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Bacteriology and pathological anatomy of pneumonias in monkeys

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SUMMARY
Data on the etiological structure of potential pneumonia agents in monkeys based on postmortem findings and subsequent bacteriological tests of lung tissues collected from the organ areas showing morphological changes are presented. In the period between 2019 and the first half of 2021, 377 animals died of pneumonia. The highest pneumonia-associated mortality was observed in newborn (0–8-day-old) and baby monkeys under the age of 1 month (161 animals). Polysegmental bronchopneumonia was detected in the dead monkeys in 94.4% of cases, croupous pneumonias accounted for 4.5%. Pneumonia was typically the only disease detected in baby monkeys. The microbial landscape in pneumonia affected monkeys was characterized by a broad diversity: 899 bacteria of different taxonomic groups were isolated from the lung tissues. Staphylococci (23.8%) prevailed among Gram-positive bacteria, *Escherichia coli* (32.1%) – among Gram-negative bacteria. *Streptococcus pneumoniae* made up 0.3%. Based on data from bacteriological tests, the proportion of pneumonias of undetermined etiology was 0.7%. Besides, bacterial associations, two- or three-component ones as a rule, were detected in the tests of lung tissue samples. The most frequent combinations of associative pathogens were the following: *Escherichia coli* + *Proteus* spp. (24.7%), *Staphylococcus aureus* + *Escherichia coli* (19.6%), *Staphylococcus* spp. + *Enterococcus* spp. + *Escherichia coli* (35.5%), *Staphylococcus* spp. + *Escherichia coli* + *Proteus* spp. (21.2%). Almost all the enterobacteria detected have a high associativity coefficient and occur mainly in the form of associations. The analysis of the study results showed that practically any microorganism alone or in combination can cause pneumonia in an animal with a weakened immunity; therefore, the effect of microbiota should not be underestimated. Also, significance of associative microbes in the development of pneumonia in captive monkeys is increasing.

Keywords: monkeys, pneumonia, polysegmental bronchopneumonia, croupous pneumonia, bacterial agents, microbial associations

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Бактериология и патологическая анатомия пневмоний у обезьян

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РЕЗЮМЕ
Приведены данные по этиологической структуре потенциальных возбудителей пневмоний у обезьян согласно данным патолого-анатомической картины с последующими бактериологическими исследованиями тканей легких, взятых из морфологически измененных участков органа. В период с 2019 по 1-е полугодие 2021 г. от пневмоний погибло 377 животных. Наибольшая гибель от пневмоний отмечена у новорожденных (0–8 сут) и детенышей до 1 месяца (161 особь). В 94,4% случаев у погибших обезьян была выявлена полисегментарная бронхопневмония, доля крупозных пневмоний составила 4,5%. У детенышей пневмония чаще являлась единственным заболеванием. Микробный пейзаж при пневмониях обезьян отличался широким разнообразием. Из легочной ткани выделено 899 бактерий разных таксономических групп, из грамположительной микрофлоры преобладали стафилококки (23,8%), из грамотрицательной – *Escherichia coli* (32,1%). Доля *Streptococcus pneumoniae* составила 0,3%. Удельный вес пневмоний неустановленной этиологии, по данным бактериологического исследования, был равен 0,7%. При исследовании образцов легочной ткани также выявлены бактериальные ассоциации, как правило, двух- и трехкомпонентные. Среди патогенов-ассоциантов чаще встречались следующие комбинации: *Escherichia coli* + *Proteus* spp. (24,7%), *Staphylococcus aureus* + *Escherichia coli* (19,6%), *Staphylococcus* spp. + *Enterococcus* spp. + *Escherichia coli* (35,5%), *Staphylococcus* spp. + *Escherichia coli* +

Proteus spp. (21,2%). Практически все выявленные энтеробактерии имеют высокий коэффициент ассоциативности, встречаясь в основном в виде ассоциаций. Анализ результатов исследования показал, что практически любой микроорганизм изолированно или в комбинации может привести к развитию пневмонии при ослаблении иммунитета животного, поэтому нельзя недооценивать влияние микрофлоры. Также возрастает роль микробов-ассоциантов в развитии пневмонии у обезьян, содержащихся в условиях неволи.

