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Biofilms of pathogenic bacteria in pig industry

O.V. Kolchyk*, A.I. Buzun[1], A.P. Paliy[1], L.I. Nalivayko[2], O.M. Chekan[3], N.P. Grebenik[3], I.V. Bondarenko[3], H.I. Rebenko[3], V.Yu. Kushnir[4], N.I. Todorov[4]

[1]*National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", 83 Pushkinska St, Kharkiv, 61023, Ukraine*

[2]*Luhansk National Agrarian University, 68 Slobojanska St, Starobilsk, Luhansk region, 92703, Ukraine*

[3]*Sumy National Agrarian University, 160 G. Kondratieva St, Sumy, 40021, Ukraine*

[4]*Odessa state agrarian university, 13 Panteleimonovskaya St, Odessa, 65012, Ukraine*

**Corresponding author E-mail: kolchyk-elena@ukr.net*

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In the industrial pig farm for 26 thousand heads, the analysis of the influence of a forage factor on bacteria carriers of a uterine pig population in connection with mass morbidity of dairy piglets on anaerobic enterotoxemia is carried out. *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp., *Candida albicans, Aspergillus niger* which are able to form biofilms, were isolated from five samples of "SK-1" compound feed for pregnant sows and from the blood of animals (n=20) fed with this compound feed. The structural basis of the most stable biofilms *in vitro* were the aerobic fungi *Aspergillus niger* and *Candida albicans*. Biofilm-forming variants of these bacteria showed multidrug resistance to 30 antimicrobial drugs (synthetic penicillins, cephalosporins, fluoroquinolones, aminoglycosides, tetracyclines, combination drugs). Isolates of associative microflora isolated from the blood of sows were pathogenic for 30% of laboratory mice. It was found that probiotic agent No1 (composition based on Bischofite with probiotics) showed the universal bactericidal activity against the bacteria *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp.

Key words: biofilms, compound feed, microbial contamination of feed, multiresistance, sows.

Introduction

The development of the pig industry on the basis of concentration, specialization and agro-industrial integration with the introduction of industrial technologies is a natural process for all developed countries. The profitability of the industry directly depends on the epizootic well-being and productive characteristics of the livestock. Low productivity, morbidity and death of farm animals are often associated with contamination of raw materials and feed by pathogenic microorganisms (salmonella, pasteurellosis, listeria, hemophilia, enteropathogenic types of *Escherichia coli*, clostridia), which pose a threat to the health of not only animals but also humans (Crump et al., 2002; Bintsis, 2018).

Despite the successes of recent years in optimizing the feeding of farm animals, the role of microbial contamination of feed in reducing production efficiency requires additional attention and careful and comprehensive study (Maciorowski et al., 2007; Mahami et al., 2019).

Quantitative and qualitative composition of the microflora of pig feed is very diverse and is formed under the influence of many factors (Pereyra et al., 2010). First of all, if the feed is a product of processing of vegetable raw materials, epiphytic microflora of raw materials is largely transferred to the finished product, as well as microorganisms on the processing equipment, in storage shops, warehouses and so on. A special group consists of phytopathogenic microorganisms, including mold fungi (Donlan & Costerton, 2002; Flemming & Wingender, 2010). They affect plants in the field and can produce diverse in chemical nature mycotoxins (Shirokikh et al., 2017).

In recent years, laboratory studies of microbial contamination of feed have shown that a significant amount of animal feed did not meet the requirements of regulatory documentation by the degree of contamination by pathogenic microflora, microscopic fungi, including live saprophytic, pathogenic and opportunistic microorganisms (Pereira et al., 2019). Bacterial associations isolated from feed are characterized by a high level of antibiotic resistance and have the ability to withstand adverse environmental factors (Abdullahi et al., 2016), such as temperature changes, acidity changes, the presence of bactericidal substances and even disinfectants. Their presence in feed is directly related to chronic diseases of animals of productive species, so sanitary requirements for animal feed should be as strict as for food (Tamang et al., 2016; Ali et al., 2018; Machado-Moreira

