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Avian colibacillosis – current aspects

A. O. Gerasimova[1], O. B. Novikova[2,3], A. A. Savicheva[1]

[1] All-Russian Research Veterinary Institute of Poultry Science – Branch of the Federal Scientific Center "All-Russian Research and Technological Poultry Institute" of Russian Academy of Sciences, Saint Petersburg, Lomonosov, Russia

[2] Saint-Petersburg State University of Veterinary Medicine, Saint Petersburg, Russia

[3] Saint-Petersburg State Agrarian University, Saint Petersburg, Russia

ABSTRACT

Colibacillosis is a bacterial disease of humans, animals and birds caused by *Escherichia coli*, pathogenic gram-negative bacillus. Despite its secondary nature, colibacillosis widely affects poultry farms and causes significant economic losses. The disease spread is closely associated with antibiotic resistance problem because a diseased carrier bird may be a reservoir of antibiotic-resistant *Escherichia coli* strains. In addition, genes of virulence and resistance have been proven to be transferred from avian *Escherichia* strains to extra intestinal pathogenic strains that are dangerous to humans. Colibacillosis is transmitted aerogenically, alimentally, rarely transovarially, with droppings, mucus, feed, water, handling tools and operating personnel. Birds are most susceptible at the age of 1–14 days and at the onset of laying period. The disease may present as acute, subacute and chronic forms and is most often manifested by catarrhal hemorrhagic enteritis with profuse foamy diarrhea, respiratory tract lesions, fibrinous peritonitis and polyserositis, as well as a significant decrease in weight gains, stunting, egg laying decrease or complete cessation. Colibacillosis is diagnosed comprehensively taking into account the epizootic situation, findings of clinical examination and postmortem examination of dead or emergency-slaughtered poultry as well as laboratory test and bioassay results. Bacteriological, serological and molecular genetic methods are used for the disease diagnosis. Colibacillosis prevention includes improvement of poultry keeping practice (control of feed and water quality, disinfection, pest control, microclimate control) as well as timely complex vaccination of all poultry. The disease shall be treated taking into account primary etiological factors and bacteria sensitivity to antimicrobials.

Keywords: review, colibacillosis, poultry farming, epizootiology, *Escherichia coli*

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For correspondence: Alina O. Gerasimova, Postgraduate Student, Microbiology Department, All-Russian Research Veterinary Institute of Poultry Science – Branch of the Federal Scientific Center "All-Russian Research and Technological Poultry Institute" of Russian Academy of Sciences, 198412, Russia, Saint Petersburg, Lomonosov, ul. Chernikova, 48, *e-mail: gerasimova.alina.20@yandex.ru*.

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Колибактериоз птиц – актуальные вопросы

А. О. Герасимова[1], О. Б. Новикова[2,3], А. А. Савичева[1]

[1] Всероссийский научно-исследовательский ветеринарный институт птицеводства – филиал Федерального научного центра «Всероссийский научно-исследовательский и технологический институт птицеводства» Российской академии наук (ВНИВИП – филиал ФНЦ «ВНИТИП» РАН), г. Санкт-Петербург, г. Ломоносов, Россия

[2] ФГБОУ ВО «Санкт-Петербургский государственный университет ветеринарной медицины» (ФГБОУ ВО СПбГУВМ), г. Санкт-Петербург, Россия

[3] ФГБОУ ВО «Санкт-Петербургский государственный аграрный университет» (ФГБОУ ВО СПбГАУ), г. Санкт-Петербург, Россия

РЕЗЮМЕ

Колибактериоз – бактериальная болезнь человека, животных и птиц, вызываемая патогенной грамотрицательной палочкой *Escherichia coli*. Несмотря на то что болезнь носит секундарный характер, колибактериоз затрагивает птицеводческие хозяйства повсеместно, нанося значительный экономический ущерб. Распространение болезни тесно связано с проблемой антибиотикорезистентности, поскольку больная птица-носитель может являться резервуаром штаммов *Escherichia coli*, устойчивых к действию антибактериальных средств. Кроме того, доказана возможность передачи генов вирулентности и резистентности от птичьих штаммов эшерихий внекишечным патогенным штаммам, опасным для человека. Колибактериоз передается аэрогенно, алиментарно, реже – трансовариально через помет, слизь, корма, воду, предметы обихода и обслуживающий персонал. Наиболее восприимчивы птицы в возрасте 1–14 сут и в период начала яйцекладки. Болезнь протекает в острой, подострой и хронической форме и чаще всего сопровождается катарально-геморрагическим энтеритом с профузной пенистой диареей, поражением респираторного тракта, фибринозным перитонитом и полисерозитом, а также значительным снижением привесов, отставанием в росте, снижением или полным прекращением яйценоскости. Диагноз «колибактериоз» ставится комплексно с учетом эпизоотической ситуации, данных клинического осмотра и патолого-анатомического вскрытия павшей или вынужденно убитой птицы, а также результатов лабораторных исследований и постановки биопробы. Для диагностики заболевания применяют бактериологические, серологические и молекулярно-генетические методы. Профилактика колибактериоза достигается путем улучшения условий содержания птицы

(контроль качества корма и воды, дезинфекция, дератизация, контроль параметров микроклимата), а также своевременной комплексной вакцинацией всего поголовья. Лечебные мероприятия должны планироваться, основываясь на первичных этиологических факторах и чувствительности бактерий к противомикробным средствам.

Ключевые слова: обзор, колибактериоз, птицеводство, эпизоотология, *Escherichia coli*

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Для корреспонденции: Герасимова Алина Олеговна, аспирант отдела микробиологии ВНИВИП – филиала ФНЦ «ВНИТИП» РАН, 198412, Россия, г. Санкт-Петербург, г. Ломоносов, ул. Черникова, 48, *e-mail: gerasimova.alina.20@yandex.ru.*

INTRODUCTION

Colibacillosis (escherichiosis) is a zooanthroponotic bacterial septicaemic disease of poultry and wild birds, affecting mainly gastrointestinal and respiratory tracts, causing high mortality and serious economic losses [1].

It is generally believed throughout the world that avian colibacillosis occurs as a secondary disease when the immunity is suppressed and overall body resistance is decreased. This is most often associated with shortages in poultry keeping and feeding practices, serious poultry management shortcomings, as well as the presence of viral (avian influenza, Newcastle disease, acute infectious bronchitis, infectious bursal disease, etc.) and bacterial (pasteurellosis, *Haemophilus* infection, staphylococcosis, mycoplasmosis, etc.) diseases [2, 3, 4]. *Escherichia coli* infections cause losses due to embryonated egg and chick deaths, poor development of convalescent poultry, reduced egg laying and weight gain, culling of carcasses, spread of pathogenic and antibiotic-resistant strains among adult poultry [1, 2, 3]. "Colibacillosis" diagnosis is the most common in veterinary reporting since the disease is not a "quarantine disease", and *E. coli* are found in the vast majority of outbreaks and have no negative impact on the holding reputation [4].