Ключевые слова: обезьяны, пневмония, полисегментарная бронхопневмония, крупозная пневмония, бактериальные возбудители, микробные ассоциации

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INTRODUCTION

Respiratory tract pathology is a widespread group of diseases. Pneumonia belonging to this group is an inflammation in lung structures that develops against the backdrop of different factors. In most cases, it occurs as a result of aspiration of opportunistic oropharyngeal microbiota into the lower airways. Therefore, it can be said that pneumonia is a pluricausal disease of mostly bacterial, bacterial-viral or viral etiology. Most common bacterial agents are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Mycoplasma pneumoniae*. Along with these pathogenic microorganisms, opportunistic bacteria (enterobacteria, coagulase-negative staphylococci, etc.) today have become more common in the etiological structure of significant agents. However, among many microorganism species colonizing upper airways, only a few can enter the lungs and cause an inflammatory response. Different pathogens can cause pneumonia either alone, or in association with other microorganisms, that is why some authors underscore the polymicrobial nature of this disease [1, 2]. Pneumonias caused by *St. aureus* and *K. pneumoniae* account for the largest percentage of deaths [3]. Given the infectious nature of pneumonia, it is important to study the variety of the disease agents.

Pneumonia affects all animals, but typically cattle, small ruminants, horses, cats, cetaceans. The reasons for the disease occurrence are living conditions (keeping animals in poor conditions), hypothermia, vitamin deficiency, malnutrition and, as a consequence, weakened body defences. The disease most commonly affects newborns and young animals under the age of one year.

Pneumonia is currently one of important problems in primatology, which is not solely due to its high occurrence in monkeys in captivity, but also due to high mortality rates, especially in baby animals under the age of 1 month. Pneumonia kills up to 20–50% of monkeys in the breeding facilities and zoos worldwide [4, 5]. Pneumonia can occur as a primary disease, as well as complicate other diseases. Specific features of pneumonia etiology and pathogenesis in captive monkeys require further detailed examination. There are reports in literature that highlight the close similarity between pneumonia in humans and in monkeys [6].

The aim of the study is to carry out the analysis of pneumonia-associated deaths of monkeys, to identify the range of bacterial pathogens being potential infectious agents.

MATERIALS AND METHODS

In the period from 2019 to the first half of 2021, 866 dead monkeys of both sexes, belonging to sixteen species (Table 1) that had lived in the breeding facility of the FSBSI "Research Institute of Medical Primatology" (FSBSI "RIMP") were necropsied. The age of the dead animals submitted to the necropsy department ranged from 0 days (newborns) to 35 years. Based on the necropsy results, a total of 355 monkeys died in 2019, 316 monkeys – in 2020, and 195 monkeys – in the first half of 2021. Stillbirths, decomposed carcasses and euthanized animals were not taken into consideration in this study.

Based on pathomorphological findings, pneumonia was identified as a principal or secondary diagnosis in 377 animals. Preliminary diagnosis was made based on macroscopic findings from the necropsy. In most cases, it was confirmed, with some additions and refinements, by histology.

Pieces of lungs collected from the organ areas showing morphological changes served as necropsy material used for examination. A part of the material was fixed in the 10% buffered formalin. Hematoxylin-eosin stained histological samples were prepared. The material was examined using a light microscope.

In parallel, bacteriological tests were carried out. For bacterial microbiota detection, lung material was inoculated by replica plating method, as well as to sugar broth, from which the resulting colonies were inoculated to differential diagnostic media after 24 hours. Salt Agar with egg yolk emulsion, Endo Agar, 5% Blood Agar were used for the isolation of different microorganism groups, as previously described [7]. The isolated cultures were identified based on the morphology and biochemical properties.

