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THE PARTICIPATION OF WILD MIGRATORY BIRDS IN THE SPREAD OF RIEMERELLOSIS AND THE MICROBIAL BIOCENOSIS OF THE WILD DUCKS

Yakimova E.A.[1], **Kapustin A.V.**[1], **Smirnov D.D.**[1], **Savinov V.A.**[1],
Laishevtcev A.I.[1,2]*, **Plygun S.A.**[2,3]
[1]Federal Scientific Centre VIEV, Moscow, Russia
[2]Laboratory of Biological Control and Antimicrobial Resistance, Orel State University named after I.S. Turgenev, Orel, Russia
[3]European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland

*E-mail: a-laishevtsev@bk.ru

ABSTRACT

The results of microbiological screening of various organs and tissues obtained from wild migratory birds (ducks) in order to detect the presence of *Riemerella anatipestifer*, as well as study the total microbial biocenosis, are presented in the work. During the research, 90 species of cultivated bacteria were identified as part of the biocenosis of the studied birds. This diversity was established due to the use of routine bacteriological methods and time-of-flight mass spectrometry - MALDI-ToF for the species identification of bacteria. The results obtained in the course of this work indicate that the migratory bird is the carrier and possible reservoir of the causative agent of riemerellosis - *Riemerella anatipestifer*.

KEY WORDS

Infectious pathologies, migratory birds, microbiological risks, riemerellosis, pasteurellosis, *Riemerella anatipestifer.*

Riemerellosis for Russian agricultural producers is a relatively new infectious pathology not previously officially registered in the country, which is why domestic data on the disease are absent despite the fact that infection in the number of foreign countries is actively controlled [7, 8, 11]. Of course, we can assume that the examined pathogen has been actively circulating in the Russian Federation for quite a long time, but due to the complexity of diagnosing this disease and the lack of state control over it it's impossible to assess the full extent of the damage caused by the infection. We can say that the pathogen circulates on the territory of the Russian Federation based on scientific data from the CIS countries, in particular the Republic of Belarus, where this problem has already been encountered and is actively struggling with it [3]. In our previous works, cases of the development of riemerellosis among industrial-type poultry in various regions of the Russian Federation are presented. In this work, we reflect the results of migratory birds studying, in terms of their possible participation in the spread of the pathogen [1, 2, 4].

Since the present work was originally focused on the study of specific pathogen dangerous for waterfowl, namely *Riemerella anatipestifer*, the concomitant bacterial flora was simultaneously determined and studied by us. Setting this task, we expected to obtain results not only describing the association of bacteria in the body of the bird, but also to obtain information about the possible joint course of riemerellosis with other pathogens [9, 14, 15, 13, 6, 10].

MATERIALS AND METHODS OF RESEARCH

Scientific work was performed in the period of 2016-2019 on the basis of the Federal State Budget Scientific Institution “Federal Scientific Centre VIEV” (FSC VIEV) at the Mycology and Antibiotics Laboratory named after A. Kh. Sarkisov, as well as at Laboratory of Microbiology with the Museum of Typical Cultures.

Samples of material from wild migratory birds were obtained from Vladimir, Vologda, Kaluga, Pskov, Tula, Rostov, Ryazan, Tambov, Kursk, Lipetsk, Belgorod regions, as well as the Republic of Tatarstan, Bashkortostan, Krasnodar and Stavropol Territories. The shot birds got in the laboratory with a whole body and / or separately selected organs.

Bacteriological research followed by study of the morphological, tinctorial, cultural, biological and serological properties of *Riemerella* was carried out in the Microbiology Laboratory with the Museum of Typical Cultures of FSC VIEV with an experimental base on Lisiy Island, Vyshnevolotsky District, Tver Region.

Selected field isolates of microorganisms, bacteria strains of *Flavobacteriaceae* family from the collection and museum of FSC VIEV, as well as reference cultures for monitoring nutrient media and diagnostics, were used to carry out scientific work. In addition, differential diagnostic and elective culture media, chemical reagents, laboratory glassware and equipment, animals, as well as epizootological, bacteriological, serological, statistical, pathological, clinical research methods, biotechnology methods and molecular biology methods were used.

Complex bacteriological diagnostics was carried out using the following solid nutrient media: Bile Esculin Agar (Modified), MacConkey Agar, Phenol Red Agar, Columbia Agar, Brain Heart Infusion, Trypticase Soy Agar, Meat Peptone Agar, Cystine Tryptone Agar, Tryptone Soy Agar; as well as using the following broth media: Nitrate Broth, Trypticase Soy Broth, Andrade Peptone Water, Tryptone Soy Broth, Bromocresol Purple Broth, Eugonic Broth, Meat Peptone Broth, Hottinger Broth from LLC Himedia (India) and Oxoid (UK). Carbohydrates in disks were used to study the proteolytic and saccharolytic properties of bacteria with the aim of their generic and specific identification: adonite, arabinose, galactose, D-glucose, dulcite, inositol, inulin, xylose, maltose, mannitol, mannose, raffinose, rhamnose, salicit, sorbitol, sucrose, trehalose, fructose, cellobiose from LLC Himedia (India). Additionally, species identification of microorganisms was carried out using MALDI-ToF analysis.