Colibacillosis poses a serious threat both to birds and humans. The uncontrolled use of chemotherapeutics in poultry and animal farming industry resulted in formation of a reservoir of antibiotic-resistant *E. coli* strains capable of being transmitted to humans through processed products produced by commercial agricultural establishments [5, 6]. Whole genome sequencing has shown that human extra-intestinal pathogenic *E. coli* (ExPEC) is genetically similar to avian pathogenic *E. coli* strains. Virulence and antibiotic resistance genes are proven to be horizontally transferred. Moreover, avian ExPEC-specific ColV (colicin V) plasmid have been detected in human ExPEC isolates that suggests a possible zoonotic transmission of pathogenic *Escherichia* from poultry to humans [7, 8].

In February 2017, the World Health Organization published a list of 12 species of bacteria resistant to antimicrobials and posing the most threat to human health. *E. coli* was classified to category 1 comprising bacteria with critical antibiotic resistance together with other *Enterobacteriaceae* family members, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, resistant to carbapenems and producing extended-spectrum beta-lactamases [9].

The scientific novelty of the presented review is in description of modern methods for *E. coli* detection and identification, new trends in colibacillosis treatment and prevention.

The work was aimed at summarizing scientific literature data on avian colibacillosis and highlighting the most important aspects of its investigation.

AGENT CHARACTERIZATION

Avian colibacillosis is caused by *E. coli* (avian pathogenic *Escherichia coli*, APEC), the most widespread member of *Enterobacteriaceae* family that was named after T. Escherich, German pediatrician, who isolated the said pathogen from intestinal contents in children for the first time in 1885 [10]. Straight or slightly curved Gram-negative rods that are motile due to flagella and peritrichial cilia and occur singly less often in pairs in smears (Fig. 1).

On dense media *E. coli* bacteria form smooth round colonies of medium size (1.5–2.5 mm) (Fig. 2). Furthermore, they actively ferment glucose, lactose, mannitol, arabinose, galactose with acid and gas production; they typically do not ferment sucrose and dulcite; produce indole, do not produce hydrogen sulfide; reduce nitrates

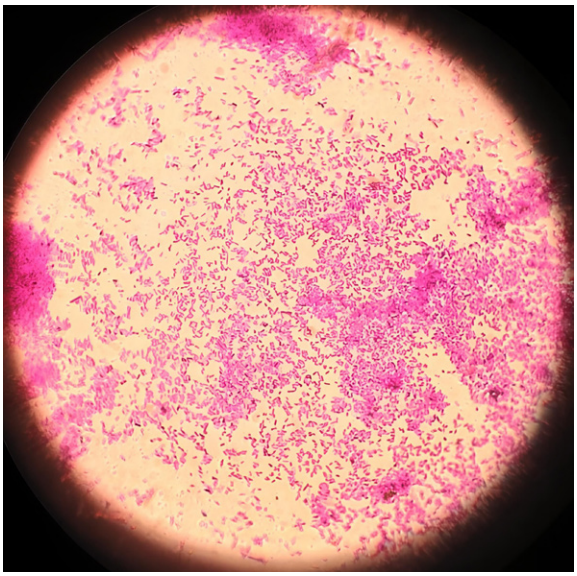

Fig. 1. Morphology of Gram-stained E. coli (magnification 40×)

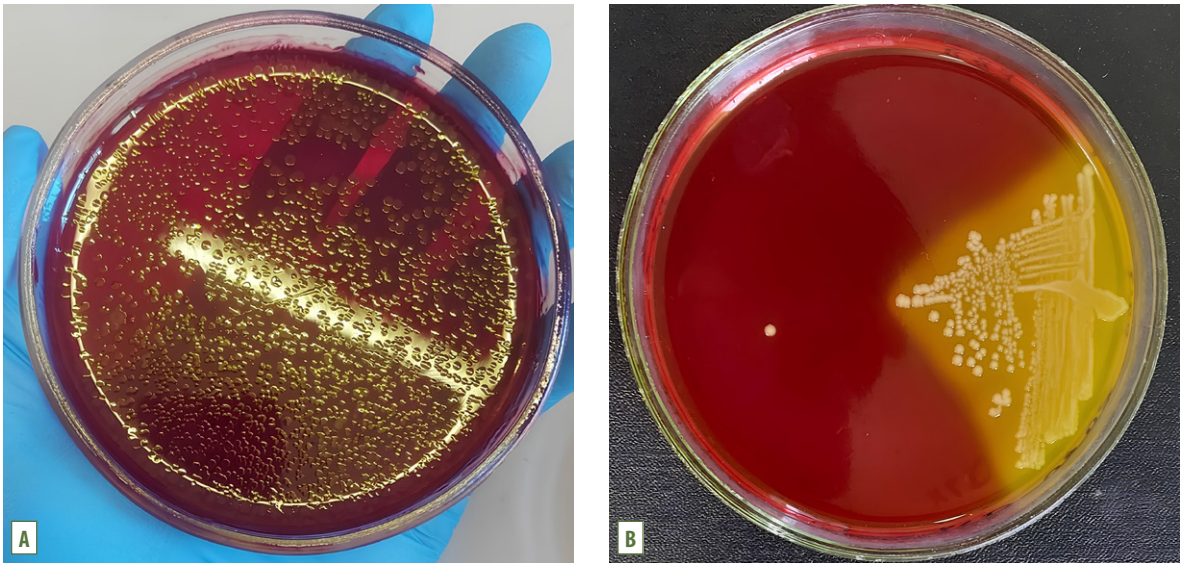

Fig. 2. E. coli growth on dense nutrient media:
A – E. coli colonies with characteristic metallic sheen on Endo agar; B – E. coli colonies on XLD-agar

to nitrites, do not dilute gelatin, do not grow on a citrate-containing medium, do not digest urea; demonstrate positive methyl red test reaction and negative Voges–Proskauer test reaction (Fig. 3) [11, 12].

E. coli bacteria are rather persistent in the environment. The pathogen is decontaminated by heating to 60 °C for 15 minutes and decontaminated instantly by boiling at 100 °C. The bacteria are able to survive in the environment (water, soil, household items) for several months, in food – for more than 30 days. In addition, *E. coli* are highly susceptible to most disinfectants (formaldehyde, chlorine preparations, sodium hydroxide, etc.) and antibiotics (tetracyclines, aminoglycosides, rifampicin, etc.), however, they are able to acquire resistance to antimicrobial drugs through R-plasmids. Horizontal transfer of resistance plasmids promotes the spread of resistance within the bacterial population [10, 13, 14, 15].