Table 1
Characteristics of dead monkeys

Monkey species	Age						Total
	under 1 month	1 month –1 year	1–3 years	3–10 years	10–15 years	15 years and over	
Macaca mulatta	29	19	43	87	49	63	290
Macaca fascicularis	48	21	19	70	29	41	228
Macaca nemestrina	5	2	–	5	5	6	23
Chlorocebus sabaeus	6	3	4	10	4	7	34
Papio anubis	18	3	10	27	12	10	80
Papio hamadryas	69	23	16	43	18	22	191
Macaca assamensis	–	–	–	–	1	1	2
Macaca arctoides	–	–	–	–	–	2	2
Macaca maura	–	–	–	–	–	1	1
Cercopithecus mona	–	–	–	–	–	1	1
Lophocebus aterrimus	–	1	–	–	–	–	1
Cebus apella	2	–	2	–	–	1	5
Cebus capucinus	2	–	–	–	–	–	2
Macaca sylvanus	–	–	–	–	–	1	1
Erythrocebus patas	–	–	2	1	–	1	4
Hylobates	–	–	1	–	–	–	1
Total	179	72	97	243	118	157	866

Associativity coefficient (AC) used as a criterion for determination of microbial association frequency and bacterium involvement in them was calculated:

$$AC = \frac{\text{number of associative cultures of a certain species}}{\text{total number of cultures of this species}} \times 100\%.$$

When AC is less than 50% (low criterion), microorganisms mainly occur in monocultures; when AC is 50–79% (medium criterion) – more frequently as associative microorganisms; when AC is 80–100% (high criterion) – mainly in the form of associations.

RESULTS AND DISCUSSION

Out of the total number of monkeys that had died between 2019 and the first half of 2021, 377 (43.5%) were diagnosed with pneumonia based on pathomorphological findings; that was evidenced by various manifestations of inflammation and the location of the organ lesions: lung tissue hardening, darkened areas, catarrhus of tracheal and bronchial mucosa (Table 2). Most frequently, chronic atrophous gastroenterocolitis, in presence of general emaciation and dehydration of the body, was identified as a secondary diagnosis in adult animals affected with pneumonia.

Based on the number of dead monkeys by species, the highest pneumonia-associated mortality percentages were observed in *Papio hamadryas* (28.4%), *Macaca fascicularis* and *Macaca mulatta* (27.3 and 24.9%, respectively) (Table 2). Observations showed that mortality in the animals does not depend on their sex. Specifically, 187 male and 190 female monkeys died during the said period. The analysis of seasonal dynamics demonstrated the absence of a sharp pneumonia-associated mortality increase in monkeys in the course of a year.

According to the data presented in Tables 1 and 3, pneumonia-associated mortality in baby monkeys aged under 1 month was 90% (161 out of 179 animals). Pneumonia also killed more than half of baby monkeys under the age of 1 year (42 out of 72 animals) and 42.3% of adolescent monkeys aged under 3 years (41 out of 97 animals). Pneumonia-associated mortality in young monkeys aged 3 to 10 years (55 out of 243 animals) is more than twice lower than that in adolescent monkeys, and pneumonias are, as a rule, concurrent diseases in such cases. Adult and old animals again demonstrate higher mortality, with pneumonia being the main cause of deaths. Pneumonia-associated mortality regardless of species was found to be the greatest (42.7%) in newborn and baby monkeys aged from several days to 1 month (Table 3). In particular, *Papio hamadryas* demonstrated the highest mortality percentage (37.3%). In the said age group, high mortality was also observed in baby *Macaca fascicularis* (30.4%). In baby *Macaca mulatta*, mortality becomes higher starting from the age of 1 month to 1 year (23.8%). Pneumonia was typically the only disease detected macroscopically in baby monkeys under the age of 1 year. Mortality rates in the adult and old monkeys of the genus *Macaca* (*Macaca mulatta* and *Macaca fascicularis*) are approximately the same, whereas mortality in baboons decreases with the increase of years.

In most cases, the dead monkeys were diagnosed with bilateral polysegmental bronchopneumonia (94.4%). Lobar pneumonia was detected in 4.5% of cases.

Necropsy revealed that mucous membranes of trachea and large bronchi were swollen, engorged and with multiple petechias (Fig. 1).

Microscopic examination revealed the presence of such cellular elements as neutrophilic leukocytes, lymphocytes, squamous alveolar cells and erythrocytes in different proportions (Fig. 2).

