

ANALYSIS OF PNEUMONIA-ASSOCIATED MORTALITY OF MONKEYS IN CAPTIVITY AND THE ROLE OF METHICILLIN-SUSCEPTIBLE *STAPHYLOCOCCUS AUREUS* (MRSA) IN THE RECOVERED MICROFLORA SPECTRUM

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SUMMARY

Pneumonia-associated mortality of different monkey species in captivity in 2017 has been analyzed. The animal death frequency and seasonality was demonstrated. Pneumonia-associated mortality in baby monkeys and old monkeys exceeds the mortality of juveniles and mature animals. The maximum pneumonia-mortality rate in monkeys was observed in February, April and May. The spectrum of microorganisms recovered from lungs of dead animals was identified. The prevailing bacterial flora detected in case of pneumonia was *Staphylococcus aureus* (43.1%) and *Escherichia coli* (34.9%), which were detected both in monoculture and communities. One of the peculiarities of pneumonia in monkeys is high occurrence of polymicrobial communities. Most frequently *S. aureus* is observed in combinations with *E. coli* (52.5%). All isolated *S. aureus* cultures were methicillin-susceptible and did not have gene *mecA* in their genome. During performance of bacteriological tests of autopsy material the lung tissue samples can get contaminated with the foreign flora that's why it's quite difficult to speak about the role of these and those microorganisms as major disease agents.

Key words: monkeys, pneumonia, *Staphylococcus aureus*, enterobacteria, microbial communities.

INTRODUCTION

Pneumonia is one of the most common diseases in the world. It is a pluricausal disease of bacterial, viral as well as viral and bacterial etiology. Pneumonia can be caused by a wide range of agents both pathogenic and opportunistic [1, 6]. The research performed during the previous decades shows that the most common disease agents in humans are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus* (*S. aureus*) [3, 5, 6, 8–10, 12]. According to different publications in the present time *S. aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), is still among the most common pathogens causing dangerous invasive diseases in humans and animals [11]. However, lately, the role of gram-negative bacteria such as *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp., *Pseudomonas aeruginosa* in pneumonia etiology has increased [5, 9]. Besides, pneumonia can be caused by such agents as legionella, micoplasma, chlamydia. At the same time some authors specify that etiology of up to 50% of pneumonia is still unknown [6].

In veterinary medicine pneumonia issue is till under-investigated but is quite important as pneumonia is frequently reported in mammals (felines, canines, equids) [3, 12]. The relevance of the investigation of pneumonia agents in monkeys in captivity stems from the high morbidity resulting in death of animals.

Monkeys of different species comprising together with the human being one primate order, are widely used for experimental studies of the most important problems of medicine and biology [7]. During the last decades due to drastic reduction in monkey population in natural habitats and capture ban the main source of laboratory primates are monkey nurseries. In Russia it is Adler nursery of FSBSI "NII PM" in Sochi. Monkeys are susceptible to all bacterial, viral and parasitic diseases of humans and some animals and birds [2, 4, 7], herewith pneumonia is one of the most frequent causes of animal death.

It is registered as an independent process and often develops as a concurrent secondary disease [4, 7]. In foreign publications there is no information on the role of

Table
Age groups of monkeys of different species died of the disease

Monkey species	Age, year old (absolute number/pneumonia)					
	Up to 1	1–3	3–10	10–15	Older than 15	Total
Rhesus macaque	34/21	8/2	31/5	26/7	19/10	118/44
Javanese macaque	24/19	15/1	26/4	9/5	7/3	81/32
Pig-tailed macaque	1/1	–	1/–	1/1	3/3	6/5
Barbary macaque	–	–	–	1/1	–	1/1
Marmoset verdant	4/2	2/1	1/–	3/2	1/–	11/5
Savanna baboon	10/10	2/–	6/2	1/1	–	19/14
Hamadryas baboon	26/18	6/3	17/8	2/1	6/2	57/32
Total	99/71	33/7	82/19	43/18	36/18	293/133

S. aureus, including MRSA and other bacterial diseases, in development of spontaneous pneumonia in monkeys.

The paper is aimed at analysis of monkey death of pneumonia and frequency of *S. aureus* in lung tissue microflora.