et al., 2019). The association of microorganisms is represented by biofilms, which in turn are more dangerous in terms of epidemiology and epizootology (Lasa et al., 2005; Schillaci & Vitale, 2012). Bacterial biofilms are structured communities of bacterial cells organized into a self-made polymer matrix attached to the surface.
Within the biofilm, bacteria are able to interact with each other through intracellular communication, and thus quickly adapt to changing environmental conditions (Clutterbuck et al., 2007). Bacteria in biofilms can resist the immune responses of the macroorganism and are much less susceptible to antibiotics and disinfectants compared to their planktonic counterparts (Jacques et al., 2010). Biofilm formation is determined not only by the nature of the attachment surface, but also by the characteristics of the bacterial cell and environmental factors (Van Houdt & Michiels, 2010).
Over the last decade, veterinary medicine has conducted research to determine the role of biofilm infections in various animal pathologies (Gardner et al., 2011), but the role of biofilm-forming properties of feed contaminants is insufficiently studied, methodological approaches to assess their harmfulness, their role in animal pathology (first of all with chronic infections) are not developed. Due attention is not paid to the development of methods for the control of microbial biofilms in feed, in particular the creation of a methodological basis for their destruction (Abdullahi et al., 2016).
This is especially true for industrial livestock of precocious species, such as pigs and poultry. Therefore, we joined in solving the problems of control of film-forming bacteria in feed to prevent chronic infectious diseases in pig breeding. The aim of our work was to study the species composition and properties of biofilm-forming microorganisms in feed and to develop practical approaches to their disposal.

Materials and methods

Microbiological studies of feed were performed in the laboratory for swine diseases of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) using modern methods (Quality Control for Feed Safety at the International Level International Feed Safety Alliance (IFSA), 2018). The ability of microorganism isolates to form biofilms was studied by current methods (O'Toole & Kolter, 1998). Determination of the sensitivity of microorganisms to antimicrobial drugs was performed by the disco-diffusion method (Guidelines for Susceptibility Testing of Microorganisms to Antibacterial agents, 2018).
Sampling and delivery of compound feeds for pigs ("SK-3", "SK-5", "SK-6", "SK-31" for rearing and "SK-1" for pregnant sows) in the amount of 36 samples was carried out from a standard pig farms of Dnipropetrovsk region for 26 thousand heads, which is unfavorable for infectious pneumoenteritis (suckling pigs suffer from anaerobic enterotoxemia, animals for rearing and fattening have an associated course of pasteurellosis and hemophilosis).
Compound feeds for animals are made from the farm's own raw materials and are stored for no more than 7 days. Blood was collected from 20 animals fed with contaminated feed for microbiological testing for bacterial agents (SOP: Blood Collection in Swine/Virginia State University, 2017). Identification of isolated field isolates of bacteria was performed by cultural-morphological and biochemical properties (Bergey's Manual of Determinative Bacteriology, 1997).
Isolation, cultivation and study of cultural, morphological properties of feed microorganisms were carried out on nutrient media: pepted meat broth (PMB) with a pH of 7.2-7.4; Hottinger broth; Martin's medium; 2.5% PMB with the addition of 2.0% glucose or selective Fraser supplement (to isolate listeria); meat-peptone agar (MPA) with a pH of 7.2-7.4; Endo agar; modified Kitt-Tarozzi medium; MPC-4 medium; Blaurok's medium; agar Saburo; Olkenytsky's medium; Simons citrate; acetate agar; PALKAM agar (for identification of listeria); Mueller-Hinton agar for disco-diffusion test (DDT).
The pathogenicity of isolated field isolates of bacteria was tested on white mice weighing 16-18 g by intra-abdominal infection at a dose of 0.5 billion microbial bodies, in compliance with the Law of Ukraine "On protection of animals from cruel treatment" (No1759-VI of 15.12.2009) and norms of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes" (Strasbourg, France, 1985). Ethyl ether was used for anesthesia.
For the destruction of biofilms in forages we used drugs produced by LLC 'Sirion' (Dnipro):
- Prebiotic agent – a composition based on Bischofite (chloride-magnesium complex);
- Probiotic agent No1 – composition based on Bischofite with probiotics;
- Probiotic No 2 – concentrate of probiotic bacteria and fungi for feed disinfection.
The results were taken into account for the presence of bacterial growth and their number in colony-forming units (CFU) compared to the control. The number of mesophilic aerobic and facultative-anaerobic microorganisms (total microbial number) (MAFAnM) was also determined.