Microfocal and confluent serous pneumonia prevailed. Loose lying or phagocytized diplococci were typically detected in exudates. Bronchial disorders manifested themselves as desquamative or ulcerative bronchitis. Mixed microbiota represented by cocci was detected in the bronchial lumen. The mentioned lung lesions often occurred in combination with multifocal atelectasis. The following was detected in lobar pneumonia cases: gross lesions of one or several lung lobes, fibrinous exudate in the alveoli, fibrin deposition on the pleura (pleuropneumonia), exudate consisted predominantly of fibrin. Pneumonias in monkeys under the age of 6 months were systemic, affecting the entire lung, accompanied by bacteriaemia and purulent inflammation of meninges. Lobar pneumonia and bronchopneumonia are classical anatomical categories of bacterial pneumonia with morphological features depending on infectious agent species, and bacteriological tests play a critical role in identifying the etiology of infectious processes in such cases.

Table 2
Number of monkeys (by species) that died of pneumonias of different etiology between 2019 and the first half of 2021

Monkey species	Number (%) of animals			Total
	2019	2020	the first half of 2021	
Macaca mulatta	42 (44.7)	30 (31.9)	22 (23.4)	94 (24.9)
Macaca fascicularis	42 (40.8)	43 (41.7)	18 (17.5)	103 (27.3)
Macaca nemestrina	6 (35.3)	9 (52.9)	2 (11.8)	17 (4.5)
Chlorocebus sabaeus	5	5	–	10 (2.7)
Papio anubis	19 (54.2)	8 (22.9)	8 (22.9)	35 (9.3)
Papio hamadryas	42 (39.3)	40 (37.4)	25 (23.3)	107 (28.4)
Macaca assamensis	2	–	–	2 (0.5)
Macaca sylvanus	1	–	–	1 (0.3)
Erythrocebus patas	2	–	–	2 (0.5)
Cebus apella	1	1	2	4 (1.1)
Cebus capucinus	–	2	–	2 (0.5)
Total	162 (43.0)	138 (36.6)	77 (20.4)	377

A total of 899 microorganism cultures were detected in bacteriological tests of monkey lung samples, with Gram-positive microbiota accounting for 45.1% (405 cultures) and Gram-negative microbiota – for 54.9% (494 cultures). There was no growth on nutrient media in 0.7% of cases (7 lung samples). The analysis of microbial

Table 3
Age structure of monkeys that died of pneumonias

Monkey species	Number (%) of animals aged					
	under 1 month	1 month – 1 year	1–3 years	3–10 years	10–15 years	15 years and over
Macaca mulatta	24 (14.9)	10 (23.8)	15 (36.6)	20 (36.4)	13 (34.2)	12 (30.0)
Macaca fascicularis	49 (30.4)	12 (28.6)	8 (19.5)	14 (25.5)	8 (21.1)	12 (30.0)
Macaca nemestrina	5 (3.1)	1 (2.4)	–	5 (9.1)	3 (7.9)	3 (7.5)
Chlorocebus sabaeus	5 (3.1)	–	–	2 (3.6)	–	3 (7.5)
Papio anubis	15 (9.3)	–	4 (9.8)	5 (9.1)	8 (21.1)	3 (7.5)
Papio hamadryas	60 (37.3)	18 (42.8)	11 (26.8)	8 (14.5)	5 (13.1)	5 (12.5)
Macaca assamensis	–	–	–	1 (1.8)	–	1 (2.5)
Macaca sylvanus	–	–	–	–	1 (2.6)	–
Erythrocebus patas	–	–	2 (4.9)	–	–	–
Cebus apella	1 (0.6)	1 (2.4)	1 (2.4)	–	–	1 (2.5)
Cebus capucinus	2 (1.3)	–	–	–	–	–
Total	161 (42.7%)	42 (11.1%)	41 (10.9%)	55 (14.6%)	38 (10.1%)	40 (10.6%)

