EFFECTIVENESS OF ECOLOGICALLY SAFE DISINFECTANTS AGAINST PSEUDOMONAS AERUGINOSA AND THE POULTRY MAIN BACTERIOSIS PATHOGENS

Andriy Berezovskyi, Tetiana Fotina, Yevheniia Vashchyk, Dmytro Berezhnyi, Dmytro Morozenko

The aim: study of the effectiveness of environmentally safe disinfectants against P. aeruginosa and pathogens of the main bacteriosis of poultry at test facilities.

Materials and methods. To study the antimicrobial action of the investigated disinfectants against a mixture of epizootic cultures of E. coli, P. aeruginosa, S. aureus, S. typhimurium, isolated from poultry, bacteriological studies were carried out on test objects: galvanized iron, wooden bars (painted and unpainted), red brick, cutouts from plaster, size 10×10 cm.

Results. The working solutions of the new disinfectant "Dezsan" were studied in comparison with the control agents: "Virocid" and "Bi-dez" at a concentration of 0.01; 0.1; 0.25; 0.5; 1; 1.5 % in relation to suspension cultures of E. coli, P. aeruginosa, S. aureus, S. typhimurium. In this case, it was established, that the "Dezsan" agent showed an antimicrobial effect on rough test objects after exposure for 3 hours at a concentration of 0.1 %, and at a concentration of 0.25 % - for 1 hour. On smooth surfaces, the agent neutralized bacterial cultures at a concentration of 0.1 % after exposure for 1 hour. The preparation "Bi-dez" at a concentration of 0.25 % was effective on smooth surfaces after exposure for 1 hour, on rough surfaces (brick, plaster) - at a concentration of 0.5 % after exposure for 3 hours or more. The working solution of 1 % concentration neutralized bacterial cultures on all types of surfaces after exposure for 1 hour or more. "Virocid" agent after exposure for 1 hour neutralized bacterial cultures on smooth surfaces in concentrations of 0.25 % and higher; on rough surfaces, the growth of cultures was not detected when using a 0.5 % solution.

Conclusions. Environmentally safe disinfectants "Dezsan" and "Shumerske sryblo" compared to the control ones ("Bi Dez" and "Virotsid") show an active antimicrobial effect at a concentration of 0.25 % and 3 %, respectively, against the suspension of epizootic cultures of E. coli, P. aeruginosa, S. aureus and S. typhimurium on different types of production surfaces, which justifies the feasibility of their use based on the principle of rotation of disinfectants for the prevention of bacterial pseudomonosis of poultry

Keywords: poultry, pseudomonosis, E. coli, P. aeruginosa, S. aureus, S. typhimurium, effectiveness, prevention

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1. Introduction

Poultry farming, as a branch of animal husbandry, is progressive and constantly improving. This especially applies to the prevention of bacterial diseases [1, 2]. It differs from other branches of animal husbandry in its high reproduction rate and prematurity, which makes it the main source of providing the population with proteins of animal origin [3].

The use of modern technologies allows in broiler production to reduce the period of poultry fattening to 35 days, to ensure an average daily gain of more than 50 grams for feed conversion of 1.75 kg, and to obtain more than 230 kg of broiler meat in slaughter weight per laying hen of the parent flock; in egg production, more than 340 eggs can be obtained for an average annual laying hen with feed conversion of 1.17 kg [4]. However, in conditions of intensification of production, the effect of factors, contributing to the disruption of normal microflora in farm poultry, is noted. Non-observance of veterinary and sanitary norms and zoohygiene requirements causes a violation of the balance between normal and conditionally pathogenic microflora of the gastrointestinal tract, which, against the background of constant stress and a decrease in the natural resistance of the body of birds, leads to an increase in the pathogenic and virulent properties of microorganisms and causes the development of an infectious disease [5].

The aim of the research study of the effectiveness of environmentally safe modern disinfectants against P. aeruginosa and pathogens of the main bacteriosis of poultry at test facilities.
2. Materials and methods
To study the antimicrobial action of the investigated disinfectants against a mixture of epizootic cultures of E. coli, P. aeruginosa, S. aureus, S. typhimurium, isolated from pat material from poultry, bacteriological studies were carried out on test objects in accordance with the methodological recommendations "Use of the latest means and methods of rehabilitation of poultry facilities and control of their effectiveness", 2007. The test objects were: galvanized iron, wooden bars (painted and unpainted), red brick and plaster cutouts, measuring 10×10 cm. Before applying the cultures, the test objects were subjected to heat treatment for the purpose of sanitation. The research was conducted in a bacteriological box. Using a sterile pipette, a mixture of 1 billion suspended microbial cells of bacterial cultures of E. coli, P. aeruginosa, S. aureus, S. typhimurium in an isotonic solution was applied to test objects in enameled cuvettes. An hour later, using a sprayer, aerosols of disinfectant solutions were sprayed at the rate of 10 cm³ per 10 cm² for exposure from 1 hour up to 1 day Working solutions of disinfectants "Dezsan", "Virocid" and "Bi-dez" at a concentration of 0.01 were studied: 0.1; 0.25; 0.5; 1; 1.5 %. Control test objects were irrigated with sterile distilled water.