The study of the epizootic situation of the infectious diseases of waterfowl was carried out based on our own results of recording disease cases and the results of bacteriological studies of pathological material selected and / or received from the poultry enterprises of various regions of the country.

Microbiological study with the aim of establishing a final diagnosis was carried out using routine methods of bacteriology and serology.

Microscopy of bacterial cultures and smears of pathological material was carried out in order to study the morphological and tinctorial properties of microorganisms using preliminary Gram staining and subsequent microscopy.

Studying the saccharolytic and proteolytic properties of microorganism cultures was carried out using carbohydrates and bromocresol purple broth as an indicator of acid formation, as well as commercial kits for the biochemical identification of bacteria [12, 5].

RESULTS AND DISCUSSION

The results of the determination of bacterial microflora in various organs and tissues of the free-living waterfowl are presented in table 1 (%).

Table 1 – Results of the bacteriological studies of organs and tissues of migratory wild birds (ducks)

№	Diagnostic result / number of isolates	Intestines, %	Liver, %	Lungs, %	Oviduct, %	Heart, %
	1	2	3	4	5	6
1.	Acinetobacter baumannii / 10	20.00	60.00	0.00	20.00	0.00
2.	Acinetobacter lwoffii / 3	66.67	0.00	33.33	0.00	0.00
3.	Actinobacillus ureae / 2	0.00	0.00	50.00	50.00	0.00
4.	Aerococcus urinae / 5	20.00	0.00	0.00	80.00	0.00
5.	Aerococcus vaginalis / 4	0.00	25.00	75.00	0.00	0.00
6.	Aerococcus viridans / 6	0.00	16.67	50.00	0.00	33.33
7.	Aeromonas hydrophila / 7	71.43	28.57	0.00	0.00	0.00
8.	Aeromonas salmonicida / 3	33.33	66.67	0.00	0.00	0.00
9.	Arcanobacterium haemolyticum / 6	0.00	0.00	33.33	50.00	16.67
10.	Bacillus beijingensis / 5	20.00	40.00	40.00	0.00	0.00
11.	Bacillus cereus / 8	50.00	37.50	0.00	12.50	0.00
12.	Bacillus subtilis / 6	50.00	33.33	0.00	0.00	16.67