All poultry, ornamental, exotic and wild bird species are susceptible to the disease.

Colibacillosis is transmitted alimentary, aerogenically and transovarially. The infection transmission factors include feed, bedding, handling tools, rodents, synanthropic birds, operating personnel.

Chicks at the age of 1–14 days are the most susceptible and can be affected with acute disease in the form of sepsis. Poultry during the laying period are also susceptible. Convalescent adult birds become pathogen-carriers and often serve as a reservoir of pathogenic *E. coli* strains [1, 2].

Incubation period varies from several hours to 6 days.

The disease occurs in acute (sepsis), subacute and chronic forms and characterized by hyporexia or anorexia, depression, significantly reduced weight gain, foamy yellow-green diarrhea, and when respiratory tract is affected – by rales, sneezing and profuse nasal discharges. The following postmortem lesions are found in necropsied birds: petechiae and ecchymoses on mucous membranes and internal organs, catarrhal hemorrhagic enteritis, pancreatitis, splenitis, fibrinous peritonitis and polyserositis; cecum is often swollen (Fig. 4) [1, 16, 17].

CURRENT ASPECTS OF DIAGNOSIS

Bacteriological methods. Samples are inoculated on dehydrated Endo's or Levin's medium and incubated at temperature of 37–38°C for 24 hours for the bacteria isolation and differentiation. Then the most characteristic *E. coli* colonies are selected and smears are prepared, Gram-stained and examined under microscope. When

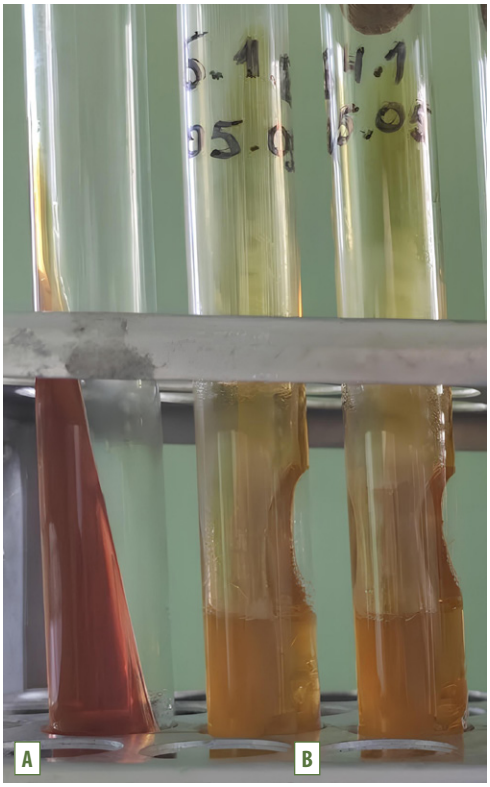

Fig. 3. Biochemical properties of E. coli on triple sugar iron agar: A – control sample containing medium not inoculated with the culture; B – test samples with E. coli: the medium slant turns yellow due to lactose fermentation, and glucose and sucrose fermentation with acid and gas formation discolors the medium column with butt splitting

Gram-negative rounded rods are detected in the smears, the colonies are re-seeded on Olkelnitsky's triple sugar agar, Gissa's, Simmons media, Kligler agar for testing them for biochemical properties. Paper indicator discs are used for the same purpose [17, 18]. The diagnosis is made based on *E. coli* isolation from cardiac blood, bone marrow, liver, spleen and pericardium tissues. Isolation of *E. coli* from intestine is considered diagnostically insignificant [19].

Serological methods. *E. coli* has 900 known serotypes. They are differentiated by examination of bacteria antigenic properties.

Somatic O-antigen is a lipopolysaccharide-protein complex of outer membrane of bacterial wall and defines the serological group of the bacteria; O-antigen-lacking strains form rough colonies of R-form and most often are non-virulent. To date, 175 O serogroups have been identified. Prevailing serogroups are O1, O2 and O78 but they account for only 15 up 60% of isolates depending on testing [20].

Motile *Escherichia* have flagellar H-antigen consisting of the flagellin protein and are thermolabile. Totally, there are about 70 H-antigen variants.

Some bacteria possess capsular mucopolysaccride thermostable K-antigen (Vi-antigen) located outside the somatic antigen. This allows bacteria to block agglutination reaction to specific O-serum. About 100 K-antigen variants and 3 classes among them: A, B and L. Antigens of class A are thermostable and antigens of class B and L are thermolabile.

Also, 17 types of fimbrial F-antigens required for bacteria adhesion have been described. F-antigens are classified into mannose-sensitive and mannose-resistant depending on whether agglutination is inhibited in the presence of mannose or not.

There are several diarrheagenic groups within known *E. coli* serotypes: entertoxicogenic (ETEC); enteroinvasive (EIEC); enteropathogenic (EPEC); enterohemorrhagic (EHEC); enteroaggregative (EA) и diffusely

