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Bacteriological profile in falcon-like birds under aviary captive conditions

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Abstract. Bacteriological studies of material from the pharynx and cloaca from clinically healthy saker falcons and eastern imperial eagles under aviary captive conditions were carried out. Indicated are eleven cultures of pathogenic and conditionally-pathogenic microorganisms represented in the association. Microscopic examination of blood in erythrocytes revealed hemosporidia. Conducted macro – and microscopic, as well as helminthological study of feces. The invasion rate was 32.6%. Revealed 2 types of helminths. The obtained results may indicate persistent carriage of the causative factor of infections and the formation of foci of helminthiasis and haemosporidia.

1. Introduction

To date, many species of fauna have drastically reduced their numbers or are on the verge of extinction. Measures for their protection and restoration have become an urgent need to ensure biological self-regulation. In this regard, diurnal birds of prey are of particular importance, which, with a small number and biomass, play a key role in the ecosystem [1].

Literature data indicate that in areas with a high density of falcon-like birds, the likelihood of occurrence of natural focal infections, such as haemorrhagic fever with renal syndrome and some other serious diseases, decreases, and damage to agriculture and forestry decreases [4, 3, 6].

On the territory of our Moscow region, 28 species of falcon-like birds inhabit (including 12 registered in the Red Book). The list of birds is extensive, but the number of birds is small. Birds of prey are highly susceptible to anthropogenic factors and require special protection measures. Environmental legislation has created a sufficient legal framework to ensure the protection of birds, including birds of prey. These acts define the requirements to ensure the prevention of diseases and the death of wildlife [5].

Despite the program of measures aimed at preserving and restoring the number of diurnal birds of prey in the territory of many countries and the Russian Federation in particular, the priority and only full-fledged measure in the conservation of rare species was their breeding in artificial conditions with subsequent reintroduction into nature [3, 6].

In recent years, a number of specialized farms have appeared on the territory of the Russian Federation, successfully implementing environmental programs that aim to preserve the gene pool and the population of birds of prey. First of all, this is – a scientific and methodological work aimed at



increasing the nesting suitability of the territory for feathered predators, monitoring of the population and living conditions, measures to ensure successful breeding and preventing the death of birds associated with anthropogenic factors [1, 3].

The creation of such farms means the transition from passive measures to protect the wildlife to active. An integral part of ensuring the quality work of farms is veterinary care, the primary task of which is the monitoring of infectious diseases with subsequent treatment and prevention [3].

Implementation of monitoring control prevents the entry of avian pathogens into synanthropic biocenosis and prevents epidemic outbreaks of those diseases that are considered common with humans [4, 3, 6].

Currently, issues of veterinary medicine of wild and exotic birds are being actively developed. In Europe, America, countries in Asia and the Middle East, a large number of specialists work in this area, there are specialized clinics and rehabilitation centres, this area of research in the Russian Federation has been developed [1, 2, 3].

Therefore, the study of the nosological profile of infectious pathology in falcon-like birds is relevant.

2. The aim

Aim of our work was to study the species representation of pathogenic microbiota and parasites of the digestive system of clinically healthy falcon-like birds in aviary captive conditions to establish the helminthobacteriological profile and its epizootic value.

As tasks determined, is the indication, identification and study of the biological properties of pathogenic bacteria - potential pathogens of infectious diseases of animals and humans, microscopic examination of blood for the presence of parasites, parasite-helminthoscopic examination of feces.

3. Materials and methods

The object of research was falcon family birds, kept in aviaries of city zoos, sports-hunting and amateur centers and with private, individual keeping. The material came from various regions of the Russian Federation: Rostov, Moscow, Tula, Vladimir regions. For the period from 2018 to 2020 a comprehensive study was conducted on 138 clinically healthy falcon-like birds: thirty-six saker falcons, thirty eastern imperial eagles, from eight kestrel s, from ten barbary falcons, from twenty-four rough-legged buzzard, from thirty peregrine falcons. 67 samples were obtained from birds from four specialized sports-hunting farms in the Moscow and Tula regions, 16 - from two private farms in the Rostov region, 55 samples from two aviaries of city zoos in the Moscow and Vladimir regions.

Blood was taken for microscopic examination from the axillary vein. The smears were stained using the Romanovsky-Giemsa method based on eosin and methylene blue and the Diff-Quik method.

For helminthological studies, 138 samples of fresh feces from these birds were obtained.

In the course of work, clinical, microscopic, helminthoscopic, microbiological, statistical research methods were used.