n/n	1	2	3	4	5	6
13.	Bacillus thuringiensis / 6	50.00	16.67	16.67	16.67	0.00
14.	Branhamella catarrhalis / 3	0.00	0.00	33.33	66.67	0.00
15.	Brevibacterium casei / 6	16.67	16.67	33.33	0.00	33.33
16.	Brevundimonas diminuta / 2	50.00	0.00	0.00	0.00	50.00
17.	Burkholderia cepacia / 3	0.00	0.00	66.67	33.33	0.00
18.	Campylobacter coli / 3	66.67	33.33	0.00	0.00	0.00
19.	Campylobacter jejuni / 4	100.00	0.00	0.00	0.00	0.00
20.	Citrobacter diversus / 6	50.00	50.00	0.00	0.00	0.00
21.	Clostridium perfringens / 11	54.55	45.45	0.00	0.00	0.00
22.	Clostridium sporogenes / 8	37.50	62.50	0.00	0.00	0.00
23.	Corynebacterium xerosis / 9	11.11	11.11	33.33	22.22	22.22
24.	Enterobacter cancerogenus / 3	0.00	0.00	33.33	33.33	33.33
25.	Enterobacter cloacae / 13	38.46	38.46	15.38	7.69	0.00
26.	Enterococcus faecalis / 6	16.67	33.33	0.00	50.00	0.00
27.	Enterococcus faecium / 9	55.56	33.33	0.00	11.11	0.00
28.	Enterococcus gallinarum / 4	50.00	25.00	0.00	25.00	0.00
29.	Escherichia coli / 28	60.71	32.14	3.57	3.57	0.00
30.	Escherichia hermannii / 5	60.00	20.00	0.00	20.00	0.00
31.	Klebsiella mobilis / 6	0.00	33.33	16.67	33.33	16.67
32.	Klebsiella oxytoca / 9	55.56	44.44	0.00	0.00	0.00
33.	Kytococcus sedentarius / 4	0.00	25.00	50.00	25.00	0.00
34.	Lactobacillus salivarius / 5	60.00	40.00	0.00	0.00	0.00
35.	Lactococcus lactis / 7	57.14	42.86	0.00	0.00	0.00
36.	Leminorella richardii / 3	100.00	0.00	0.00	0.00	0.00
37.	Lysinibacillus sphaericus / 7	28.57	57.14	0.00	14.29	0.00
38.	Macrococcus carouselicus / 7	0.00	57.14	14.29	14.29	14.29
39.	Micrococcus cohnii / 2	100.00	0.00	0.00	0.00	0.00
40.	Micrococcus flavus / 4	25.00	25.00	50.00	0.00	0.00
41.	Micrococcus luteus / 4	0.00	25.00	75.00	0.00	0.00
42.	Micrococcus lylae / 11	18.18	18.18	27.27	36.36	0.00
43.	Moraxella lacunata / 1	0.00	0.00	0.00	100.00	0.00
44.	Morganella morganii / 7	42.86	57.14	0.00	0.00	0.00
45.	Myroides odoratimimus / 8	0.00	37.50	12.50	12.50	37.50
46.	Nocardiopsis alba / 1	0.00	0.00	100.00	0.00	0.00
47.	Oligella ureolytica / 1	0.00	0.00	0.00	100.00	0.00
48.	Paenibacillus amylolyticus / 2	0.00	0.00	50.00	50.00	0.00
49.	Pantoea agglomerans / 1	0.00	100.00	0.00	0.00	0.00
50.	Pasteurella aerogenes / 3	0.00	0.00	100.00	0.00	0.00
51.	Pasteurella multocida / 7	0.00	0.00	71.43	0.00	28.57
52.	Pediococcus argentinicus / 7	28.57	42.86	0.00	14.29	14.29
53.	Pediococcus pentosaceus / 3	66.67	33.33	0.00	0.00	0.00
54.	Plesiomonas shigelloides / 3	33.33	33.33	0.00	33.33	0.00
55.	Prevotella oris / 4	25.00	75.00	0.00	0.00	0.00
56.	Proteus mirabilis / 3	33.33	0.00	0.00	33.33	33.33
57.	Proteus vulgaris / 7	28.57	28.57	14.29	28.57	0.00
58.	Providencia alcalifaciens / 3	66.67	0.00	33.33	0.00	0.00
59.	Pseudomonas aeruginosa / 7	28.57	42.86	14.29	14.29	0.00
60.	Pseudomonas flavescens / 4	50.00	0.00	25.00	25.00	0.00
61.	Pseudomonas putida / 11	45.45	27.27	9.09	18.18	0.00
62.	Riemerella anatipestifer / 1	0.00	0.00	0.00	0.00	100.00
63.	Rothia endophytica / 3	0.00	33.33	33.33	33.33	0.00
64.	Salimicrobium halophilum / 3	33.33	33.33	33.33	0.00	0.00
65.	Serratia marcescens / 3	33.33	0.00	33.33	0.00	33.33
66.	Shigella boydii / 1	0.00	100.00	0.00	0.00	0.00
67.	Shigella sonnei / 3	33.33	66.67	0.00	0.00	0.00
68.	Staphylococcus agnetis / 7	28.57	14.29	57.14	0.00	0.00
69.	Staphylococcus aureus / 4	0.00	75.00	0.00	25.00	0.00
70.	Staphylococcus chromogenes / 5	20.00	40.00	0.00	20.00	20.00
71.	Staphylococcus cohnii / 9	55.56	11.11	11.11	22.22	0.00
72.	Staphylococcus equorum / 4	25.00	25.00	25.00	25.00	0.00
73.	Staphylococcus gallinarum / 3	33.33	66.67	0.00	0.00	0.00
74.	Staphylococcus haemolyticus / 9	33.33	22.22	22.22	22.22	0.00
75.	Staphylococcus massiliensis / 2	0.00	0.00	50.00	50.00	0.00
76.	Staphylococcus simulans / 2	0.00	0.00	0.00	50.00	50.00
77.	Staphylococcus xylosus / 5	20.00	20.00	40.00	20.00	0.00
78.	Streptococcus alactolyticus / 3	0.00	33.33	33.33	0.00	33.33
79.	Streptococcus entericus / 6	66.67	16.67	16.67	0.00	0.00
80.	Streptococcus iniae / 9	22.22	55.56	11.11	0.00	11.11
81.	Streptococcus minor / 2	0.00	50.00	50.00	0.00	0.00
82.	Streptococcus mitis / 1	0.00	100.00	0.00	0.00	0.00
83.	Streptococcus pasteurianus / 5	0.00	20.00	20.00	20.00	40.00
84.	Streptococcus rubneri / 2	0.00	50.00	0.00	50.00	0.00
85.	Streptomyces albogriseolus / 1	0.00	0.00	100.00	0.00	0.00
86.	Streptomyces sampsonii / 1	0.00	100.00	0.00	0.00	0.00
87.	Streptomyces thermocarboxydus / 2	0.00	0.00	100.00	0.00	0.00
88.	Trueperella pyogenes / 5	0.00	0.00	20.00	60.00	20.00
89.	Vagococcus fluvialis / 1	0.00	0.00	0.00	0.00	100.00
90.	Yersinia enterocolitica / 1	0.00	100.00	0.00	0.00	0.00