MATERIALS AND METHODS

In 2017 293 dead monkeys were tested, and 133 of them were diagnosed with pneumonia. 7 monkey species died of pneumonia: rhesus macaque ($n = 44$), Javanese macaque ($n = 32$), pig-tailed macaque ($n = 5$), Barbary macaque ($n = 1$), marmoset verdant ($n = 5$), savanna baboon ($n = 14$), Hamadryas baboon ($n = 32$). Most dead animals diagnosed with pneumonia were baby monkeys under 12 months of age – 72.7%, juveniles – 21.2%, young animals (3–10 years old) – 23.2%, 10–15 year old monkeys – 41.9% and old monkeys (15–35 y.o.) – 50%.

The material for testing – pieces of lung tissue, 2 × 2 cm, collected from the outbreak, was put in a sterile container and delivered to the laboratory. Bacteriological tests were performed using standard nutrient media (Endo's, Ploskirev's), 5% blood agar (for detection of hemolytic properties), egg-yolk Salt Agar (for detection of lecithinase activity). The inoculated cultures were incubated in a thermostat for 24 hours at 37 °C. The type of pure bacterial cultures was identified by biochemical characteristics using "MMT S" and "MMT E24" test-systems (OOO NPO «Immunotex», Russia), based on determining microbial substrate-active

enzymes. Staphylococcus cultures were examined for phenotypic characteristics: morphology of colonies, their pigmentation, Gram staining, capacity for plasma coagulation and lecithinase activity. Serological identification of *S. aureus* (MRSA) was performed using Dryspot Staphytest Plus (Oxoid, GB) latex agglutination test kit.

The obtained *S. aureus* cultures were tested with RT-PCR. Total staphylococcus DNA extraction was performed from bacterial suspensions ($1,5 \times 10^8$ CFU/ml equivalent to McFarland standard 0.5), prepared from day-old *S. aureus* agar cultures and suspended in 100 µl NaCl using a set of reagents "DNA-sorb-B" ("InterLabServis", Russia) according to the manufacturer's recommendations. Amplification was performed using commercial test-system "AmpliSens" MRSA-screen-titre-F" («InterLabServis», Russia) and Rotor-Gene Q PCR cyler (Germany) according to the Instruction. The commercial test system identifies *S. aureus* and detects genetic determinants of antimicrobial resistance responsible for resistance to β-lactam antibiotics of penicillin group (gen *mecA*).

The frequency of different bacteria occurrence was estimated by constancy value calculated using the following formula

$$C = \frac{p \times 100}{P},$$

where C – constancy;
 p – number of samplings containing the same genus (species);
 P – general number of samplings.

RESULTS AND DISCUSSION

133 monkeys died of pneumonia which is 45.4% of the total number of animals that died in 2017, herewith pneumonia was reported in males in 46.6% of cases and in females – in 53.4% of cases. The frequency of death from the disease in monkeys of different species and age groups is given in the table below.

Apparently, the highest mortality rate was observed in baby monkeys under 12 months of age (71.7%), and in juveniles (under 3 years old) – the lowest rate of 21.2%. The highest rate of pneumonia occurrence was observed in sa-