Microbiological studies were performed according to generally accepted methods [7]. Sowing was carried out directly on a dense medium in a cup, turning the swab on all sides, rubbing the material. When sowing into a liquid nutrient medium, the swab was immersed and left for 15-20 min. at room temperature. Then the tube was intensively scrolled between the palms, the swab was pressed against the walls of the tube in its lower part and removed. After this, sowing was made from a test tube onto a solid nutrient medium in a cup. To isolate pure cultures, a swab with the material to be studied was introduced into the cup and its contents were rubbed into the surface of the medium in a circular motion, while rotating the tampon and cup, as well as sowing of the loop.

For bacteriological studies of enterobacteria, pathogen identification kits were used "Analytical Profile Index (API) for *Enterobacteriaceae*". Determination of antigen and sero-identification was carried out by staging an agglutination reaction with specific polyvalent and monovalent serum.

Confirmation of microorganisms belonging to *S. aureus* was carried out by sowing on thioglycolic medium, salt broth. The pathogenicity of the isolated isolates of staphylococci was studied by plasma coagulation test and sowing on Zeissler blood agar. For the study of coagulase-negative staphylococci,

the STAPHYtest 16 was used. The lecithinase test of pathogenic *S. aureus* was determined on nutrient medium № 10-GRM (A).

A bacteriological study of *S. pneumoinae* was carried out by sowing on glucose-blood agar, MPB with 1% glucose and 15-20% serum. To differentiate pneumonia streptococcus from other α -hemolytic streptococci, the following properties were studied: bile lysis, fermentation of sorbitol, mannitol and raffinose.

To isolate *P. aeruginosa*, we determined the activity of cytochrome oxidase, aerobic oxidation of glucose and the absence of its decomposition under anaerobic conditions (under a layer of liquid petroleum jelly) in Hugh-Leifson medium, growth in broth at 43 ° C, synthesis of acetone.

A bacteriological study of *C. perfringens* was carried out by sowing on Kitt-Tarozzi medium, Wilson-Blair medium. Part of the tubes was heated at 80 ° C for 30 min to destroy non-spore-forming bacteria. Of all the tubes with turbidity and gas formation, smears were made and Gram stained. Identification was carried out using a test to determine toxigenicity, lecithinase activity. The hemolytic properties of microorganisms were determined by sowing on Zeissler blood agar. To study the virulent properties of the selected cultures, infection was carried out in the abdominal muscles of guinea pigs in a dose of 0.5-0.1 ml. Observation of animals was conducted up to 8 days.

The pathogenicity of the selected cultures from the pharynx and cloaca was studied by bioassay on 2-4 white mice by intraperitoneal and subcutaneous administration of the studied agar cultures at a dose of 500 thousand micro cells followed by isolation of the original cultures from the dead laboratory animals.

The sensitivity of the isolated microorganisms was determined by the disk diffusion method using an E-test strip containing a gradient of antibiotic concentrations from maximum to minimum. At the intersection of the ellipsoidal growth suppression zone with the E-test strip, the minimum inhibitory concentration (MIC) value was obtained. The agar plates were pre-dried to form a clear edge of the growth suppression zone. The studies were carried out making sure that the medium ensured the proper growth of the control strain of the microorganism of the same species. For the preparation of Streptococcus pneumonia suspension, cultures grown on chocolate agar were used. Disks with antibiotics were used by factory-made Agat-Med LLC. The results of determining the sensitivity of microorganisms to antibiotics were considered according to the guidelines [6].

Helminthoscopic examination was carried out by flotation method according to G. A. Kotelnikov and V. M. Khrenov using a saturated solution of ammonium nitrate [5].

4. Research results

Data on the species affiliation of the studied birds were obtained by visual morphological characters, as well as according to registration data. The research results are presented in table 1.

Table 1. The species and age of the studied bird of prey contained in aviaries (n = 138).

Species		Age of birds	
		1-2 years	over 2 years
Peregrine (<i>F. peregrinus</i>)	30	12	18
Rough-legged buzzard (<i>B. lagopus</i>)	24	7	17
Saker (<i>F. cherrug</i>)	36	6	30
Kestrel (<i>F. tinnunculus</i>)	8	3	5
Barbary falcon (<i>F. biarmicus</i>)	10	2	8
Eastern imperial eagles (<i>A. heliaca</i>)	30	6	24
Total	138	36	102

Analysing the data of table 1, we see that the majority is dominated by Saker Falcons older than two years. This can be explained by the fact that falconry hunters prefer saker falcons due to their uniqueness.