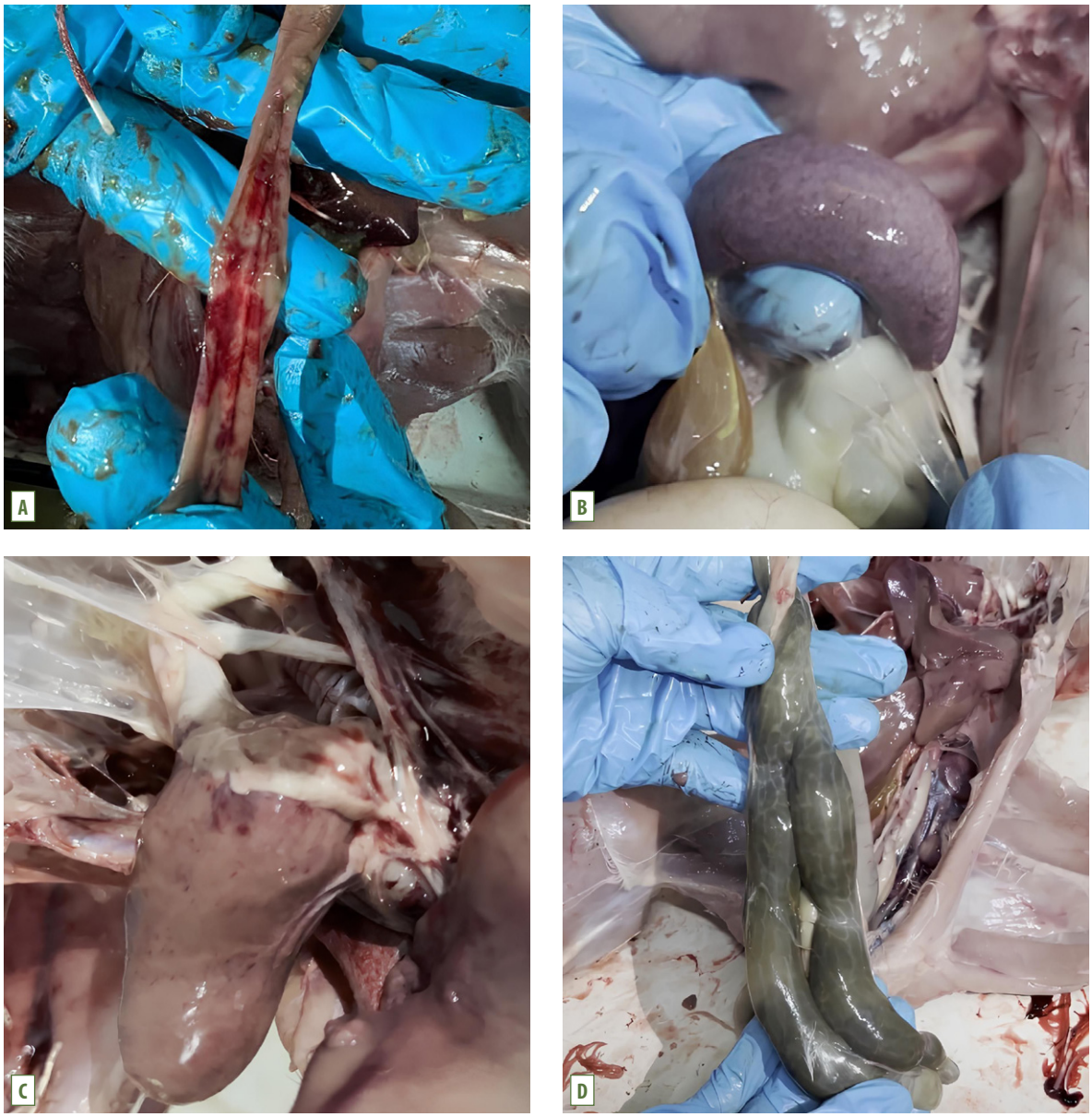

Fig. 4. Postmortem lesions detected in turkey poults infected with E. coli culture:
A – multiple hemorrhages in intestine mucosa; B – splenitis; C – hemorrhages in epicardium; D – cecum swelling

adherent (DAEC) *E. coli*. Colibacilloses are classified based on different pathogenicity factors possessed by the agent [11, 16, 18, 21].

Slide or tube agglutination test with *E. coli*-specific sera is performed for serological *Escherichia* differentiation. At first, suspension is prepared by adding 0.85% sodium chloride solution to concentration of 2–3 billion microbial cells/cm^3, and then it is heat treated – heated in a water bath at 100 °C for 1 hour and autoclaved at 1 atm for 2 hours to destroy the surface K-antigen [16]. Serotyping remains the most frequently used diagnostic method in laboratories, but it only allows the identification of a limited number of APEC strains. Therefore, this method cannot be used as an effective diagnostic tool, particularly since serotype does not reflect the virulence trait [20].

Molecular-genetic methods. Tests by polymerase chain reaction (PCR) are used for rapid pathogen detection in clinical samples. DNA from tracheal and intestinal mucosa scrapings, parenchymal organ and red bone marrow pieces are used for the PCR. Control sample and negative control are concurrently tested with PCR [22]. Colibacillosis is diagnosed when at least one of the determinants of pathogenicity is detected in samples: P-fimbrial protein (*papC*), temperature-sensitive hemagglutinin (*tsh*), iron-binding protein (*fyuA/irp 2*), aerobactin (*iutA/iucD*), increased serum survival protein (*iss*), colicin V plasmid (*cva/cvi*), enteroaggregative *E. coli* heat-stable toxin (*astA2*), vacuolating autotransporter toxin (*vat*) [8, 23]. Some other virulence genes involved in the development of avian colibacillosis have also been identified: genes encoding adhesins (*F1, P* and *Stg fimbriae, curli* and *EA/I*), host immunity protection factors (*OmpA*, lipopolysaccharide and K1), iron acquisition systems (*Iro* proteins, yersiniabactin and iron acquisition *Sit* locus), autotransporters (*AatA*), phosphate transport system, sugar metabolism and *IbeA* protein.

Numerous studies have demonstrated that these virulence factors (VFs) are rarely all present in the same isolate, showing that APEC strains constitute a large heterogeneous group. Different isolates may harbor different associations of virulence factors, each one is able to induce avian colibacillosis [20].

Today, double digest selective label (DDSL) technique is one of the most accurate and highly specific methods of genotyping. It allows pathogen DNA to be detected in samples as well as the infection pathways to be tracked, close related strains and mutations to be identified. The accuracy of this method determined based on discrimination index, exceeds the accuracy of pulsed-field gel electrophoresis – gold standard for genotyping [24].

CURRENT ASPECTS OF THERAPY

Colibacillosis therapy includes use of antibacterial agents. Mass monitoring carried out in 2015–2020 has shown that *E. coli* strains are currently susceptible to almost all classes of antibiotics, except for carbapenems [8]. Preliminary testing of *Escherichia* for susceptibility to antibiotics followed by selection of appropriate drug is an important therapy stage [23]. The Committee for Veterinary Medicinal Products of the European Medicines Agency actively revises guidelines for use of antibiotics in animal farming industry including dosing regimen optimization to prevent expansion of the resistome (a set of antibiotic resistance genes) among animals and, as a result, in humans [25].

Antimicrobial peptides, phytobiotics, probiotics, bacteriophages are alternatives widely used for bacterial disease therapy. According modern studies, they are found to be highly effective for both *in vitro* and *in vivo* colibacillosis control [26, 27, 28].

Antimicrobial peptides (AMPs) are a promising natural alternative to traditional antibiotics. AMPs are characterized by rapid and highly selective antimicrobial effect, as well as a low drug resistance tendency and easy synthesizability. Moreover, they demonstrate immunomodulatory and anti-endotoxin activity, inhibit proinflammatory reactions, stimulate chemotaxis and differentiation of immune cells [29, 30, 31]. Kathayat D. et al. during their study performed in 2021 found that AMPs derived from *Lactobacillus rhamnosus* probiotic bacteria were active against resistant *E. coli* serotypes isolated from birds, as well as reduced the number of *Enterobacteriaceae* in chicken caeca, with minimal impact on intestinal microbiota [32].