Results and discussion

According to the results of bacteriological studies it was found that the total contamination of 36 samples of 5 types of feed (SK-1, SK-3, SK-5, SK-6, and SK-31) is within acceptable limits and corresponds to veterinary and sanitary quality (Table 1).
However, it should be noted that all 5 studied samples of feed "SK-1" for pregnant sows contained an association of pathogenic microorganisms, namely *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria sicca, Candida albicans, Aspergillus niger* in the form of continuous growth on nutrient media.
Actinobacillus pleuropneumonia microorganisms were cultured at 37.0±0.5 °C for 24 hours on MPA with the addition of 10% yeast extract. The culture of *A. pleuropneumonia* had the form of small, convex, round, with smooth edges and mucous consistency colonies with a diameter of 0.5-1.5 mm. When culturing actinobacilli in PMB with yeast extract, bacterial growth was observed in the form of uniform turbidity of the medium with the formation of a white precipitate at the bottom, which

was easily broken by shaking; on 5% blood agar with the addition of 10% yeast extract the growth of small translucent colonies with a diameter of 0.2-1.5 mm with smooth edges surrounded by a transparent zone of β-hemolysis was observed. According to the biochemical properties of the bacterium *A. pleuropneumonia* catabolized D-glucose and fructose with the formation of acid, were positive for β-galactosidase and negative for methyl red and for the formation of indole. During microscopy of smears in the field of view we noted gram-negative, small short rods, located singly.

Table 1. Microbiological content of pig feed (n=36)

Polluting substance	Maximum allowable content, CFU, norm	SK-3	SK-5	SK-6	SK-31	SK-1
Total bacterial contamination (MAFAnM), CFU in 1 g at 37.0±0.5 °C	not more 5×10^5	3×10^5	4×10^5	2×10^5	4×10^5	34×10^5
Enterobacteria	not more 300	–	–	–	–	–
Salmonella in 50 g	are not allowed	–	–	–	–	–
Pathogenic strains *E. coli*	are not allowed	–	–	–	–	–
Sulfite-reducing clostridia in 1 g	are not allowed	–	–	–	–	–
Pathogenic yersinia in 50 g	are not allowed	–	–	–	–	–
Coagulase-positive *S. aureus*	are not allowed	–	–	–	–	–

Isolates of *Pasteurella multocida* during cultivation on the PBM after 24 h at a temperature of 37.0±0.5 °C formed a uniform turbidity of the medium. At the bottom of the tube a precipitate of a mucous nature was formed, which when shaken rose in the form of a tape. The obtained isolated colonies were subcultured on a dense nutrient medium (MPA) to obtain a pure culture, and the growth of small grayish colonies of mucous consistency was observed. On Hotinger's agar 24 h after cultivation, the appearance of round, convex, with a smooth, moist surface, smooth edges translucent colonies with a diameter of 1-3 mm was noted; on blood agar with the addition of 5% sheep blood hemolysis zone was absent. According to the biochemical properties bacterium *P. multocida* catabolized D-glucose and fructose with the formation of acid, were oxidase- and catalase-positive, reduced nitrate to nitrite, samples with methyl red and for the formation of acetoin (Voges-Proskauer), as well as for lysinecarboxylase, arginine dihydrolase and gelatinase were negative. Microscopy of smears from colonies of cultures revealed gram-negative cells, coccoid-ovoid and ovoid forms with a pronounced bipolarity. There was no mobility.

Cultures of *Neisseria sicca* after 24 h of cultivation at a temperature of 37.0±0.5 °C on the MPA formed small transparent colonies with a blue tinge; turbidity was registered in the PMB; small transparent colonies resembling dewdrops appeared on the serum agar. Colonies of diplococci cultures on blood agar were small, round, transparent, surrounded by an α-zone of hemolysis (green zone). According to the biochemical properties of the bacterium *Neisseria sicca* oxidase- and catalog-positive, formed carbonic anhydrase, reduced nitrite. During microscopy, gram-negative cocci in the form of pairs 0.6-0.8 μm in size were observed in the field of view of the microscope.

Isolates of *Clostridium perfringens* on Kitt-Tarozzi medium after 24 h of cultivation at a temperature of 37.0±0.5°C formed turbidity with gas formation. Round smooth grayish colonies were recorded on 5% blood agar, which gradually turned green and were surrounded by a β-zone of hemolysis. Blackening and rupture of agar were recorded on Wilson-Blair medium after 1 h of cultivation. Clostridia fermented lactose, glucose, sucrose, maltose to form acid and gas, slowly diluted gelatin, curdled litmus milk with the formation of a brick-colored clot and complete enlightenment of whey. Reduced nitrates to nitrites, indole was not formed. Polymorphic rod-shaped gram-positive bacteria were observed in the field of view of the microscope. Spores were oval, centrally or subterminally located, immobile.