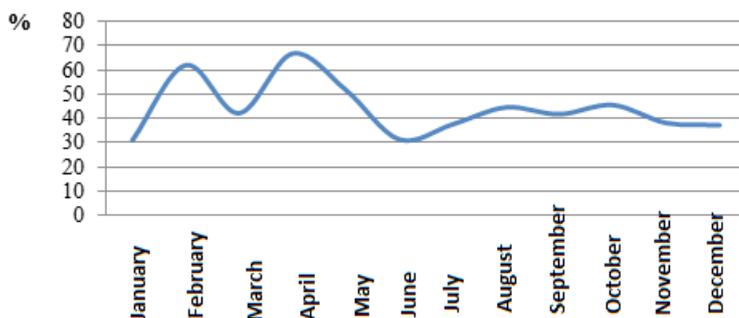


Fig. 1. Seasonality of monkey mortality from pneumonia

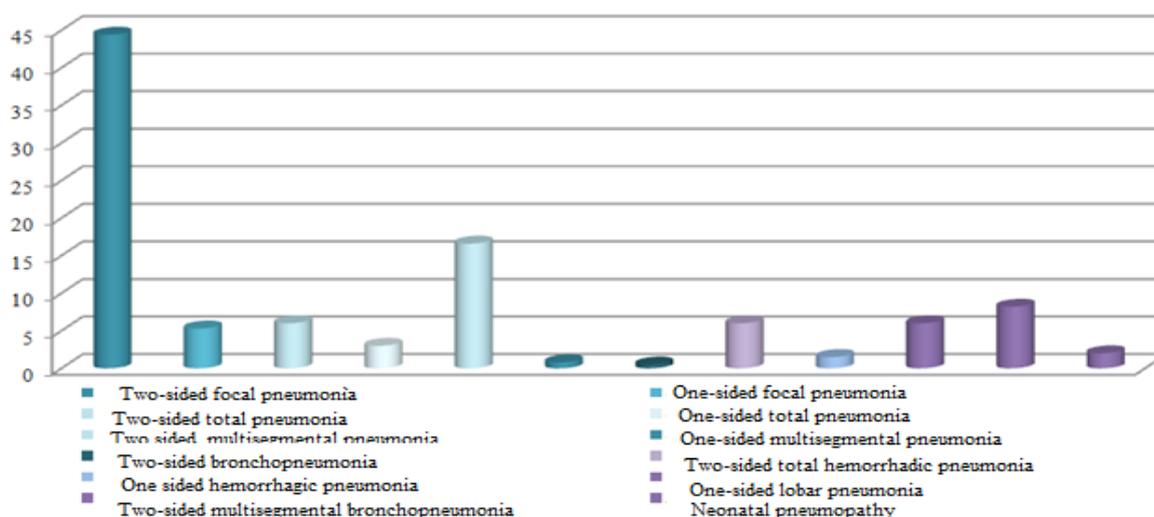


Fig. 2. Types of pneumonia in monkeys by the disease localization and its nature

vanna baboons (73.7%) and Hamadryas baboons (56.2%). Five out of six pig-tailed macaques were diagnosed with pneumonia. In other species pneumonia-associated death frequency was less than 50%.

Analysis of pneumonia-associated mortality within a year demonstrated seasonality (Fig. 1).

The highest frequency of pneumonia occurrence is observed in winter and spring (49.7%). In summer the mortality rate decreased by 1.4 times (35.1%). The peak of monkey mortality was in February (61.9%), April (66.7%), and May (51.7%).

Most often pneumonia in monkeys was characterized by inflammation in both lungs (83.1%), in 16.9% of cases inflammation was observed in one (right-sided pneumonia – 59%, left-sided pneumonia – 41%). According to information in Figure 2, more frequently monkeys demonstrated two-sided focal pneumonia (44.4%), two-sided multisegmental pneumonia was observed in 16.6% of cases. Neonatal pneumopathy was diagnosed in 2% of newborns.

It is well known that pneumonias are infections that's why it is important to study the range of agents. During tests of 133 monkeys that died of pneumonia 218 bacterial cultures were detected, 49.1% – gram-positive flora (94 cultures – *S. aureus* (43.1%) and 13 cultures – *Enterococcus* spp. (6%). The proportion of gram-negative bacteria was 50.9%, herewith in 48.6% of cases enterobacteria were detected, in 2.3% – *Pseudomonas aeruginosa* (Fig. 3). Most frequently occurred enterobacterium was *E. coli* – 76 cultures (34.9%), 11% – representatives of *Proteus* genus (24 cultures). There were detected single cases of *Klebsiella* spp. (1.4%), *Citrobacter diversus* (0.9%), *Yersinia pseudotuberculosis* (0.4%). In 5.3% of cases no bacteria growth in nutrient media was observed.

The microbiota is represented both by monoculture and by microbial communities. 46 species of microorganisms were isolated from the monoculture: *S. aureus* (54.4%) – 25 isolates, *E. coli* (30.4%) – 14 isolates, *Enterococcus* spp. (6.5%) – 3 isolates, *Proteus vulgaris* – 1 isolate, *Proteus penneri* (2.2%) – 1 isolate, and *Klebsiella pneumoniae* – 1 isolate. In 63.5% of cases mixed microflora was isolated. Microbial communities included combination of *S. aureus* with enterobacteria as well as *Enterobacteriaceae* combinations. *S. aureus* combinations are represented by two-,

three-, four-component communities. The most frequent combination is combination *S. aureus* + *E. coli* (52.5%). The frequency of microbe detection in two- and three-component combinations is not high: *S. aureus* + *Proteus* spp. (10%), *S. aureus* + *Enterococcus* spp. (7.5%), *S. aureus* + *Pseudomonas aeruginosa* (2.5%); *S. aureus* + *E. coli* + *Proteus* spp. (6.3%), *S. aureus* + *E. coli* + *Enterococcus* spp. (2.5%), *S. aureus* + *E. coli* + *Pseudomonas aeruginosa* (1.1%).