As can be seen from the data presented in table 1, the species composition of bacterial agents, isolated from free-living waterfowl in various regions of the Russian Federation, is represented by 90 species of cultivated bacteria isolated from various parenchymal organs and tissues of the bird. So, the generic composition of the microflora of waterfowl organism was represented by bacteria of 26 families (the number of generic representatives is indicated in parentheses): *Actinomycetaceae (2) – Arcanobacterium, Trueperella; Aerococcaceae (1) – Aerococcus; Aeromonadaceae (1) – Aeromonas; Alcaligenaceae (1) – Oligella; Bacillaceae (3) – Bacillus, Lysinibacillus, Salimicrobium; Brevibacteriaceae (1) – Brevibacterium; Burkholderiaceae (1) – Burkholderia; Campylobacteraceae (1) – Campylobacter; Caulobacteraceae (1) – Brevundimonas; Clostridiaceae (1) – Clostridium; Corynebacteriaceae (1) – Corynebacterium; Dermacoccaceae (1) – Kytococcus; Enterobacteriaceae (13) – Citrobacter, Enterobacter, Escherichia, Klebsiella, Leminorella, Morganella, Pantoea, Plesiomonas, Proteus, Providencia, Serratia, Shigella, Yersinia; Enterococcaceae (2) – Enterococcus, Vagococcus; Flavobacteriaceae (3) – Myroides, Riemerella; Lactobacillaceae (1) – Lactobacillus; Lactobacillaceae (1) – Pediococcus; Micrococcaceae (2) – Micrococcus, Rothia; Moraxellaceae (3) – Acinetobacter, Branhamella, Moraxella; Nocardiopsaceae (1) – Nocardiopsis; Paenibacillaceae (1) – Paenibacillus; Pasteurellaceae (2) – Actinobacillus, Pasteurella; Prevotellaceae (1) – Prevotella; Pseudomonadaceae (1) – Pseudomonas; Staphylococcaceae (2) – Macrococcus, Staphylococcus; Streptococcaceae (2) – Lactococcus, Streptococcus; Streptomycetaceae (1) – Streptomyces*.

For clarity, the data on the excretion of bacterial agents from different organs and tissues of the free-living waterfowl were reflected in the form of figure 1.

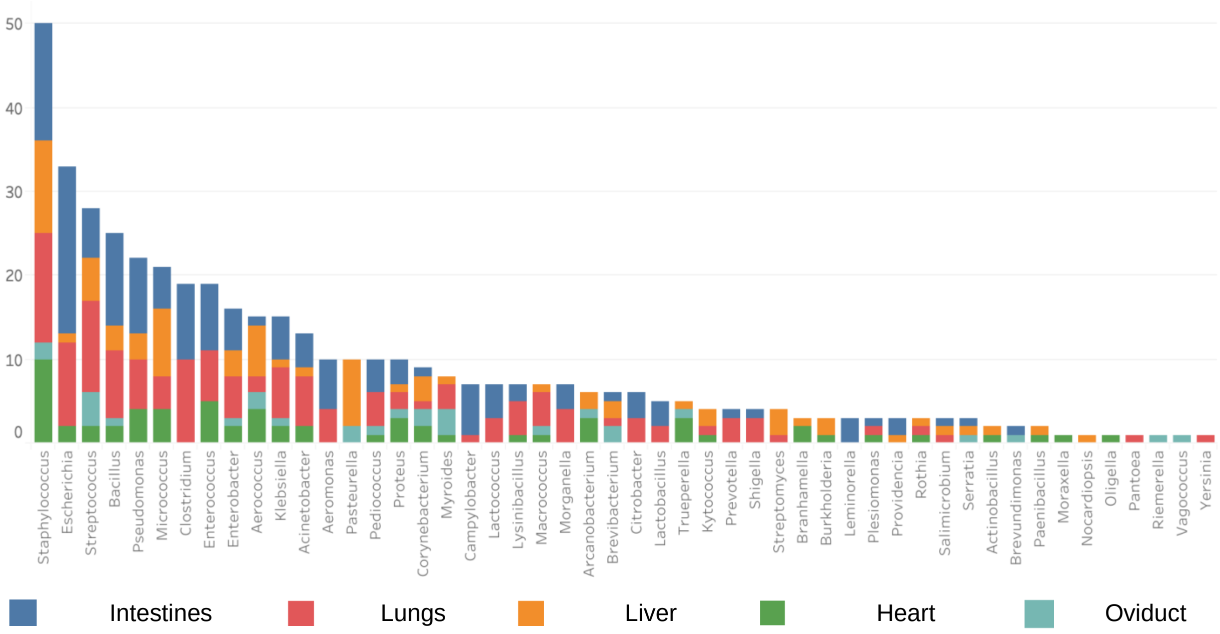

Figure 1 –Results of the bacterial microflora generic determination in various organs and tissues of wild waterfowl

The most etiologically significant species were saved in the microorganisms' collection of FSC VIEV in lyophilized form for the purpose of further study.

The full structure of the microbiome, determined during study of various organs and tissues of the free-living waterfowl (by families), is shown in Figures 2 – 6.