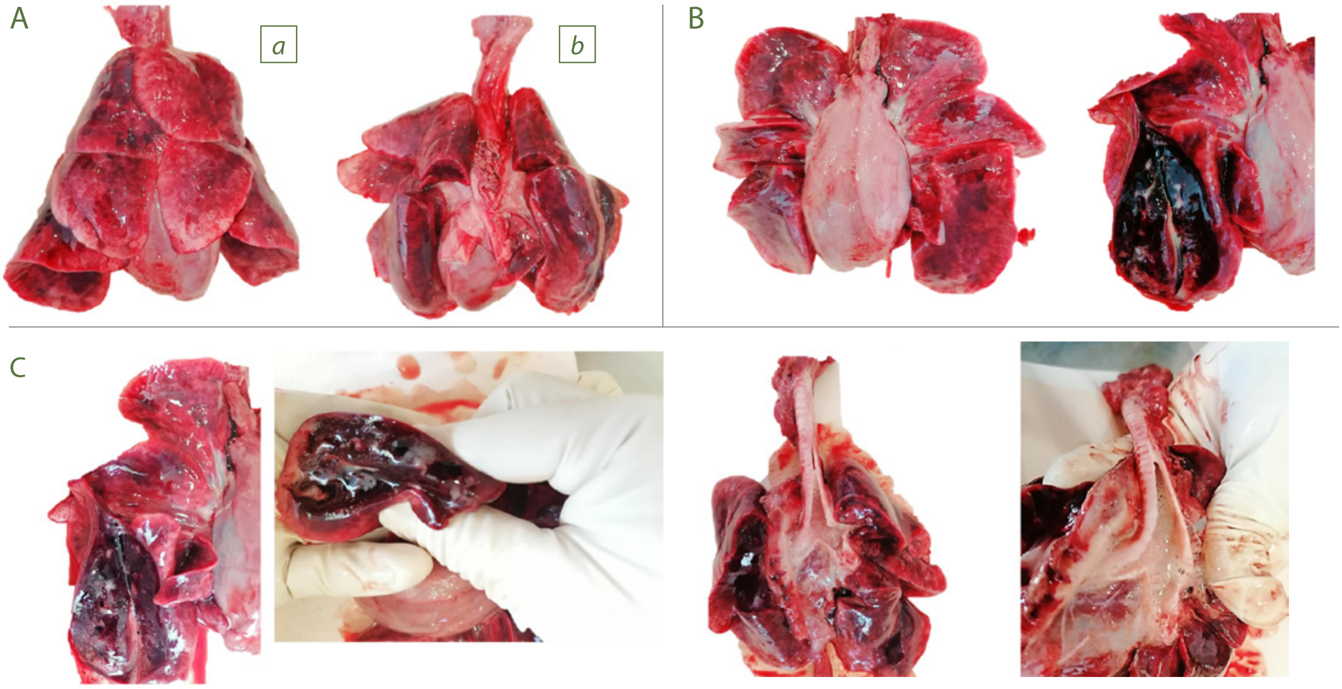

Fig. 1. Bilateral polysegmental bronchopneumonia (gross lung lesions) in a 4-year-old male Macaca mulatta
A – ventral (a) and dorsal (b) surfaces of lungs;
B – lower lobe of the right lung;
C – pulmonary alveolar edema

landscape showed that the representatives of the family *Enterobacteriaceae* (54.1%) were detected most frequently, Gram-negative non-fermenting bacteria were detected in 0.2%, and *Pseudomonas aeruginosa* – in 0.7% of cases. Among coccal flora, staphylococci (23.8%) occurred most frequently, *St. aureus* accounted for 16.8%, *Enterococcus* spp. – for 15.2%, *St. pneumoniae* bacteria were detected in as little as 0.3% of cases. Among enterobacteria, *Escherichia coli* were detected most frequently (32.1%). The percentages for other bacteria were low (Fig. 3).

Enterobacteria of the genus *Klebsiella* (2.6%) isolated from pneumonia cases were represented by three species: *K. pneumoniae* (10 isolates), *K. oxytoca* (11 isolates), *K. ozaenae* (2 isolates). Among the members of the genus *Enterobacter* (2.5%), the most frequently detected ones were *E. cloacae* (7 isolates) and *E. aerogenes* (6 isolates); *E. gergoviae* (3 isolates), *E. agglomerans* (4 isolates) were detected less frequently. The bacteria of the genus *Citrobacter* were detected in 1.2% of cases, with the number of *C. freundii* isolates being 8, *C. diversus* – 2 and *C. farmeri* – 1. The representatives of the genus *Providencia* were detected in 0.7% of cases: *P. stuartii* (5 isolates), *P. rettgeri* (1 isolate). Other enterobacteria were detected in individual cases: *Erwinia* spp. (2 isolates), *Hafnia alvei* (2 isolates), *Serratia* spp. (1 isolate). The proportion of detected *Bacillus* spp. was 0.6%, that of other unidentified Gram-positive rod-shaped bacteria – 4.3%. As Table 4 shows, the isolated microorganisms were represented