The composition of the "Bi-dez" product: 100 cm³ of the drug contains active substances (g): polyhexamethylenebiguanide hydrochloride – 6.5; dodecylpropylene triamine – 6.5. Excipients: glutamic acid, cocoamidopropyl betaine, demineralized water (NVF BROVAFARMA LLC, Ukraine).

The composition of "Dezsan": 100 cm³ of the drug contains active substances: alklyldimethylbenzylammonium chloride – 4.8 %, octyldodecylmethylammonium chloride – 3.6 %, dioctyldimethylammonium chloride – 1.44 %, dodecylmethylammonium chloride – 2.16 %, glutaraldehyde – 10 % (BROVAFARMA Ltd., Ukraine).

"Virocid" contains a composition of two quaternary ammonium compounds: alklyldimethylbenzylammonium chloride – 17.06 %, dodecylmethylammonium chloride – 7.8 %, glutaraldehyde – 10.7 %, isopropanol – 14.6 %, turpentine derivative – 2.0 % ("CID LINES NV/SA", Belgium).

To determine the quality of disinfection with sterile cotton swabs that were previously immersed in tubes with MPB, washings were taken from the surfaces of the test objects and again placed in tubes with MPB, which were incubated in a thermostat at a temperature of +38 °C, followed by recording the growth of the culture after 12, 24 and 48 hours. The presence of the development of signs of culture growth (turbidity, change in the color of MPB, the formation of a film on the surface and sediment at the bottom of the test tube) indicated the absence of antimicrobial action of the studied disinfectant.

3. Research results
According to the results of our monitoring bacteriological studies of pathological material and washings from the production surfaces of poultry premises, on average, in all farms of different technological direction, escherichia prevailed – 37.58 % and Pseudomonas aeruginosa – 22.98 %, coccus microflora was detected in 20.23 % of cases. The number of Proteus, Campylobacter, Enterobacter, Citrobacter, Klebsiella, Yersinia, and Clostridium cultures was 19.21 %. Thus, the frequency of isolation of P. aeruginosa and E. coli was 3 times higher than the cases of coccal microflora isolation and 3.15 times higher than the frequency of other causative agents of poultry bacteriosis.

Our results and the data of other researchers regarding the isolation of bacterial flora from poultry farms differed somewhat, which can be explained by climatic features, different methods and focus of research. A higher percentage of P. aeruginosa isolation confirms the effectiveness of our proposed medium, which was used to isolate the pathogen from pat material and facilities of poultry farms. Thus, according to the results of studies by Stegnii B.T., Gliebova K.V., Petrenchuk E.P. et al. [6], the percentage of productive poultry, affected by salmonella, is 7.7 % of the total number of poultry examined. The share of pathogenic E. coli cultures accounts for 14.7 % of the number of isolated pathogens. The frequency of isolation of Enterobacter, Citrobacter, and Proteus cultures is 3.9 %, 15.6 %, and 8.2 %, respectively. The percentage of poultry, infected with representatives of the Staphylococcus, Pseudomonas, Neisseria and Ornithobacterium families, is not significant [7].

Disinfection measures, aimed at destroying pathogens in the environment and preventing their penetration into the bird's body, are an integral part of the effective fight against bacterial infections. Effectively carried out disinfection measures in premises for growing poultry, hatcheries allow to prevent the spread of pathogens and the occurrence of epizootic outbreaks. Long-term use of disinfectants of the same chemical group causes the development of microflora resistance to antimicrobial and disinfectant drugs. There is a need to constantly search for new effective antimicrobial substances. The environmental aspect is extremely important when choosing disinfectants. Effective, but aggressive substances (formalin, caustic soda, chlorine-containing disinfectants, etc.) are recognized by the world as ecologically dangerous, have an irritating and carcinogenic effect, so they are abandoned in many countries [8, 9].

Epizootic well-being in the economy directly depends on timely and regular disinfection with effective disinfectants. The competitiveness of modern poultry enterprises is determined by many factors, including the quality of disinfection measures. The main purpose of disinfection is not only to improve the health of the livestock industry, but also to prevent infectious diseases in healthy farms. Therefore, preventive disinfection is gaining more and more popularity as a combination of disinfection measures, carried out in the absence of infectious diseases, its purpose is to prevent the occurrence and spread of infections [10–12].