According to the data reflected on graphic figures 2 - 6, the following conclusions can be made:

In the structural composition of bacterial agents, located in the intestines of the free-living waterfowl, the *Enterobacteriosisae* family prevails, the percentage ratio of which was

32.64%; then, by the frequency of excretion, were bacteria of the family *Bacillaceae* and *Staphylococcaceae* – 9.72%; then *Streptococcaceae* – 6.94%; *Clostridiaceae* and *Pseudomonadaceae* – 6.25%; *Enterococcaceae* – 5.56%; *Lactobacillaceae* – 4.86%; *Aeromonadaceae* and *Campylobacteraceae* - 4.17%; *Micrococcaceae* – 3.47%; *Moraxellaceae* – 2.78%; *Aerococcaceae*, *Brevibacteriaceae*, *Caulobacteraceae*, *Corynebacteriaceae* and *Prevotellaceae* - 0.69% respectively.

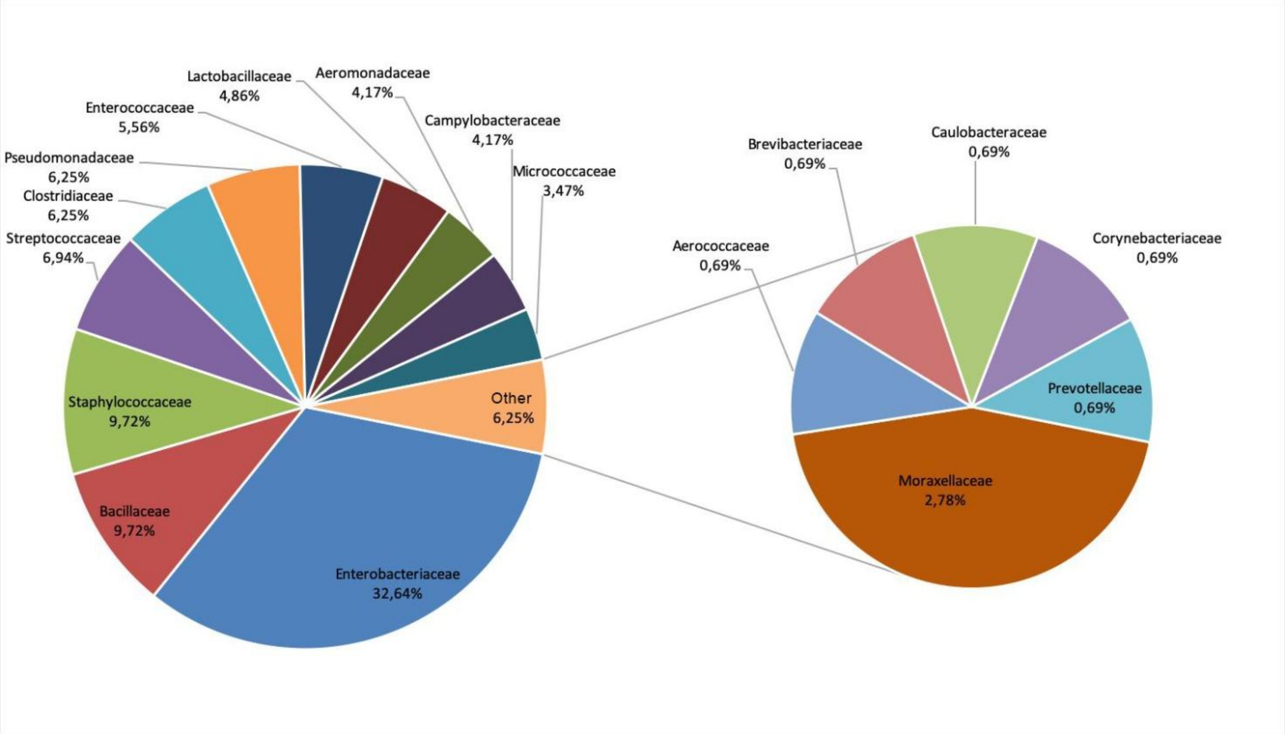

Figure 2 – Bacterial microflora structure isolated from the intestines of the free-living waterfowl

