15 0%	0/15 0%	n/d	n/d	0/15 0%
Papio hamadryas	0/4 0%	0/4 0%	0/4 0%	0/4 0%	n/d	n/d	0/4 0%
Total	17/41 41.5%	0/41 0%	0/41 0%	0/41 0%	n/d	n/d	2/41 4.9%
Monkeys that died in 2023–2024							
Macaca mulatta	0/33 0%	0/33 0%	0/33 0%	0/33 0%	0/33 0%	8/33 24.2%	4/33 12.1%
Macaca fascicularis	1/18 5.6%	0/18 0%	0/18 0%	0/18 0%	0/18 0%	9/18 50.0%	1/18 5.6%
Chlorocebus aethiops ssp.	0/4 0%	0/4 0%	2/4 50.0%	0/4 0%	1/4 25.0%	2/4 50.0%	1/4 25.0%
Papio anubis	0/23 0%	0/23 0%	3/23 13.0%	0/23 0%	0/23 0%	8/23 34.8%	2/23 8.7%
Papio hamadryas	0/7 0%	0/7 0%	0/7 0%	0/7 0%	0/7 0%	3/7 42.9%	1/7 14.3%
Subtotal	1/85 1.2%	0/85 0%	5/85 5.9%	0/85 0%	1/85 1.2%	30/85 35.3%	9/85 10.6%
Total	18/126 14.3%	0/126 0%	5/126 4.0%	0/126 0%	1/85 1.2%	30/85 35.3%	11/126 8.7%

* positive serum samples / number of tested serum samples; n/d – no data.

microbiota of monkeys. Over six years, 1,660 microorganisms were isolated during bacteriological tests, the majority of which were representatives of the family *Enterobacteriaceae* (58.5%). Gram-positive cocci were detected in 36.6% of cases, the proportion of non-fermenting bacteria, including *Pseudomonas aeruginosa*, and non-differentiated Gram-positive rods was 1.0 and 3.8%, respectively (Table 5).

Statistical analysis showed significant changes in the detection rates of certain microorganisms over the years of the study ($p < 0.0001$), which indicated shifts in lung microbiota composition.

The number of representatives of tribe *Proteeae* varied without any clear tendency to increase or decrease, which was indicative of a dynamic change in the role of these microorganisms ($p = 0.0002$). The number of *Klebsiella* spp. detections decreased over the years ($p = 0.0048$), potentially indicating a decline in the pathogen's role in pneumonia development. The number of *Citrobacter* spp. ($p = 0.0344$) and *Bacillus* spp. ($p = 0.0024$) detections varied over the years. Notwithstanding the high isolation rate of *Staphylococcus* spp., a significant decline has been recorded in recent years, from 123 to 25 cases ($p < 0.0001$). Gram-positive rods detection rate also decreased ($p < 0.0001$). Non-fermenting Gram-negative rods were rarely isolated from the lungs, however, they were detected in 6 cases ($p < 0.0001$) in 2024. The total level of the isolated microorganisms decreased over the years (from 410 in 2019 to 206 in 2024), which was confirmed by statistical data ($p < 0.0001$).

As for microbial landscape, the leading position was occupied by *E. coli*, which were found in the lungs of 66.1% of monkeys, the second position was occupied by *Staphylococcus* spp., including *S. aureus* (41.1%), followed by representatives of tribe *Proteeae* (*Proteus* spp., *Providencia* spp., *M. morganii*) and *Enterococcus* spp. (34.8 and 27.5%, respectively). Other microorganisms were isolated rarely. During the tested period, *S. aureus* was more often isolated

as compared to other main bacterial pathogens of pneumonia – in 267 monkeys (31.5%), other pathogens were found in some cases, namely: *K. pneumoniae* – in 19 monkeys (2.2%), *Ps. aeruginosa* – in 7 monkeys (0.8%), *St. pneumoniae* – in 5 monkeys (0.6%). No bacterial growth was observed on the nutrient media inoculated by the samples from 7 cases (0.8%). Also, no materials were collected for examination from 17 monkeys with pneumonia due to postmortem decomposition. Bacterial associations were observed, however, the number of detected associations decreased during the tested period. In 2019, 89% of the detected microbial isolates were associations, compared to 52% in 2024, with the frequency of 4-component associations apparently decreasing.

Any analysis of the etiological role of the isolated microorganisms in monkey pneumonia shall account for the fact that, in the majority of cases, the disease has arisen concurrently with gastrointestinal pathology and immunosuppression. This was evidenced by the detection of coliforms in lungs, as well as the prolonged pneumonia course with subtle or non-specific clinical signs. *S. aureus* represented an exception, based on previous molecular genetic evidence indicating its high pathogenic potential and lung tissue tropism of some strains [29, 30].

When 126 monkeys with diagnosed pneumonia who died in 2021–2024 were tested for AVRI indicators, IgG antibodies indicative of post-infection immunity against the following viruses were detected: parainfluenza virus type 1 and 3 (14.3 and 4.0% of the monkeys, respectively), RSV (1.2%), and adenovirus (8.7%). IgA and IgM antibodies indicative of acute infection period were detected only against RSV (35.3% of the monkeys). No AVRI pathogen RNAs/DNAs were detected with polymerase chain reaction (PCR) in the lung parenchyma (Table 6).

In monkeys that died in 2021–2022 ($n = 41$), IgG antibodies against parainfluenza virus type 1 (41.5%) only and adenovirus (4.9%) were detected, while no IgG antibodies against parainfluenza virus type 3 were detected. Also, no serological indicators of acute infection were detected.

In animals with diagnosed pneumonia that died in 2023–2024 ($n = 85$), IgM antibodies against RSV (35.3%) were detected in their sera, while the no IgA antibodies against parainfluenza virus type 1 and 3 were detected. In addition, IgG antibodies indicative of postinfection immunity were detected: IgG against parainfluenza virus type 1 and 3 (1.2 and 5.9%, respectively), RSV (1.2%), and adenovirus (10.6%).

CONCLUSIONS

1. Pneumonia is the most common cause of death of monkeys kept in the Kurchatov Complex of Medical Primatology breeding centre (46.5%) and often occurs concurrently with gastrointestinal diseases in weakened animals, which is consistent with previously published data.

2. The highest mortality due to pneumonia was observed in baby monkeys under the age of one month (92.9%) and during the first year of life (65.4%). Consequently, captive monkeys from birth to 1 year of age were the most susceptible to pneumonia.

3. The most frequent finding was bilateral polysegmental bronchopneumonia, and less frequently, lobar fibrinous pneumonia resembling human croupous pneumonia, with predominant involvement of the right lung.

4. The bacterial microflora isolated from pneumonia-affected lungs consisted of enterobacteria (58.5%) and Gram-positive cocci (36.6%), with *S. aureus* being isolated in 41.1% of cases. Microscopic examination also revealed cocci in the lung tissues.

5. Circulation of adenovirus, human parainfluenza virus types 1 and 3, as well as RSV in monkeys who died from pneumonia was shown. A high percentage of IgM against RSV indicated the possible involvement of this virus in the respiratory pathologies in monkeys.

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