They can freeze in the air, rushing to prey, and can catch up with it like a hawk. Saker Falcon is also interesting for breeders in order to preserve the species and maintain the population size.

Microscopic examination of blood smears in one case from four eastern imperial eagles revealed the presence of several ookinetes (5 pcs) in the erythrocytes, which caused the damage of the erythrocyte. We attributed these parasitic protozoa to the Haemoproteus coccidia order based on the following diagnostic criteria: Haemoproteus gametocytes are present only in erythrocytes; schizonts are absent in smears from peripheral blood; the presence of multiple pigment granules in an erythrocyte with a displacement of the cell nucleus.

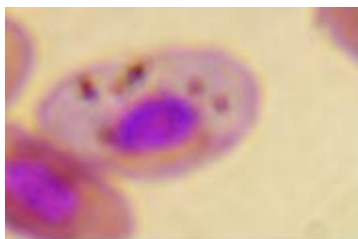


Figure 1. Erythrocyte damage by haemosporidia.

Microscopic examination of feces revealed that 45 out of 138 birds examined were infested. However, no clinical signs of helminthiasis were detected.

In total, 2 species of helminths were revealed in the studied birds under aviary captive condition, including the nematode *Capillaria falconis*, trematode *Strigea falconis*. The infestation rate was 32.6%. The same distribution of helminth fauna by bird species can be explained by common cleaning equipment and same cleaning staff. In this case, infection occurs directly through contaminated helminth eggs feed and the external environment.

During bacteriological examination, samples of material from the cloaca and pharynx were sown on nutrient media to indicate cultures.

As a result of this work, isolates of 11 types of microorganisms were isolated: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, *Yersinia enterocolitica*, *Bacillus subtilis*, *Clostridium perfringens* in associations. Identification of the selected cultures was carried out using biochemical studies.

In bacteriological studies, selected were bacteria belonging to the families of zoo-pathogenic or conditionally-pathogenic (opportunists). All symbionts were not considered.

The isolated cultures of *E. coli* possessed characteristic morphology and cultural properties. Four isolates from the pharynx and eight from the cloaca exhibited α -hemolysis. Serological identification made it possible to divide the isolated isolates into serogroups O78-7; 055 - 5 isolates.

Isolated *S. aureus* cultures were tested for virulent properties. Six *S. aureus* isolates from the pharynx had plasma coagulative properties, folding plasma for two hours. Two of them showed α -hemolysis, four isolates - β -hemolysis. All six cultures fermented mannitol under anaerobic conditions, which confirms the pathogenicity of the cultures.

Five cultures of *S. pneumoniae* with α -, β -hemolytic activity were isolated from the pharynx; 2 cultures showing β -hemolytic properties were indicated from the cloaca.

Seven *P. aeruginosa* cultures were isolated from cloaca and pharynx. The isolated cultures formed pigment pyocyanin on dense and liquid nutrient media.

Six cultures of *C. perfringens* isolated from cloaca had β -hemolytic activity. A study of the toxigenic properties of *C. perfringens* cultures was carried out by infecting guinea pigs. Isolates caused their death in six hours, followed by isolation of the original culture from internal organs.

The next stage of our research was the study of the biological properties of the isolated cultures of the microorganisms *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, *Yersinia enterocolitica*, *Bacillus subtilis*, *Clostridium perfringens* from clinically healthy

representatives of falcons.

In the study of the pathogenicity of *E. coli* cultures on white mice weighing 16-18 grams with intraperitoneal infection in a volume of 0.5 cm³ at a dose of 500 thousand micro cells in 1 cm³. The virulence of four isolates from the pharynx and eight isolates from the cloaca was established.

A study of the pathogenicity of *P. aeruginosa* cultures was carried out on white mice weighing 16-18 grams with subcutaneous infection in a volume of 0.5 cm³ at a dose of 500 thousand micro cells in 1 cm³. The pathogenicity of seven cultures of cloaca and pharynx was established.

The studies revealed the pathogenicity of twelve isolates of *E. coli* from cloaca and pharynx, six isolates of *S. aureus* from pharynx, seven cultures of *S. pneumoniae* from cloaca and pharynx, six cultures of *C. perfringens*, seven cultures of *P. aeruginosa* from cloaca and pharynx.