Inhibition of *Escherichia* quorum sensing (QS), a phenomenon underlying the bacteria social behavior, ability to regulate gene expression in response to changes in the microorganism population density, has become a new strategy for avian colibacillosis control. This regulation mechanism serves to enhance bacteria survivability. Quorum sensing inhibitors (QSI) are targeted to bacteria signaling circuit disruption and population virulence reducing. The use of QSI for avian colibacillosis treatment is described. Some studies showed that QSI significantly reduced the mortality of chickens caused by the infection. Moreover, some inhibitors (QSI-8 and QSI-10) have beneficial effects on the intestinal microbiota through increasing the number of *Butyricicoccus* spp. and *Lactobacillus* spp. [7, 33, 34, 35].

The use of bacteriophages for avian colibacillosis treatment has been widely described. Their principle of action is a highly specific effect on bacterial cells causing their lysis. Some studies demonstrated successful application of lytic bacteriophages for inhibition of pathogenic *E. coli* strain development. At the same time, many authors note a decrease in the effectiveness of biologicals *in vivo* [36, 37, 38, 39]. Sørensen P. E. et al. indicate the prospects of investigations related to the use of bacteriophages in poultry farming, however, they emphasize the problem of bacteriophage-resistant mutant APEC strain emergence. Bacteria are able to develop phage resistance through various mechanisms: spontaneous mutations, acquisition of restriction-modification (R-M) systems, adaptive immunity associated with clustered regularly interspaced short palindromic repeats (CRISPRs) [40].

CURRENT ASPECTS OF PREVENTION

Nonspecific prevention of avian colibacillosis relies on good management practice including appropriate veterinary and sanitary measures: use of all-in-all-out management practice and sanitary breaks before restocking, good egg acceptance and incubation hygiene practice, timely disinfection and pest control, use of pathogenic *Escherichia*-free feedstuffs and feedstuffs protected from rodents and wild birds, microclimate maintenance [23, 41, 42].

Current trend in poultry farming industry is introduction of alternative feed additives having immunostimulating

effect. Such additives include: prebiotics, probiotics, synbiotics, essential oils. Lactic acid bacilli are known to synthesize bacteriocins and bacteriocin-like factors that are antagonists of putrefactive, pathogenic and opportunistic microflora due to their ability to produce lactabiotics, lactacins having antimicrobial activity, as well as hydrogen peroxide, lysozyme, interleukins, interferons, etc. [23, 26, 43].

The effect of biologicals inoculated *in ovo* is being actively studied. To date, it has been found that the injection of probiotics and phage cocktails into the amniotic fluid prevents colibacillosis at the stage of chick hatching, but is ineffective for mass long-term prevention on large poultry farms [44, 45].

Specific prevention includes timely vaccination of poultry contributing to disease freedom maintaining.

There are live and inactivated vaccines, monovaccines against colibacillosis, as well as combined vaccines against several diseases. Live vaccines in poultry farming industry are most often administered to group of poultry with drinking water, by coarse and fine spraying. Whereas, inactivated vaccines are administered to each bird parenterally: intramuscularly, subcutaneously in the middle third of the neck, cutaneously, intranasally, intraocularly or cloacally [16, 23, 24, 41, 42, 43, 46].

Studies conducted in Japan in 2017 [47] showed high effectiveness of live attenuated vaccine against colibacillosis caused by *E. coli* serovar O78. Attenuated *E. coli* strain was constructed in 2012 by the allelic exchange procedure based on the mutant AESN1331strain containing a deletion in the C-reactive protein (*crp*) gene, lacking virulence-associated genes (*iss, tsh, cvaA* and *papC*) and susceptible to many antimicrobials, except for nalidixic acid [48].

Non-cellular vaccines based on bacterial outer membrane vesicles (OMVs) – proteolipid nanostructures secreted by Gram-negative bacteria and enriched with various immunoactive molecules (cell wall components, membrane proteins, cytoplasmic proteins and bacterial nucleic acids) are described. Such vaccines confer strong cross-immunity against several *E. coli* serogroups through enhancement of nonspecific serum immune factors, specific antibody response and spleen and peripheral blood lymphocyte proliferation. Studies carried out by R. Hu et al. in 2020 demonstrated the effective use of OMVs vaccines based on *E. coli* O1, O2 and O78 serogroups, that reduced bacterial load and stimulated proinflammatory cytokine production [49, 50].

Non-live vaccines based on bacterial ghosts (BGs) – cell envelopes devoid of genetic and cytoplasmic components and produced by controlled expression of lysis E gene of PhiX 174 bacteriophage are currently of scientific and practical interest. Bacterial ghosts are proven to possess adjuvant properties and also show tropism to host antigen-presenting cells, allowing the induction of humoral and cellular immune responses (in particular, they maintain high levels of IgY, IgA and IFN-γ, as well as increase the production of proinflammatory IL-6, IL-1β and TNFSF15 cytokines). At the same time, the bacterial ghosts do not have any endotoxicity and exhibit antigenic properties of living bacteria. Antigenic epitopes are transferred in inner or outer membrane proteins, as well as in flagella, fimbria or periplasm. Bacterial ghost-based vaccines advantages include simplicity of production method, safety, long shelf life without need for a cold chain, possible needle-free administration and universality [51, 52].

CONCLUSIONS

Russian and foreign scientific literature review has allowed us to make the following conclusions.

1. Currently, colibacillosis is of great concern for poultry industry for many reasons. Firstly, colibacillosis causes severe economic losses on farms, affects poultry performance and compromises poultry welfare. Secondly, convalescent carrier birds are able to form reservoirs of resistant *E. coli* strains. Thirdly, APEC strains are genetically similar to with extra-intestinal pathogenic human *E. coli* strains, that makes transfer of virulence and antibiotic resistance genes between them possible.

2. Bacteriological, serological and molecular genetic test methods are used for *E. coli* detection and identification. Bacteriological methods enable coliform isolation from pathological materials but cannot be used for pathogenicity determinant identification and serotyping. Serological diagnostic methods using *E. coli*-specific sera are used for serotyping. The said methods are considered less informative for avian colibacillosis diagnosis since they do not allow for bacteria virulence determination. Molecular genetic diagnostic methods are more specific and informative since they allow for detection of bacteria in pathological materials as well as identification of pathogenicity determinants, determination of the genetic relationship between isolates and the infection pathways.