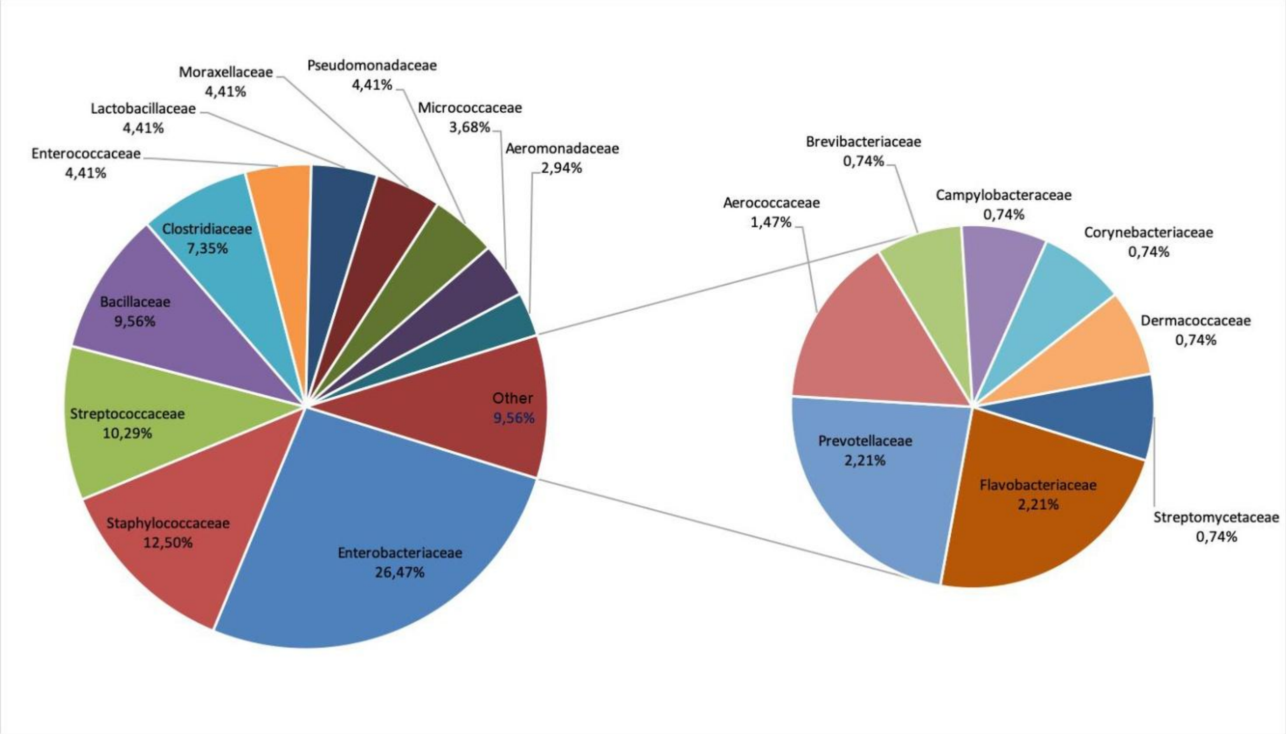

Figure 3 - Bacterial microflora structure isolated from the liver of the free-living waterfowl

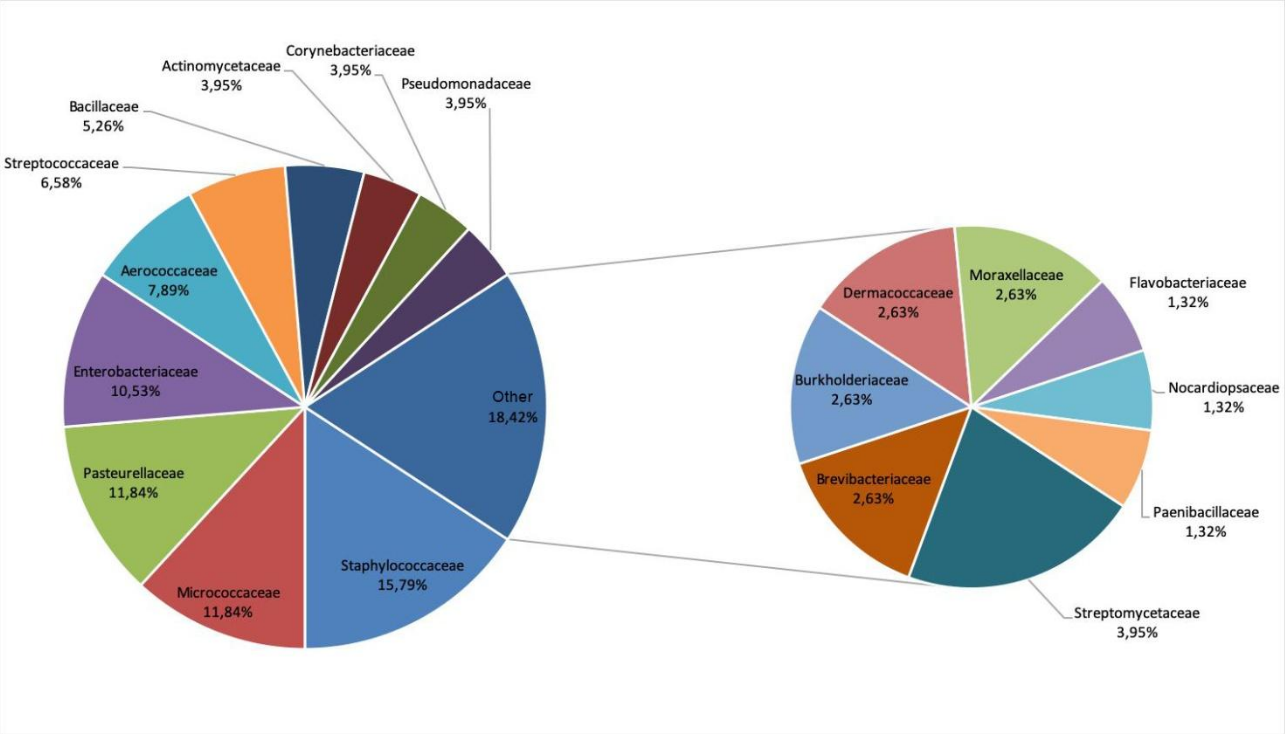

Figure 4 – Bacterial microflora structure isolated from the lungs of the free-living waterfowl