The *Candida albicans* culture on wort agar at 22.0±0.5 °C after 3 days formed round, shiny, flat or convex, colonies with smooth edges. Colonies on glucose-peptone medium were white-cream with a dull sheen, smooth, moist. On microscopy, *Candida albicans* looked like rounded or slightly elongated budding cells. To determine the species of *Candida albicans*, a test for the formation of germinal (embryonic tubes) was performed. A colony of a 24-hour yeast culture was added to a test tube with 0.5 ml of sterile sheep serum and kept for 3 hours at 37.0±0.5 °C. After 3 hours of incubation of the samples in a thermostat at 37.0±0.5 °C, the contents of the tube were placed on a glass slide and examined under a microscope. In the field of view of the microscope, a characteristic absence of cell narrowing at the base of the germ tubes was observed, where it is formed from the mother cell, which is characteristic of *Candida albicans* (Saigal et al., 2011). These microorganisms fermented glucose, maltose, sucrose, galactose, did not ferment lactose and raffinose.

Microscopic fungi *Aspergillus niger* have been identified by morphological and biochemical properties (McClenny, 2005). Thus, *Aspergillus niger* on wort agar at a temperature of 37.0±0.5°C after 3 days formed a branched, multinucleate mycelium of black color. On Chapek's agar, *A. niger* agar colonies under microscopy had the appearance of filamentous fungi, the hyphae of which resembled the structure of a plant and formed smooth and colorless conidiophores with conidial heads (beads) with black or dark brown spores. On glucose agar, *A. niger* isolates isolated citric acid and according to the test for the decomposition of starch to glucose contained the enzyme glucoamylase (Geiser et al, 2007).

In the study of blood of sows fed with this feed, 20 of the 60 samples contained the same bacterial associations as the feed samples.

Isolated from feed and blood isolates of bacteria *Actinobacillus leuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria sicca* had the ability to form biofilms *in vitro*, ie were biofilm-forming. For the ability to form biofilms, the

association of bacteria was cultured in 96-well polystyrene plates (O'Toole, 1998). After culturing, planktonic cells were removed from the wells by washing with phosphate-buffered saline. A 0.1% solution of gentian violet was added to the wells for staining. After incubation for some time, the dye was decanted, washed with distilled water and extracted with ethanol. Optical density was measured on a microplate spectrophotometer, evaluating by its ability to biofilm formation. The optical density of the associated microflora reached > 4 optical units, which is the highest level of density (Stepanovic et al., 2007).

Biofilm-forming isolates of bacteria *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria sicca, Candida albicans, Aspergillus niger* from feed and blood showed multidrug resistance, up to 30 antimicrobials (synthetic penicillins, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines, combined drugs); only the combined drug bromodox (bromhexine + doxycycline) was clinically effective (Table 2).

Table 2. Sensitivity of biofilm-forming bacteria to antimicrobial drugs

Antimicrobial drug	Content in disk, mcg	Diameter of zones of growth inhibition, mm		
		Resistant (R)	Moderately sensitive (M)	Sensitive (S)
Synthetic penicillins				
Amoxiclav	10	≤13	–	–
Amoxicillin	20	≤13	–	–
Oxacillin	1	≤11	–	–
Ampicillin	10	≤11	–	–
		Cephalosporins		
Ceftiofur	30	≤14	–	–
Cefuroxime	30	≤14	–	–
Cefoxitin	30	≤14	–	–
Cefipim	30	≤14	–	–
		Tetracyclines		
Doxycycline	30	≤12	–	–
Oxytetracycline	30	≤14	–	–
Remacycline	30	≤14	–	–
		Aminoglycosides		
Kanamycin	30	≤13	–	–
Gentamicin	10	≤12	–	–
Spectinomycin	30	≤12	–	–
Neomycin	30	≤12	–	–
		Fluoroquinolones		
Ofloxacin	5	≤12	–	–
Marbofloxacin	5	≤14	–	–
Gatifloxacin	5	≤14	–	–
Levofloxacin	5	≤13	–	–
		Combined drugs		
Gentamicin sulfate + ofloxacin	30	≤12	–	–
Colistin sulfate + doxycycline	30	≤10	–	–
Colistin sulfate + thiamulin	30	≤8	–	–
Colistin sulfate + enrofloxacin	30	≤8	–	–
Tilmicosin + bromhexine	30	≤10	–	–
Bromhexine + doxycycline	30	≤7	–	≥16
Trimethoprim + sulfadimesine	30	≤6	–	–
Colistin sulfate + levofloxacin	30	≤7	–	–
Tylosin + doxycycline	30	≤10	–	–
Colistin sulfate + amoxicillin	30	≤12	–	–
Lincomycin + Spectinomycin	30	≤12	–	–

Antibiotic resistance of microorganisms in biofilms is due to the ability of bacteria to accumulate in the matrix extracellular enzymes that destroy antimicrobial substances (Stacy et al., 2014).