All isolated cultures (n = 38) were tested for sensitivity to 11 antibacterial drugs from the groups of fluoroquinolones, aminoglycosides, tetracyclines, β -lactams, sulfanilamides, cephalosporins, complex antibiotics (paratil, ribavex), which are available in the clinics of the studied zoos and hunting centers. Criteria for interpreting the results of determining the sensitivity of the diameters of growth suppression zones (mm) were carried out according to EUCAST (version 8.0. valid from 01.01.2018). The research results are presented in table 2.

Table 2. Sensitivity of selected cultures to antibiotics.

Isolates	Antibiotics	Drug	Number of resistant strains/%
<i>E. coli</i> (n=12)	Fluoroquinolones	Enroxil	4/33.4
	Penicillins	Amoxicillin with clavulanic acid	9/75
		Ceftriaxone	7/58.4
	Aminoglycosides	Gentamicin	4/33.4
	Tetracyclines	Doxycycline	5/41.7
	Complex antibiotics	Paratil	2/16.7
		Ribavex	3/25.0
<i>S. pneumonia</i> (n=7)	Cephalosporins	Ceftriaxone	2/8.0
	Fluoroquinolones	Enroxil	6/85.7
	Sulfonamides	Ditrim	2/8.0
	Complex antibiotics	Paratil	4/42.8
<i>P. aeruginosa</i> (n=7)	Fluoroquinolones	Enroxil	2/28.5
	Cephalosporins	Ceftriaxone	3/42.8
	Aminoglycosides	Gentamicin	2/28.5
	Complex antibiotics	Paratil	1/14.2
<i>S. aureus</i> (n=6)	Fluoroquinolones	Enroxil	6/100.0
	Penicillins	Amoxicillin with clavulanic acid	3/50.0
		Ceftriaxone	2/33.4
	Aminoglycosides	Gentamicin	5/83.4
	Tetracyclines	Doxycycline	2/33.4
	Complex antibiotics	Lincospectin	3/50.0
		Paratil	5/83.4
<i>C. perfringens</i> (n=6)	Tetracyclines	Doxycycline	3/20.0
	Penicillins	Amoxicillin with clavulanic acid	6/33.34
		Lincomycin	1/6.7

Analyzing the results shown in table 2, it is obvious that ceftriaxone, gentamicin, paratil (combined antibiotic), ribavex (combined antibiotic) had a high antibacterial effect on *E. coli* cultures. Antibiotics ceftriaxone and ditrim from the group of sulfonamides, paratil, had a bactericidal effect on *S. pneumonia* isolates. On the *P. aeruginosa* isolates, the greatest bactericidal effect was exerted by the antibiotic enrofloxacin from the group of fluoroquinolones and paratil (a complex antibiotic) and ceftriaxone. Isolates of *S. aureus* showed resistance to enroxil, paratil, amoxicillin. *C. perfringens* isolates were sensitive to lincomycin and doxycycline.

As can be seen from the data presented in table 2, there is a multiple antibiotic resistance in selected microorganisms, which, with a decrease in resistance, poses a danger to birds of prey.

5. Conclusion

Timely and systematic monitoring of infectious nosology and microbe-helminth-carrier in birds of prey is necessary in order to predict the epizootic situation in hunting farms, aviaries, nurseries, zoos and other places of keeping Falconiformes. The results of bacteriological studies can serve as reliable markers of the initial assessment of the health status of birds under aviary captive conditions and the potential spread of pathogens.

When studying the microbiota of the pharynx and intestines, a wide representation of associations of pathogenic and opportunistic microflora was established. The absence of clinical signs of infectious diseases indicates persistent carriage with the possible development of the disease with a decrease in resistance. The inapparent manifestation of mixed infections in falconiformes was revealed under aviary captive conditions on the background of Helminthiasis *Capillaria falconis* and *Strigea falconis* (the invasion rate was 32.6%) of such bacterioses as Escherichiosis, perfringenosis, pseudomonosis, streptococcosis pneumonia, staphylococcus aureus. Moreover, variations in associations are - as a rule, with double pathogenic representation. In 4 individuals, hemosporidiosis parasitism was noted.

In this regard, it should be noted that for birds of prey in aviary there is a risk of the development of clinically pronounced helminthiasis and hemoproteosis in connection with the formation of a stable focus of invasion, complicated by ubiquitous bacterial pathogens. Most isolates of pathogenic microorganisms have multiple antibiotic resistance (lack of sensitivity or low sensitivity to drugs of three or more groups).

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