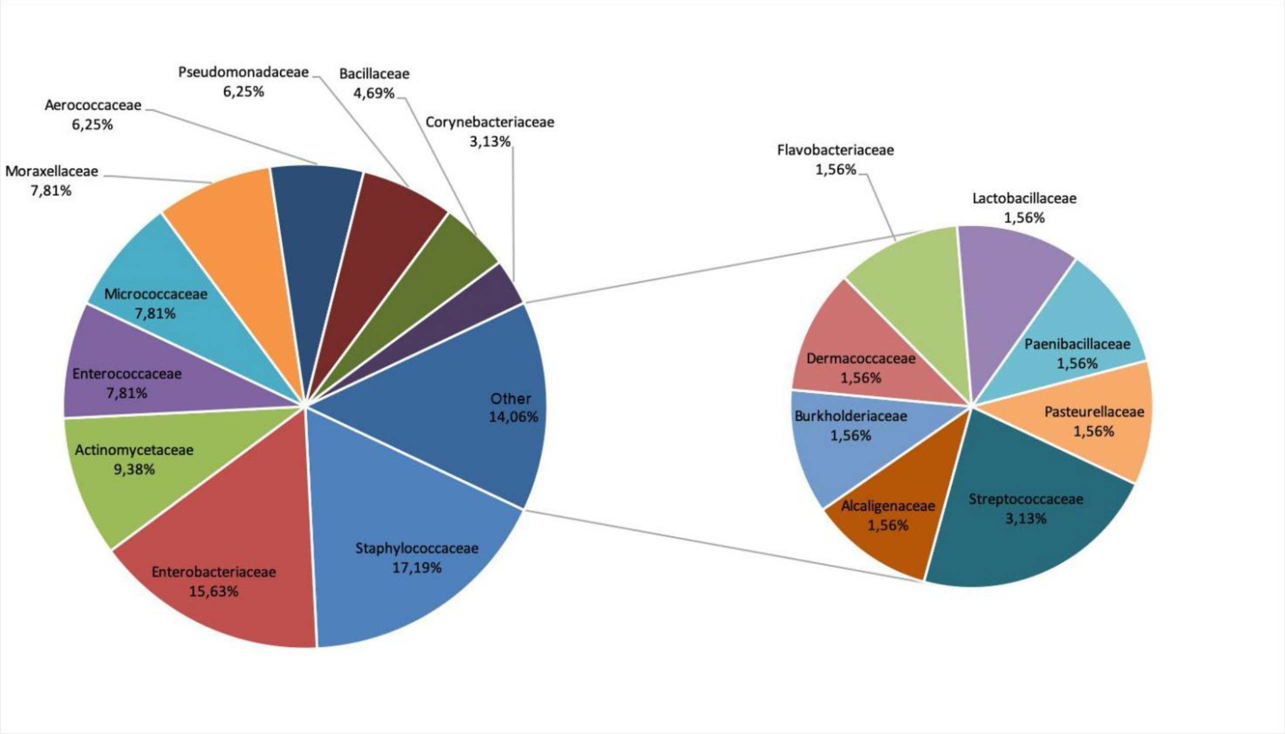

Figure 5 – Bacterial microflora structure isolated from the oviduct of the free-living waterfowl

In the structural composition of bacterial agents, located in the liver of the free-living waterfowl, the *Enterobacteriosisae* family prevails – 36%; *Staphylococcaceae* – 17%; *Streptococcaceae* – 14%; *Bacillaceae* – 13%; *Clostridiaceae* – 10%; *Enterococcaceae*, *Lactobacillaceae*, *Moraxellaceae* and *Pseudomonadaceae* – 6%; *Micrococcaceae* – 5%; *Aeromonadaceae* – 4%; *Flavobacteriaceae* and *Prevotellaceae* – 3%; *Aerococcaceae* – 2%; *Brevibacteriaceae*, *Campylobacteraceae*, *Corynebacteriaceae*, *Dermacoccaceae* and *Streptomycetaceae* – 1% respectively.