No deaths were recorded during infection of white mice with biofilm-forming isolates of *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp., isolated from compound feed. While biofilm-forming isolates of the same bacteria infected laboratory animals, up to 30% of mice died: that is, variants of biofilm-forming "feed bacteria" adapted to pigs showed weakly virulent properties. However, this indicates that biofilms that enter the body of animals with food undergo passage, acquire virulence properties and, as a consequence, cause complications with a chronic course and are antibiotic-resistant.

Since the grain for the studied batches of feed came from forage lands fertilized with manure from the examined pig complex, it can be assumed with high probability that the biofilms of bacteria *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp. can successfully overcome the modern technological chain "from the field - to the feeder" in the composition of manure, which fertilizes the forage lands. This may be the basis for the formation and rooting of stationary foci of infection. The next stage of research was to determine the antagonistic properties of prebiotic and probiotic drugs, ie their suitability for the destruction of biofilms of pathogenic bacteria in feed (Fig. 1).

a b c D

Fig. 1. Biofilms of microorganisms: **a**) biofilms of *A. pleuropneumonia, P. multocida, Neisseria* spp., *Candida albicans*, in compound feed before treatment with drugs; **b**) colonies of *A. pleuropneumonia* after treatment with the drug 'Prebiotic agent'; **c**) continuous growth of colonies of *P. multocida, Neisseria* spp., *Candida albicans* after treatment with the drug 'Probiotic agent No 2'; **d**) colonies of *P. multocida* after treatment with the drug 'Probiotic agent No 1'.

After treatment of biofilms of microorganisms, the Prebiotic agent (Fig. 1b) showed bactericidal activity against *P. multocida, Neisseria* spp., *Candida albicans*, but did not act on *A. pleuropneumonia*. Probiotic agent No 2 (concentrate of probiotic bacteria and fungi for food disinfection) on the contrary destroyed *A. pleuropneumonia*, and bacteria *P.multocida, Neisseria* spp.; *Candida albicans* showed resistance to its action (Fig. 1c). At that time, Probiotic agent No 1 (Bischofite-based composition with probiotics) destroyed the bacterial biofilm, but did not act on *P. multocida* (Fig. 1d). Thus, it is proved that each of the presented drugs acts on a certain type of bacteria, but they all destroy the fungi *Candida albicans*, which create the framework and conditions for the creation of biofilms with pasteurella, neisseria and actinobacillus.

Microorganisms in biofilms show an altered phenotype in terms of growth rate and gene transcription compared to freely existing forms of the same organisms. Subsequent studies have shown that the phenotype of the biofilm can be described using genes of expressed cells (Donlan & Costerton, 2002). Biofilms are clinically important in both human and veterinary medicine, and have the ability to form on both objects and living tissue (Paterson, 2017).

The ability to form biofilms is now considered a universal attribute of microorganisms (Jacques et al., 2010). Typically, biofilms are found in chronic diseases that oppose the host's immune response and antibiotic treatment (Hall-Stoodley & Stoodley,

2009). Thus, biofilm formation can be considered an important virulence factor (Landini et al., 2010). Bacteria in biofilms are able to protect against stress, including resistance to antibiotics, disinfectants and humoral and cellular parts of the animal's immune system (Bujold & MacInnes, 2015).

The high ability of *Pseudomonas aeruginosa* to form biofilms has been proven, but no significant differences were found between isolates from animals and from humans (Milivojevic et al., 2018). One of the most important food pathogens is *Campylobacter jejuni*, which also has the ability to form a biofilm on stainless steel, glass or polyvinyl chloride (Moe et al., 2010). Other studies suggest that *A. pleuropneumoniae* has the ability to form biofilms under appropriate growth conditions, and the transition from a biofilm-positive to a biofilm-negative phenotype is reversible (Labrie et al., 2010).

