Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2020, 10(5), 29-35, doi: 10.15421/2020_202

RESEARCH ARTICLE

Influence of antibacterial agents on vaccine strains of Anthrax

H. A. Zaviryukha[1], U. M. Yanenko[1*], T. B. Vasylieva[1], N. I. Kosyanchuk[2], N. H. Sorokina[2], H. V. Kozlovska[2], O. L. Kravtsova[3], O. O. Marchuk[3], L. M. Muzykina[4]

[1]*State Centre of Innovation Biotechnologies, Kyev,*
[2]*National University of Life and Environmental Sciences of Ukraine*
[3]*State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine*
[4]*Institute of Veterinary Medicine The National Academy of Agrarian Sciences of Ukraine, Kyev, Ukraine*
Corresponding author E-mail: ulyanakuzyk@ukr.net
Received: 14.09.2020. Accepted: 14.10.2020

Neutralization of spore-forming pathogenic microflora is carried out by solutions of disinfectants repeatedly. Antibacterial agents when used uncontrolled can reduce the sensitivity of microorganisms and as a result cause severe complications. Of considerable interest is the combined use of antibiotics with antibacterial drugs of plant origin. The use of antimicrobial agents of plant origin is due to their low toxicity, the possibility of long-term use, greater availability and ability to biodegradation, while synthetic drugs: antibiotics, fluoroquinolones, antiseptics have strong activity. Therefore, of great interest is the combined use of antibiotics with antibacterial drugs of plant origin. The object of our study were vaccine strains of the anthrax pathogen: *Bacillus anthracis* K-79Z, *B. anthracis* 34F2 and anthrax-like bacilli *B. cereus* 8035, in spore form, disinfectants – sterilium (classic pur), ethanol 96%, alcohol solutions chlorophyllipt (1%) and propolis (7%). Studies have shown that bacteria of the strain *B. cereus* 8035 were insensitive to disinfectants of non-vegetable origin in the native and diluted state at exposures of 30, 60, 120 minutes and 24 hours of incubation, as evidenced by the intensive growth of the culture on meat-peptone agar (MPA). In the study of the disinfecting effect of 96% ethanol and sterile on the *B. anthracis* K-79Z strain, it was found that after exposure for 30, 60, 120 minutes and 24 hours, bacterial growth is recorded when using the native and disinfectant. Strains *B. anthracis* K-79Z and *B. cereus* 8035 were found to be more resistant to the action of disinfectants of plant and synthetic origin. In the future, we plan to continue the study of strains of the anthrax pathogen and anthrax bacilli on the sensitivity to disinfectants and to determine the relationship between the toxigenic characteristics of the strains.
Keywords: *Bacillus anthracis*; Ethanol 96%; Sterilium; Propolis; Chlorophyllipt

Introduction

Anthrax – an acute zoonotic infectious disease that most often affects herbivores, it is a potential problem for the veterinary and health care system (Counotte et al., 2016). The pathogen is transmitted to humans by contact with sick animals and products of animal origin (Dixon et al., 1999, Hanna, 1998). *Bacillus anthracis* is the causative agent of anthrax, a gram-positive bacterium that forms spores. Under favorable and extreme environmental conditions, for a long period of time, anthrax spores remain viable for more than a hundred years (Shadomy et al., 2016; Hatami et al., 2010, 2015; Cizauskas et al., 2014). Infection of humans occurs through spores that affect the skin, respiratory system and gastrointestinal tract. Infection through inhalation of anthrax spores without timely treatment is the most lethal to humans, this feature of infection with the pathogen can be used as a biological weapon (Owen et al., 2015; Swartz, 2002; Keim et al., 2000).

Inactivation of anthrax spores takes place in two hours with the use of 1% formalin solution. In a few hours, a 0.5% solution of carbolic acid inactivates bacteria. The following solutions have a detrimental effect on the pathogen: formaldehyde (4%), caustic soda (10%), sulfuric carbolic acid (10%), chlorinated lime (containing at least 5% active chlorine). The vegetative form of the anthrax pathogen is unstable to disinfectants (Prokopishyn, 2015).

Veterinary facilities are periodically treated against pathogenic microflora with solutions of chemically active compounds. To neutralize the pathogenic microflora use 2–4% solutions of formalin, sodium hydroxide and chlorine-containing drugs (chlorinated lime, chloramine, etc.). Solutions of these disinfectants adversely affect animal and human health and the quality of livestock products. (Yashchenko et al., 2009). The most common are disinfectants developed on the basis of Quaternary

ammonium compounds (QAC), aldehydes, phenols and alcohols, as well as oxygen-containing drugs. The antimicrobial spectrum of action of disinfectants can be enhanced by the creation of complex drugs, which include several chemical agents. Manufacturers of disinfectants and antiseptics test drugs on collection strains, which may differ in their properties from field isolates, which can lead to errors in the choice of concentrations and exposure time with disinfectant. We observe the disinfecting effect of hydrogen peroxide and lysoformin on gram-negative bacteria that contaminate food production (Furtat et al., 2004).

Spore-forming pathogenic microflora is many times treated with aqueous solutions of disinfectants in industrial enterprises. With repeated use of disinfectants, microorganisms may lose sensitivity to them (Yashchenko et al., 2009). Today, antibacterial agents when used uncontrolled can reduce the sensitivity of microorganisms and as a result cause severe complications. Researchers point to a gradual decrease in the sensitivity of microorganisms to bactericidal agents, while the environment also affects the genetic characteristics of the strains (Cavallo et al., 2002; Salmanov & Mariievskyi, 2011).