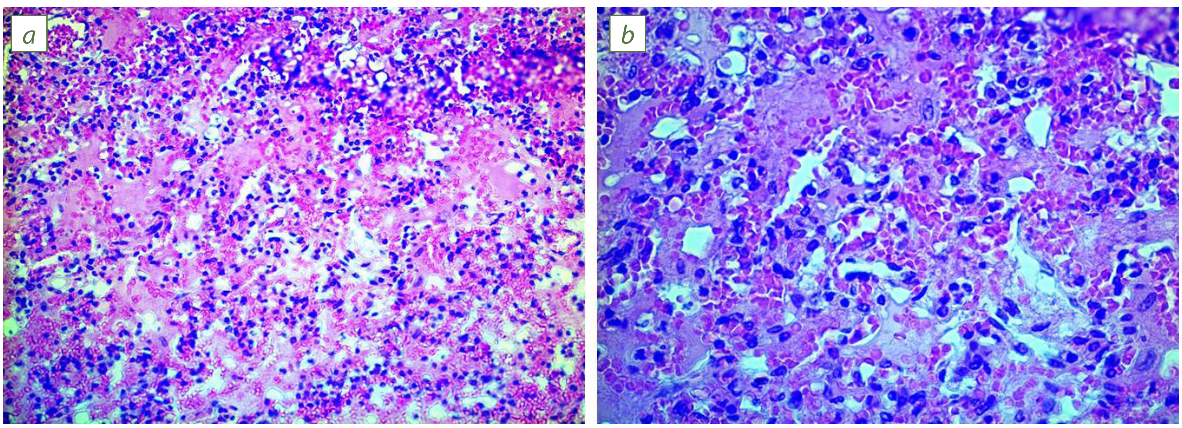

Fig. 2. Bilateral polysegmental bronchopneumonia (microscopic lung lesions) in a 4-year-old male Macaca mulatta. Hematoxylin and eosin staining, 100× (a) and 200× (b) magnification

by monocultures in 13.4% of cases, while other bacteria were part of associations (86.6%) that comprised from 2 to 6 microorganisms.

As a result of the tests performed, an increase in the number of associations was detected; in particular, in 2019, 142 associations were isolated, and in the first half of 2021 – as many as 138 microbial associations. Two-component associations (58.4%) had a leadership position.

Also, three-, four-, five-component associations (29.8, 10.3, 1.2%, respectively) and one six-component association (0.3%) were detected. Table 5 shows the number of the most frequently registered bacterial associations. As can be seen, the most frequent combination detected among two-component associations was *E. coli* + *Proteus* spp. (24.7%), among three-component associations – that of *Staphylococcus* spp. + *Enterococcus* spp. + *E. coli* (35.5%). Other combinations were present in individual cases.

When determining the frequency of microorganism occurrence as part of associations, associativity coefficient (AC) was calculated. Bacteria isolated from pneumonia cases were characterized by medium and high associativity coefficients. *E. coli* demonstrated a medium AC (58%), others had a high AC ranging from 67% for *Ps. aeruginosa* to 100% for *Providencia* ssp. and *Citrobacter* spp.

The shortcoming of the study was the absence of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* diagnosis. However, based on the previously conducted studies, these pathogens can be present in the lung tissues of pneumonia-affected monkeys [8]. According to the data on file from the Laboratory of Infectious Virology of the FSBSI "RIMP", no respiratory viruses were detected in pneumonia-affected monkeys during the period from 2019 to June 2021. The molecular genetic studies of *St. aureus* carried out earlier make it possible to speak about their high pathogenicity and consider them as etiologically significant agents of pneumonias in monkeys [9]. The isolation of such enterobacteria as *Enterobacter* spp., *Citrobacter* spp., *Proteus* spp., *Morganella morganii*, *Providencia* spp., *Hafnia alvei*, *Serratia* spp., *Erwinia* spp., as well as of *E. coli* in most cases from lung tissues is indicative of *post mortem* contamination of the tested material, rather than of the etiological significance of these bacteria. Nevertheless, the analysis of the data obtained shows that practically any microorganism alone or in combination can cause pneumonia in an animal with a weakened immunity; in view of this, the effect of microbiota must not be underestimated, and it can be said that significance of associative microbes in the development of pneumonia is increasing [2].