3. Antibiotic resistance is a problem attracting attention of researchers and medical and veterinary practitioners around the world. Therefore, search for safe and environment-friendly alternatives to antibiotics has become of current importance for treatment of bacterial diseases (including colibacillosis). The successful use of antimicrobial peptides, quorum sensing inhibitors and bacteriophages for avian colibacillosis treatment is described. It has been experimentally proven that the above-mentioned agents are capable of suppressing the pathogenic *Escherichia* reproduction in poultry, demonstrate immunomodulatory activity and at the same time do not have a negative effect on intestinal microbiocenosis. The examination of biosafe alternatives is a promising area for further studies, and, nevertheless, antibiotics remain the first choice drugs for the treatment of bacterial diseases.

4. Widespread refuse to use in-feed antibiotics as a non-specific prevention and growth stimulation tools has resulted in the large-scale use of feed additives: prebiotics, probiotics, synbiotics, phytobiotics, essential oils, etc. Numerous studies have shown positive effect of these additives on poultry performance, intestinal microbiota, non-specific immunity response and of intestinal barrier function maintenance.

5. Wide serological *Escherichia* diversity makes difficult specific avian colibacillosis prevention. Attenuated vaccines based on mutant APEC strains lacking virulence genes but retaining susceptibility to antimicrobials are being actively developed. Moreover, synthetic vaccines based on outer membrane vesicles and ghost cells are of great scientific interest. To date, such vaccines have been found to have specific effect as well as to stimulate cellular and humoral immunity.

REFERENCES

1. Zuev N. P., Tuchkov N. S. The problem of colibacteriosis in poultry farming. *Molodye uchenye – nauke i praktike APK: materialy nauchno-prakticheskoj konferencii aspirantov i molodyh uchenyh (Vitebsk, 27–28 aprelja 2023 g.) = Early-carrier scientists' input to agro-industrial science and practice: proceedings of the Scientific and Practical Conference of Postgraduates and Early-Career Scientists (Vitebsk, 27–28 April, 2023)*. Vitebsk: EE "VSAVM"; 2023; 77–81. EDN: WLOGCX. (in Russ.)

2. Novikova O. B., Bartenev A. A. Problema kolibakterioza v pticevodstve = The problem of colibacteriosis in poultry farming. *Modern trends in science and technologies*. 2015; 8–4: 35–37. EDN: VBPOVR. (in Russ.)

3. Yarygina N. A. Poultry colibacillosis epizootic situation monitoring in the Leningrad region in 2019–2020. *Molodye uchenye – nauke i praktike APK: materialy nauchno-prakticheskoj konferencii aspirantov i molodyh uchenyh (Vitebsk, 27–28 aprelja 2023 g.) = Early-carrier scientists' input to agro-industrial science and practice: proceedings of the Scientific and Practical Conference of Postgraduates and Early-Career Scientists (Vitebsk, 27–28 April, 2023)*. Vitebsk: EE "VSAVM"; 2023; 241–243. EDN: FPIOIB. (in Russ.)

4. Vinokhodov V. V., Vinokhodov V. O., Lysenco S. N. To a question about etiology of colibactiriosys of birds. *International Bulletin of Veterinary Medicine.* 2009; 3: 17–25. EDN: LLZGXD. (in Russ.)

5. Isakova M. N., Sokolova O. V., Bezborodova N. A., Krivonogova A. S., Isaeva A. G., Zubareva V. D. Antimicrobial resistance in clinical *Escherichia coli* isolates obtained from animals. *Veterinay Science Today.* 2022; 11 (1): 14–19. DOI: 10.29326/2304-196X-2022-11-1-14-19.

6. Khong M. J., Snyder A. M., Magnaterra A. K., Young M. M., Barbieri N. L., Weimer S. L. Antimicrobial resistance profile of *Escherichia coli* isolated from poultry litter. *Poultry Science*. 2022; 102 (1):102305. DOI: 10.1016/j.psj.2022.102305.

7. Helmy Y. A., Kathayat D., Deblais L., Srivastava V., Closs G. Jr., Tokarski R. J. 2nd, et al. Evaluation of novel quorum sensing inhibitors targeting auto-inducer 2 (AI-2) for the control of avian pathogenic *Escherichia coli* infections in chickens. *Microbiology Spectrum*. 2022; 10 (3): 286–322. DOI: 10.1128/spectrum.00286-22.

8. Kathayat D., Lokesh D., Ranjit S., Rajashekara G. Avian pathogenic *Escherichia coli* (APEC): an overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. *Pathogens*. 2021; 10 (4):467. DOI: 10.3390/pathogens10040467.

9. World Health Organization. WHO publishes list of bacteria for which new antibiotics are urgently needed. Available at: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (date of access: 20.06.2023).

10. Ruiz N., Silhavy T. J. How *Escherichia coli* became the flagship bacterium of molecular biology. *Journal of Bacteriology*. 2022; 204 (9):e0023022. DOI: 10.1128/jb.00230-22.

11. Methodical Guidelines for microbiological diagnosis of enterobacterial diseases: approved by the USSR Ministry of Health of 17.12.1984. Available at: https://docs.cntd.ru/document/1200112237. (in Russ.)

12. Shepelin A. P. Nutrient media: current status & trends in design, production and application. *Bacteriology.* 2016; 1 (1): 42–47. DOI: 10.20953/2500-1027-2016-1-42-47. (in Russ.)

13. Gigani O. B., Gigani O. O. Plasmids: monograph. Moscow: Ru-science; 2017. 154 p. (in Russ.)

14. Krasilnikov N. A. Manual for identification of bacteria and actinomycetes. Moscow; Saint Petersburg: USSR Academy of Sciences; 1949. 838 p. (in Russ.)

15. Tarabai H., Wyrsch E. R., Bitar I., Dolejska M., Djordjevic S. P. Epidemic HI2 plasmids mobilising the carbapenemase gene *blaIMP-4* in Australian clinical samples identified in multiple sublineages of *Escherichia coli* ST216 colonising silver gulls. *Microorganisms*. 2021; 9 (3):567. DOI: 10.3390/microorganisms9030567.

16. Shchepetkina S. V., Novikova O. B., Zabrovskaya A. V., Terletskiy V. P., Tyshchenko V. I. Modern principles of antibiotic therapy in poultry farming. Saint Petersburg: Saint-Petersburg State Academy of Veterinary Medicine; 2015. 148 p. (in Russ.)

17. Gromov I. N. Pathomorphological and differential diagnostics of poultry diseases affecting primarily intestines. *Animal Agriculture and Veterinary Medicine*. 2020; 2 (37): 27–31. EDN: OFNXLC. (in Russ.)