The working solutions of the new disinfectant "Dezsan" were studied in comparison with the control agents: "Virocid" and "Bi-dez" at a concentration of 0.01; 0.1; 0.25; 0.5; 1; 1.5 % in relation to suspension cultures of E. coli, P. aeruginosa, S. aureus, S. typhimurium. In this case, it was established, that the "Dezsan" agent showed an antimicrobial effect on rough test objects after exposure for 3 hours at a concentration of 0.1 %, and at a concentration of 0.25 % – after 1 hour. On smooth surfaces, the agent neutralized bacterial cultures at a concentration of 0.1 % after exposure for 1 hour. The results of the study are presented in Table 1.
Veterinary research

Antimicrobial effect of modern disinfectants on suspended cultures of *E. coli*, *P. aeruginosa*, *S. aureus*, *S. typhimurium* on various test objects

<table>
<thead>
<tr>
<th>Test objects</th>
<th>Exposure, hours</th>
<th>Growth of bacterial cultures on test objects / concentration of disinfection solution, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>Galvanized iron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Painted wood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Unpainted wood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Brick</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Plaster</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: "+" – presence of growth, "-" – absence of growth

The preparation "Bi-dez" at a concentration of 0.25 % was effective on smooth surfaces after exposure for 1 hour, on rough surfaces (brick, plaster) – at a concentration of 0.5 % after exposure for 3 hours or more. A working solution of 1 % concentration neutralized bacterial cultures on all types of surfaces after exposure for 1 hour or more. The "Virocid" agent after exposure for 1 hour neutralized bacterial cultures on smooth surfaces in concentrations of 0.25 % and higher; on rough surfaces the growth of cultures was not detected when using a 0.5 % solution.

Thus, the studied disinfectants "Dezsan" and the control "Bi-dez" and "Virocid" on all types of surfaces in 3 hours of exposure showed an antimicrobial effect against the suspension of epizootic cultures of *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhimurium*, isolated from the pat material from the bird, in a concentration of 0.1 %, 0.5 % and 0.5 %, and after exposure for 1 hour – in 0.25 %, 1 % and 0.5 %, respectively.

In comparison with modern studies of other authors, it should be noted, that standard disinfection schemes in poultry farming do not give results in terms of complete destruction of pathogens. This is caused by various factors, among which the leading role belongs to antibiotic resistance, in particular, Salmonella [13]. Peracetic acid, hydrogen peroxide, and formaldehyde proved to be the best disinfectants for poultry rooms, but their effectiveness was not ideal [14]. According to foreign authors, the selection and testing of disinfection is an important problem [15], which determines the relevance and necessity of conducting our research.

**Research limitations.** The research was conducted exclusively on such test objects as galvanized iron, painted and unpainted wood, brick and plaster. Therefore, the effectiveness of the tested disinfectants on other materials cannot be guaranteed.

**Prospects for further research.** A promising direction of research is the study of other concentrations of disinfectants for further use in poultry farming for disease prevention.

**4. Conclusions**

Environmentally safe disinfectants "Dezsan" and "Shumerske sryblo" compared to the control ones ("Bi Dez" and "Virotsid") show an active antimicrobial effect at a concentration of 0.25 % and 3 %, respectively, against the suspension of epizootic cultures of *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhimurium* on different types of production surfaces, which justifies the feasibility of their use based on the principle of rotation of disinfectants for the prevention of bacterial pseudomonosis of poultry.

**Conflict of interests**

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results, presented in this article.

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Andriy Berezhkovskiy, Doctor of Veterinary Sciences, Professor, Department of Veterinary Expertise, Microbiology, Zoohygiene and Safety and Quality of Livestock Products, Sumy National Agrarian University, Gerasima Kodratieva str., 160, Sumy, Ukraine, 40000

Tetiana Fotina, Doctor of Veterinary Sciences, Professor, Head of Department, Department of Veterinary Expertise, Microbiology, Zoohygiene and Safety and Quality of Livestock Products, Sumy National Agrarian University, Gerasima Kodratieva str., 160, Sumy, Ukraine, 40000

Yevheniia Vashchyk*, Doctor of Veterinary Sciences, Associate Professor, Department of Veterinary Medicine and Pharmacy, National University of Pharmacy Pushkinska str., 53, Kharkiv, Ukraine, 61002

Dmytro Berezhnyi, PhD, Associate Professor, Department of Veterinary Medicine and Pharmacy, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Dmytro Morozenko, Doctor of Veterinary Sciences, Senior Researcher, Head of Department, Department of Veterinary Medicine and Pharmacy, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

*Corresponding author: Yevheniia Vashchyk, e-mail: yevgeniavashik@gmail.com