The following single cases of *S. aureus* combinations were observed: *S. aureus* + *E. coli* + *Citrobacter diversus* + *Proteus* spp.; *S. aureus* + *E. coli* + *Citrobacter diversus* + *Enterococcus* spp.; *S. aureus* + *E. coli* + *Klebsiella ozaenae* + *Proteus* spp. (1.3%).

S. aureus isolates were used for molecular and genetic tests in order to detect *mecA* gen, which is a genetic determinant of bacteria resistance responsible for resistance to β -lactam antibiotics of the penicillin group in staphylococci. It was determined that the specified gene was not observed in the genome of all *S. aureus* cultures isolated from the lungs i.e. they were methicillin-susceptible. Results of PCR diagnostics completely matched the latex fixation test results.

CONCLUSION

Bacteriological test is one of the basic tests used for determination of pneumonia etiology. The analysis of performed tests demonstrated that many microorganisms,

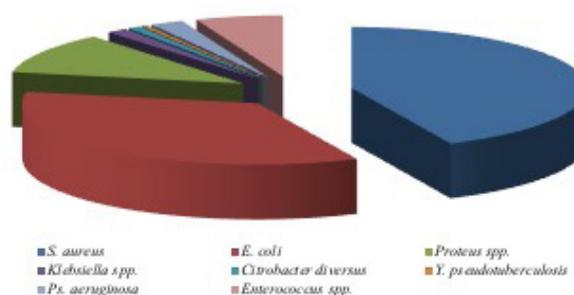


Fig 3. Proportion of microorganisms detected in lungs of monkeys infected with pneumonia

mono or in combination, can cause development of the infection in lungs of monkeys. Cases of bacteria isolation from inflammation sites are more common than cases when bacteria are not observed. The results of tests demonstrated that in the majority of cases pneumonia in monkeys is of polymicrobial etiology. The frequency of *S. aureus* and *E. coli* detection is considerably higher than of other bacteria. Prevalence of methicillin-susceptible *S. aureus* (43.1%) in monkeys infected with pneumonia reveals the leading role of this microorganism in the etiological structure of captive monkeys. *E. coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, isolated from unusual biotypes, can be considered possible disease agents. Supposedly, it is related to the low immunity and colonization resistance disorders, which influences the character of the infection process. It is known that *Enterobacteriaceae* and *Staphylococcus* bacteria, isolated as communities, more often produce different virulence factors than bacteria isolated as monocultures demonstrating their pathogenic potential during symbiosis.

Having analyzed the frequency of microbial occurrence in monkeys infected with pneumonia *S. aureus* and *E. coli* were referred to resident flora, and *Proteus* spp. – to facultative microflora and other bacteria – to transient microflora. Isolation of such bacteria as *Enterococcus* spp., *Citrobacter* spp., *Enterobacter* spp., *Yersinia pseudotuberculosis* from lungs is rather indicative of the material contamination with foreign flora than of etiological significance of these microorganisms.

Pneumonias, etiology of which is not determined, also draw attention. Unfortunately, there are still no exact data on detection of *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and viral pathogens in monkeys' lungs, however, according to publications these microbes can be among etiological factors of pneumonia. Previous diagnostics (2008–2010) of chlamydia demonstrated presence of *C. trachomatis* in monkey lung tissue (up to 30%), sometimes in combination with *C. pneumoniae*. Also, according to unpublished data of the Laboratory for Infectious Pathology FSBSI "NII MP", *Mycoplasma* spp. detection was 5%.

At the same time there is a possibility of lung tissue contamination during bacteriological tests of the autopsy material that's why it is hard to be affirmative as far as the role of these or those microbes as main disease agents is concerned.

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