18. Shonazar J. M., Mamadatokhonova G. N., Rahimov A. A., Mirakov A., Abdukosimi A. Laboratory diagnostics escherichiosis coli of poultry. *Reports of the Tajik Academy of Agricultural Sciences*. 2021; 2 (68): 50–54. EDN: HBOFRS. (in Russ.)

19. Lutful Kabir S. M. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *International Journal of Environmental Research and Public Health*. 2010; 7 (1): 89–114. DOI: 10.3390/ijerph7010089.

20. Schouler C., Schaeffer B., Brée A., Mora A., Dahbi G., Biet F., et al. Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes. *Journal of Clinical Microbiology*. 2012; 50 (5): 1673–1678. DOI: 10.1128/JCM.05057-11.

21. Sokolova E. D., Galtaeva A. M., Zamurei O. U., Didichenko O. V., Sokolova U. V., Muratova V. A., et al. Acute enteric infections polymerase chain reaction assay in pediatric practice: opportunities and challenges. *Russian Journal of Infection and Immunity.* 2016; 6 (3): 225–231. DOI: 10.15789/2220-7619-2016-3-225-231. (in Russ.)

22. Methodical Guidelines for avian disease diagnosis with polymerase chain reaction: handbook for technicians of veterinary laboratories. Vladimir: FGBI "ARRIAH"; 2015. 193 p. (in Russ.)

23. Tang Z., Tang N., Wang X., Ren H., Zhang C., Zou L., et al. Characterization of a lytic *Escherichia coli* phage CE1 and its potential use in therapy against avian pathogenic *Escherichia coli* infections. *Frontiers in Microbiology*. 2023; 14:1091442. DOI: 10.3389/fmicb.2023.1091442.

24. Novicova O. B., Terletskiy V. P., Ladanova M. A. Genotipirovanie metodom DRIM *Escherichia coli* s tsel'yu kontrolya epizooticheskoi situatsii v otnoshenii kolibakterioza v ptitsevodstve = DDSL genotyping of *Escherichia coli* for colibacillosis situation control in poultry industry. *Mechanisms of microorganisms adaptation to different habitat condition: Proceedings of All-Russian Scientific Conference with International Participation (Irkutsk, Baikal, Febriary 28 – March 6, 2022)*. Irkutsk: Irkutsk State University; 2022; 157–160. EDN: KCWBFR. (in Russ.)

25. Temmerman R., Ghanbari M., Antonissen G., Schatzmayr G., Duchateau L., Haesebrouck F., et al.

Dose-dependent impact of enrofloxacin on broiler chicken gut resistome is mitigated by synbiotic application. *Frontiers in Microbiology*. 2022; 13:869538. DOI: 10.3389/fmicb.2022.869538.

26. Lozica L., Morteza Gholi C. S., Kela A., Lošić I., Horvatek Tomić D., Gottstein Ž. Autogenous *Escherichia coli* vaccine application as an innovative antimicrobial therapy in poultry farming – a case report. *Vaccines*. 2022; 10 (9):1567. DOI: 10.3390/vaccines10091567.

27. Chodkowska K. A., Iwiński H., Wódz K., Nowak T., Różański H. *In vitro* assessment of antimicrobial activity of phytobiotics composition towards of avian pathogenic *Escherichia coli* (APEC) and other *E. coli* strains isolated from broiler chickens. *Antibiotics (Basel)*. 2022; 11 (12):1818. DOI: 10.3390/antibiotics11121818.

28. Abbas R. Z., Alsayeqh A. F., Aqib A. I. Role of bacteriophages for optimized health and production of poultry. *Animals (Basel)*. 2022; 12 (23):3378. DOI: 10.3390/ani12233378.

29. Lewies A., Du Plessis L. H., Wentzel J. F. Antimicrobial peptides: the Achilles' heel of antibiotic resistance? *Probiotics Antimicrob Proteins*. 2019; 11 (2): 370–381. DOI: 10.1007/s12602-018-9465-0.

30. Baltzer S. A., Brown M. H. Antimicrobial peptides: promising alternatives to conventional antibiotics. *Journal of Molecular Microbiology and Biotechnology*. 2011; 20 (4): 228–235. DOI: 10.1159/000331009.

31. Mahlapuu M., Håkansson J., Ringstad L., Björn C. Antimicrobial peptides: an emerging category of therapeutic agents. *Frontiers in Cellular and Infection Microbiology*. 2016; 6:194. DOI: 10.3389/fcimb.2016.00194.

32. Kathayat D., Closs G. Jr., Helmy Y. A., Lokesh D., Ranjit S., Rajashekara G. Peptides affecting the outer membrane lipid asymmetry system (MlaA-OmpC/F) reduce avian pathogenic *Escherichia coli* (APEC) colonization in chickens. *Applied and Environmental Microbiology*. 2021; 87 (17):e0056721. DOI: 10.1128/AEM.00567-21.

33. Khaitovich A. B., Mureiko E. A. Quorum sensing of microorganisms as a factor of pathogenicity. *Tavricheskiy Mediko-Biologicheskiy Vestnik*. 2018; 21 (1): 214–220. EDN: XSZOKL. (in Russ.)

34. Abaturov A. E., Kryuchko T. A. Inhibition of bacterial quorum sensing (general concept). *Zdorov'e rebenka*. 2019; 14 (1): 54–59. DOI: 10.22141/2224-0551.14.1.2019.157881. (in Russ.)

35. Peng L. Y., Yuan M., Wu Z. M., Song K., Zhang C. L., An Q., et al. Anti-bacterial activity of baicalin against APEC through inhibition of quorum sensing and inflammatory responses. *Scientific Reports*. 2019; 9 (1):4063. DOI: 10.1038/s41598-019-40684-6.

36. Sattar S., Bailie M., Yaqoob A., Khanum S., Fatima K., Altaf A. U. R. B., et al. Characterization of two novel lytic bacteriophages having lysis potential against MDR avian pathogenic *Escherichia coli* strains of zoonotic potential. *Scientific Reports*. 2023; 13 (1):10043. DOI: 10.1038/s41598-023-37176-z.

37. Yao L., Bao Y., Hu J., Zhang B., Wang Z., Wang X., et al. A lytic phage to control multidrug-resistant avian pathogenic *Escherichia coli* (APEC) infection. *Frontiers in Cellular and Infection Microbiology*. 2023; 13:253815. DOI: 10.3389/fcimb.2023.1253815.

38. Abdelrahman F., Rezk N., Fayez M. S., Abdelmoteleb M., Atteya R., Elhadidy M., El-Shibiny A. Isolation, characterization, and genomic analysis of three novel *E. coli* bacteriophages that effectively infect *E. coli* O18. *Microorganisms*. 2022; 10 (3):589. DOI: 10.3390/microorganisms10030589.