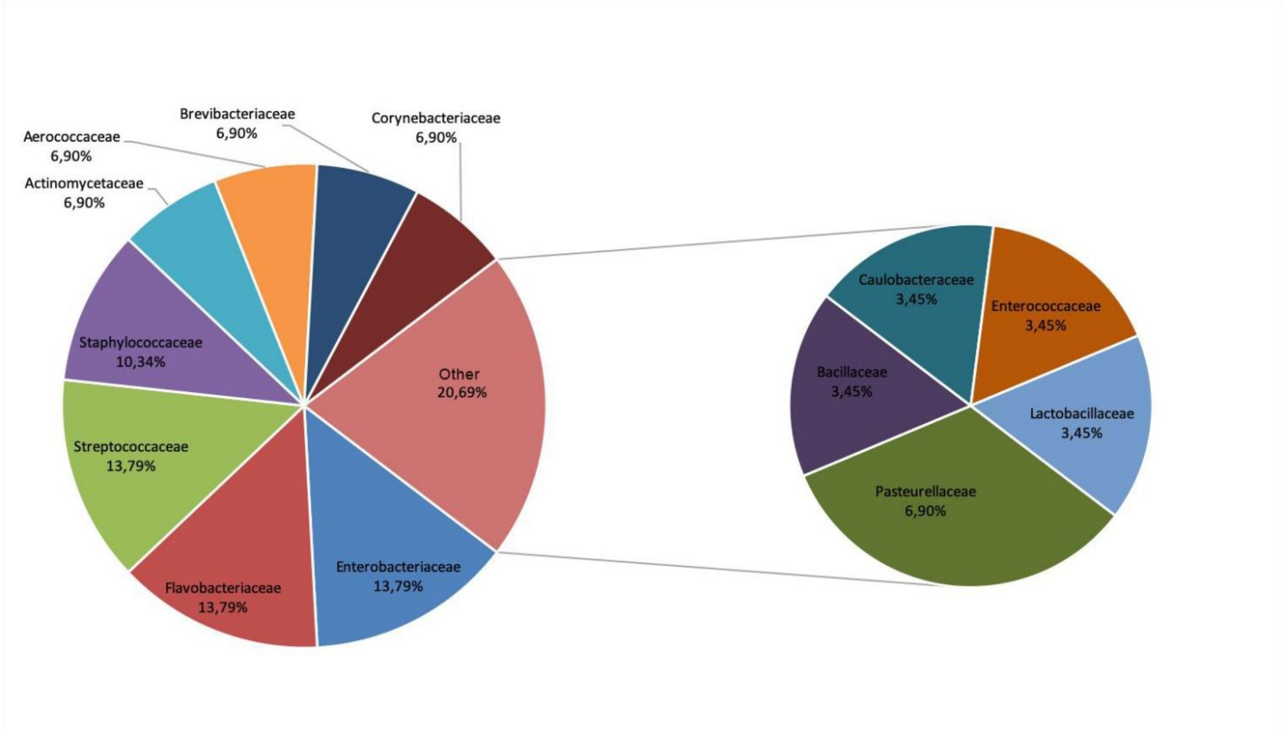

Figure 6 – Bacterial microflora structure isolated from the heart of the free-living waterfowl

In the structural composition of bacterial agents, located in the lungs of the free-living waterfowl, the *Staphylococcaceae* family prevails – 15.79%; *Micrococcaceae* and *Pasteurellaceae* – 11.84%; Enterobacteriaceae – 10.53%; *Aerococcaceae* – 7.89%; *Streptococcaceae* – 6.58%; *Bacillaceae* – 5.26%; *Actinomycetaceae*, *Corynebacteriaceae*, *Pseudomonadaceae* and *Streptomycetaceae* – 3.95%; *Brevibacteriaceae*, *Burkholderiaceae*, *Dermacoccaceae* and *Moraxellaceae* – 2.63%; *Flavobacteriaceae*, *Nocardiopsaceae* and *Paenibacillaceae* - 1.32% respectively.

In the structural composition of bacterial agents, located in the oviduct of the free-living waterfowl, the *Staphylococcaceae* family prevails - 17.19%; *Enterobacteriaceae* – 15.63%; *Actinomycetaceae* – 9.38%; *Enterococcaceae*, *Enterococcaceae* and *Moraxellaceae* – 7.81%; *Aerococcaceae* and *Pseudomonadaceae* – 6.25%; *Bacillaceae* – 4.69%; *Corynebacteriaceae* and *Streptococcaceae* – 3.13%; *Alcaligenaceae*, *Burkholderiaceae*, *Dermacoccaceae*, *Flavobacteriaceae*, *Lactobacillaceae*, *Paenibacillaceae* and *Pasteurellaceae* - 1.56% respectively.

In the structural composition of bacterial agents, located in the heart of the free-living waterfowl, the *Enterobacteriaceae, Flavobacteriaceae* and *Streptococcaceae* families prevails - – 13.79%; *Staphylococcaceae* – 10.34%; *Actinomycetaceae*, *Aerococcaceae*, *Brevibacteriaceae*, *Corynebacteriaceae* and *Pasteurellaceae* – 6.90%; *Bacillaceae*, *Caulobacteraceae*, *Enterococcaceae* and *Lactobacillaceae* – 3.45% respectively.

The results obtained in the course of this work indicate that the migratory bird is the carrier and possible reservoir of the causative agent of riemerellosis - *Riemerella anatipestifer*.

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