CONCLUSIONS

Thus, the study performed yielded the following conclusions:

1. Lower respiratory tract diseases (pneumonias) of different etiology are often reported in captive non-human primates of various species. In different years, there has been some disease dynamics in relation to sex and age, most likely associated with weather conditions and the number density of monkeys in enclosures and cages.

2. In baby monkeys, pneumonia progresses rapidly and is fatal.

3. Pneumonia is often identified as a concurrent disease in adult monkeys with gastro-intestinal disorders.

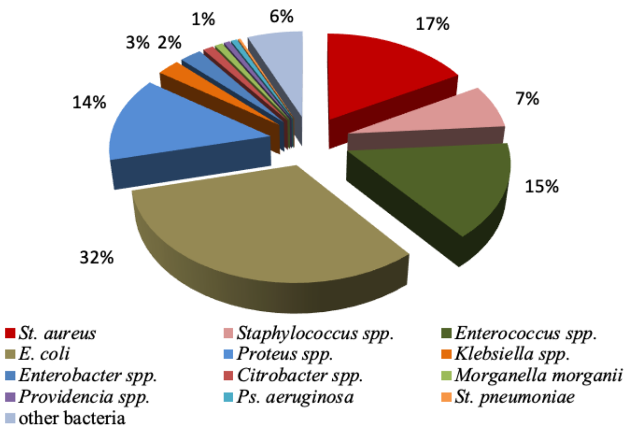

Fig. 3. Structure of bacterial cultures isolated from pneumonia-affected monkeys

Table 4
Frequency of isolation of monocultures and microbial associations from the lungs of pneumonia-affected monkeys

Microorganism	Total	Monoinfection, abs. number (%)	Associations, abs. number (%)
Staphylococcus spp.	63	10 (15.9)	53 (84.1)
St. aureus	151	4 (2.7)	147 (97.3)
E. coli	288	57 (19.8)	231 (80.2)
Proteus spp.	122	5 (4.1)	117 (95.9)
Ps. aeruginosa	6	2 (33.3)	4 (66.7)
Bacillus spp.	6	2 (33.3)	4 (66.7)
Enterococcus spp.	137	40 (29.2)	97 (70.8)

Table 5
Variants of most common combinations of microorganisms in the lungs of pneumonia-affected monkeys

Two- and three-component associations	Abs. number, (%)
E. coli + *Proteus* spp.	48 (24.7)
St. aureus + *E. coli*	38 (19.6)
St. aureus + *Enterococcus* spp.	21 (10.8)
E. coli + *Enterococcus* spp.	19 (9.8)
St. aureus + *Proteus* spp.	16 (8.3)
E. coli + *Enterobacter* spp.	10 (5.2)
Other combinations	42 (21.6)
Total	194
Staphylococcus spp. + *Enterococcus* spp. + *E. coli*	35 (35.4)
Staphylococcus spp. + *E. coli* + *Proteus* spp.	21 (21.2)
Other combinations	43 (43.4)
Total	99

4. Lobar, polysegmental processes (94.4%) prevail in the structure of lung lesions in pneumonia-affected monkeys.

5. The following classical signs of pneumonia were detected *post mortem* in dead primates: the presence of exudate, colour change (inflammatory hyperemia) and hardening of affected area. All lesions were advanced and serve as an illustrative example of classical disease progression in cases diagnosed with bilateral polysegmental bronchopneumonia and lobar pneumonia.

6. As a result of bacteriological tests of lung tissues from the monkeys that had died of pneumonias, different bacterial pathogens and their combinations were detected. The percentage of *St. aureus* within the microbial landscape structure was 16.8%, that of *Enterococcus* spp. – 15.2%, of *St. pneumoniae* – 0.3%. Among enterobacteria, *E. coli* (32.1%) were detected most frequently. The most frequently reported microbial associations were the combinations of *E. coli* + *Proteus* spp. (24.7%), *Staphylococcus* spp. + *Enterococcus* spp. + *E. coli* (35.4%). Almost all the enterobacteria detected were identified as associative ones.

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