39. Tsonos J., Oosterik L. H., Tuntufye H. N., Klumpp J., Butaye P., De Greve H., et al. A cocktail of *in vitro* efficient phages is not a guarantee for *in vivo* therapeutic results against avian colibacillosis. *Veterinary Microbiology*. 2014; 171 (3–4): 470–479. DOI: 10.1016/j.vetmic.2013.10.021.

40. Sørensen P. E., Baig S., Stegger M., Ingmer H., Garmyn A., Butaye P. Spontaneous phage resistance in avian pathogenic *Escherichia coli*. *Frontiers in Microbiology*. 2021; 12:782757. DOI: 10.3389/fmicb.2021.782757.

41. Mak P. H. W., Rehman M. A., Kiarie E. G., Topp E., Diarra M. S. Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: a review. *Journal of Animal Science and Biotechnology*. 2022; 13 (1):148. DOI: 10.1186/s40104-022-00786-0.

42. Bessarabov B. F., Vashutin A. A., Voronin E. S., et al. Infectious diseases of animals. Moscow: KolosS; 2007. 671 p. (in Russ).

43. Schepetkina S. V. Therapeutic measures in avian diseases bacterial etiology with use of biocomplexes of probiotic microorganisms. *Farm Animals*. 2015; 2 (9): 78–83. EDN: UCCBCR. (in Russ.)

44. Li T., Castañeda C. D., Miotto J., McDaniel C., Kiess A. S., Zhang L. Effects of *in ovo* probiotic administration on the incidence of avian pathogenic *Escherichia coli* in broilers and an evaluation on its virulence and antimicrobial resistance properties. *Poultry Science*. 2021; 100 (3):100903. DOI: 10.1016/j.psj.2020.11.072.

45. Nicolas M., Faurie A., Girault M., Lavillatte S., Menanteau P., Chaumeil T., et al. *In ovo* administration of a phage cocktail partially prevents colibacillosis in chicks. *Poultry Science*. 2023; 102 (11):102967. DOI: 10.1016/j.psj.2023.102967.

46. Novikova O., Pavlova M., Kryukova V. Vaccine against colibacteriosis of birds and the prevetnive method of colibacillosis of early age chickens. *International Bulletin of Veterinary Medicine*. 2018; 4: 23–27. DOI: 10.17238/issn2072-2419.2018.4.23. (in Russ.)

47. Uotani Y., Kitahara R., Imai T., Tsutsumi N., Sasakawa C., Nagai S., Nagano T. Efficacy of an avian colibacillosis live vaccine for layer breeder in Japan. *Journal of Veterinary Medical Science*. 2017; 79 (7): 1215–1219. DOI: 10.1292/jvms.17-0189.

48. Nagano T., Kitahara R., Nagai S. An attenuated mutant of avian pathogenic *Escherichia coli* serovar O78: a possible live vaccine strain for prevention of avian colibacillosis. *Microbiology and Immunology*. 2012; 56 (9): 605–612. DOI: 10.1111/j.1348-0421.2012.00482.x.

49. Hu R., Li J., Zhao Y., Lin H., Liang L., Wang M., et al. Exploiting bacterial outer membrane vesicles as a cross-protective vaccine candidate against avian pathogenic *Escherichia coli* (APEC). *Microbial Cell Factories*. 2020; 19 (1):119. DOI: 10.1186/s12934-020-01372-7.

50. Hu R., Liu H., Wang M., Li J., Lin H., Liang M., et al. An OMV-based nanovaccine confers safety and protection against pathogenic *Escherichia coli* via both humoral and predominantly Th1 immune responses in poultry. *Nanomaterials (Basel)*. 2020; 10 (11):2293. DOI: 10.3390/nano10112293.

51. Soleymani S., Tavassoli A., Hashemi Tabar G., Kalidari G. A., Dehghani H. Design, development, and evaluation of the efficacy of a nucleic acid-free version

of a bacterial ghost candidate vaccine against avian pathogenic *E. coli* (APEC) O78:K80 serotype. *Veterinary Research*. 2020; 51 (1):144. DOI: 10.1186/s13567-020-00867-w.

52. Ebrahimi-Nik H., Bassami M. R., Mohri M., Rad M., Khan M. I. Bacterial ghost of avian pathogenic *E. coli* (APEC) serotype O78:K80 as a homologous vaccine against avian colibacillosis. *PLoS One*. 2018; 13 (3):e0194888. DOI: 10.1371/journal.pone.0194888.

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Alina O. Gerasimova, Postgraduate Student, Department of Microbiology, All-Russian Research Veterinary Institute of Poultry Science – Branch of the Federal Scientific Center "All-Russian Research and Technological Poultry Institute" of Russian Academy of Sciences, Saint Petersburg, Lomonosov, Russia; *https://orcid.org/0009-0003-0503-113X, e-mail: gerasimova.alina.20@yandex.ru.*

Oksana B. Novikova, Dr. Sci. (Veterinary Medicine), Professor, Department of Poultry and Small Animal Husbandry, Saint-Petersburg State Agrarian University; Associate Professor, Department of Epizootology, Saint-Petersburg State University of Veterinary Medicine, Saint Petersburg, Russia; *https://orcid.org/0000-0003-0046-625X, e-mail: ksuvet@mail.ru.*

Alesya A. Savicheva, Postgraduate Student, Junior Researcher, Department of Microbiology, All-Russian Research Veterinary Institute of Poultry Science – Branch of the Federal Scientific Center "All-Russian Research and Technological Poultry Institute" of Russian Academy of Sciences, Saint Petersburg, Lomonosov, Russia; *https://orcid.org/0009-0007-9125-8005*.

Герасимова Алина Олеговна, аспирант отдела микробиологии ВНИВИП – филиала ФНЦ «ВНИТИП» РАН, г. Санкт-Петербург, г. Ломоносов, Россия; *https://orcid.org/0009-0003-0503-113X, e-mail: gerasimova.alina.20@yandex.ru.*

Новикова Оксана Борисовна, д-р вет. наук, профессор, кафедра птицеводства и мелкого животноводства ФГБОУ ВО СПбГАУ; доцент кафедры эпизоотологии ФГБОУ ВО СПбГУВМ, г. Санкт-Петербург, Россия; *https://orcid.org/0000-0003-0046-625X, e-mail: ksuvet@mail.ru.*

Савичева Алеся Анисовна, аспирант, младший научный сотрудник отдела микробиологии ВНИВИП – филиала ФНЦ «ВНИТИП» РАН, г. Санкт-Петербург, г. Ломоносов, Россия; *https://orcid.org/0009-0007-9125-8005.*