An important feature of bacterial biofilms is high resistance to antibacterial drugs. Antibiotic resistance of such a biofilm is due to the fact that it includes mechanisms (biofilm matrix) that prevent the penetration of antibiotics into the deep layers of the biofilm and disrupt direct contact with bacterial cells and usually show resistance to many antibiotics from different groups (Ruzicka et al., 2007; Stacy et al., 2014). Biofilm antibiotic resistance is likely to manifest as a combination of innate and induced mechanisms (Anderson & O'Toole, 2008). The problem of antibiotic resistance of pathogenic microorganisms is acute in other areas of animal husbandry (Hadzevych et al., 2019; Kasianenko et al., 2020). The mechanisms of resistance of biofilm bacteria to antibacterial agents allow them to remain viable at concentrations of antibiotics tens and hundreds of times higher than therapeutic doses that inhibit planktonic forms (Mah, 2012; Sager et al., 2015; Ramírez-Castillo et al., 2018).

Focused study of the role of biofilm-forming microorganisms in epizootology and infectious pathology of animals began only a few years ago, but it is now clear the significant difference in the properties of infectious agents between their biofilm and planktonic life forms. The above results indicate a high risk for pig farming of the presence of even trace amounts (10-12 × 10^3 CFU/g) of avirulent variants of pathogenic bacterial species in feed. They also point to the danger of insufficiently decontaminated manure, which together with microbial biofilms is traditionally exported to forage lands, as a significant risk factor for the formation of foci of enzootic diseases in pig farming. Similar problems have been identified in fish farming, which is associated with the release of a number of pathogenic microorganisms from the ponds (Nazarenko et al., 2020).

Involvement of microbial biofilms in clinical veterinary medicine provides a change in approaches to the treatment of diseases associated with biofilms, as traditional antibiotic therapy does not solve this problem. The treatment of infections associated with biofilms is very complex. This primarily applies to chronic infections. As our results show, we can increase the effectiveness of their treatment with the use of the latest probiotics, which combine high antagonistic activity and resistance to adverse environmental factors of spore probiotic bacteria with bactericidal and mycocidal properties of antiseptics and detergents.

In the biofilms, bacteria acquire qualitatively new properties compared to microorganisms in planktonic form (Romling & Balsalobre, 2012; Tremblay et al., 2013; Sanchez et al., 2013). To increase the effectiveness of counteracting biofilms of pathogenic microorganisms in feed and animals, it is necessary to determine not only the bactericidal properties of antimicrobials, but also their ability to inhibit bacterial adhesion, penetrate biofilms, inhibit their formation or contribute to extracellular matrix disorganization (Schlegelová et al., 2008; Romanko et al., 2016; Roy et al., 2018). In addition, the effectiveness of some plant components as antimicrobial compounds against bacterial biofilms has been proven (Dürig et al., 2010). Without adequate and effective diagnostic and treatment protocols for biofilm infections in animals, their impact on animal health will remain a serious problem (Richards & Melander, 2009; Gardner et al., 2011).

Along with bacterial contamination of livestock facilities, the current problem is the spread of exogenous forms of animal helminths in the environment (Paliy et al., 2018b, 2019), as well as a number of ectoparasites (Paliy et al., 2018a, 2018c). Only a comprehensive approach to the planning and organization of anti-epizootic measures will increase the culture of the livestock industry and will allow to obtain quality and safe products (Zavgorodniy et al., 2013; Paliy et al., 2020). Based on this, the immediate task for us is to study the effectiveness of the latest probiotics for disinfection of the feed chain in pig breeding from pathogenic microorganisms.

Conclusions

According to the results of the conducted researches it was established that 5 samples of compound feed for pregnant sows contained the association of biofilm-forming variants of microorganisms *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp*., Candida albicans, Aspergillus niger.* The blood of sows fed with microbially contaminated feed "SK-1" contained the same bacterial associations as feed samples.

Biofilm-forming variants of *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp., *Candida albicans, Aspergillus niger* showed multidrug resistance to 30 antimicrobial drugs (synthetic penicillins, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines, combined drugs). It was found that 30% of isolates of microorganisms isolated from the blood of sows, in contrast to isolates of the same species of bacteria induced from feed "SK-1", were pathogenic for laboratory mice.

Complex 'Probiotic agent № 1' (composition based on Bischofite with probiotics) completely destroys the microbial biofilm in the composition of *Actinobacillus pleuropneumonia, Pasteurella multocida, Clostridium perfringens, Neisseria* spp., *Candida albicans, Aspergillus niger,* however, it does not have a bactericidal effect on the planktonic form of *P. multocida*.

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