Of considerable interest is the combined use of antibiotics with antibacterial drugs of plant origin. The use of antimicrobial agents of plant origin is due to their low toxicity, the possibility of long-term use, greater availability and ability to biodegradation, while synthetic drugs: antibiotics, fluoroquinolones, antiseptics have strong activity. The advantages of herbal remedies also include the absence of dysbacteriosis and allergic reactions (Zatylnikova et al., 2010).

Plant extracts that are active against strains of microorganisms insensitive to certain antibiotics and synthetic drugs are promising objects of study. Chlorophyllipt according to the PBX classification belongs to disinfectants and antiseptics. A feature of Chlorophylliptum is its active bactericidal action against antibiotic-resistant staphylococci as well as gram-positive bacteria. Its positive effect on the pathogenetic mechanism of inflammation is confirmed (Khadzhyeva et al., 2010; Zylfykarov, 2008). High molecular weight chlorophyll compounds overcome the cell wall of *Staphylococcus spp.* and vegetative cells of bacteria of the genus *Bacillus* (Olefir et al., 2015).

Propolis exhibits significant biological activity against pathogenic microflora. This is a unique natural complex - a product of processing resinous substances collected from plants by bees. The use of propolis harmoniously affects the normalization of the body, activates internal self-rehabilitation, restores metabolic disorders, slows down the process of tumors. It does not show toxic effects after long-term use. It is established that the main antibacterial factors of propolis are fatty acids (oleic, palmitic, stearic, linoleic), as well as bioflavonoids, which inhibits the growth of a wide range of bacteria that have pathogenic properties and fungi of the genus *Candida*. Propolis solutions inhibit the viability of streptococci (*S. sobrinus, S. mutans, S. cricetus*). Antimicrobial activity of propolis against strains of *B. cereus* and *S. aureus* is registered (Karomatov, 2014; Gunar et al., 2015).

The most common antiseptics for surface and hand disinfection are alcohol and sterility. 70% ethyl alcohol has the greatest antimicrobial effect on pathogenic microflora, it has the ability to enhance the properties of other antiseptics. The antimicrobial mechanism is associated with both the dehydrating properties of alcohol and its ability to denature protein (Chekman et al., 2016).

Vegetative forms of gram-positive and gram-negative bacteria are sensitive to the action of alcohols. Bacterial spores, fungi and simple viruses are insensitive to alcohols. Cases are described when alcohol solutions served as a factor in the transmission of clostridia spores in anaerobic gas infection (Danyleichenko et al., 2017).

Modern combined products based on alcohols – Sterillium, Octeniderm, octenisept and others are widely used in veterinary medicine and medicine. General purpose disinfectants cannot be fully effective and safe. Therefore, only complex disinfectants that have a wide spectrum of antimicrobial action can be promising disinfectants. (Horzheiev, 2013).

The object of our study were vaccine strains of the anthrax pathogen: *B. anthracis K-79Z*, *B. anthracis Sterne 34F$_2$* and anthrax-like bacilli *B. cereus* 8035, in spore form, disinfectants – sterilium (classic pur), ethanol 96%, alcohol solutions chlorophyllipt (1%) and propolis (7%).

Materials and Methods

Vaccine strains of *B. anthracis K-79Z*, *B. anthracis Sterne 34F2* and anthrax-like bacilli *B. cereus* 8035 were tested according to current documents (Yakubchak et al., 2005). The strains were grown on meat peptone broth at 37°C for 48 hours. To obtain the spore material, the culture was incubated on MPA at a temperature of 37°C for 14 days. The concentration of spore biomass of experimental crops ranged from 30.0 to 35.0 × 106 colony-forming units (CFU), namely: *B. anthracis Sterne* 34F$_2$ Sterne –30.0 × 10^6 CFU, *B. anthracis K-79Z* – 35, 0 × 10^6 CFU, *B. cereus* 8035 – 32.0 × 10^6 CFU. Determined the bactericidal effect of disinfectants: propolis tincture (Tinctura propolisi), chlorophyllipt alcohol solution (Chlorophyllipt alcohol solution), sterillium classic pur. For each experimental strain, the bactericidal action of the disinfectant was determined in the native and diluted state. To determine the bactericidal effect of disinfectants on spore cultures in the first tube to the suspension of microorganisms with a pipette was added a native disinfectant solution in the amount of 1.0 cm^3. A disinfectant solution in dilution (50/50 with sterile 0.9% NaCL) in the amount of 1.0 cm^3 was pipetted into the second tube with the spore suspension of experimental cultures. The exposure was maintained for 30, 60, 120 minutes and 24 hours, after which the bactericidal loop was inoculated from each tube on the MPA, followed by cultivation at 37°C for 48 hours. After the end of the incubation period, the presence of the pathogen was determined by visual assessment of the growth of colonies on MPA. Each result was photographed, below is the effect of disinfectants (propolis, Sterillium, chlorophyllipt, 96% ethanol) at different exposure times (30, 60, 120 min) on the culture under study.

Results and Discussion

In control samples, without the addition of disinfectants, in crops on MPA, after 24 hours of incubation at 37 °C, a well-defined growth of strains *B. cereus* 8035 (Fig. 1a), *B. anthracis* 34F2 Sterne (Figure 2a) and *B. anthracis* K-79Z (Fig. 3a). The results of studies of bactericidal action of disinfectants of plant origin on anthrax and anthrax-like bacilli presented in Figs 1, 2, 3 and Tables 1 and 2.

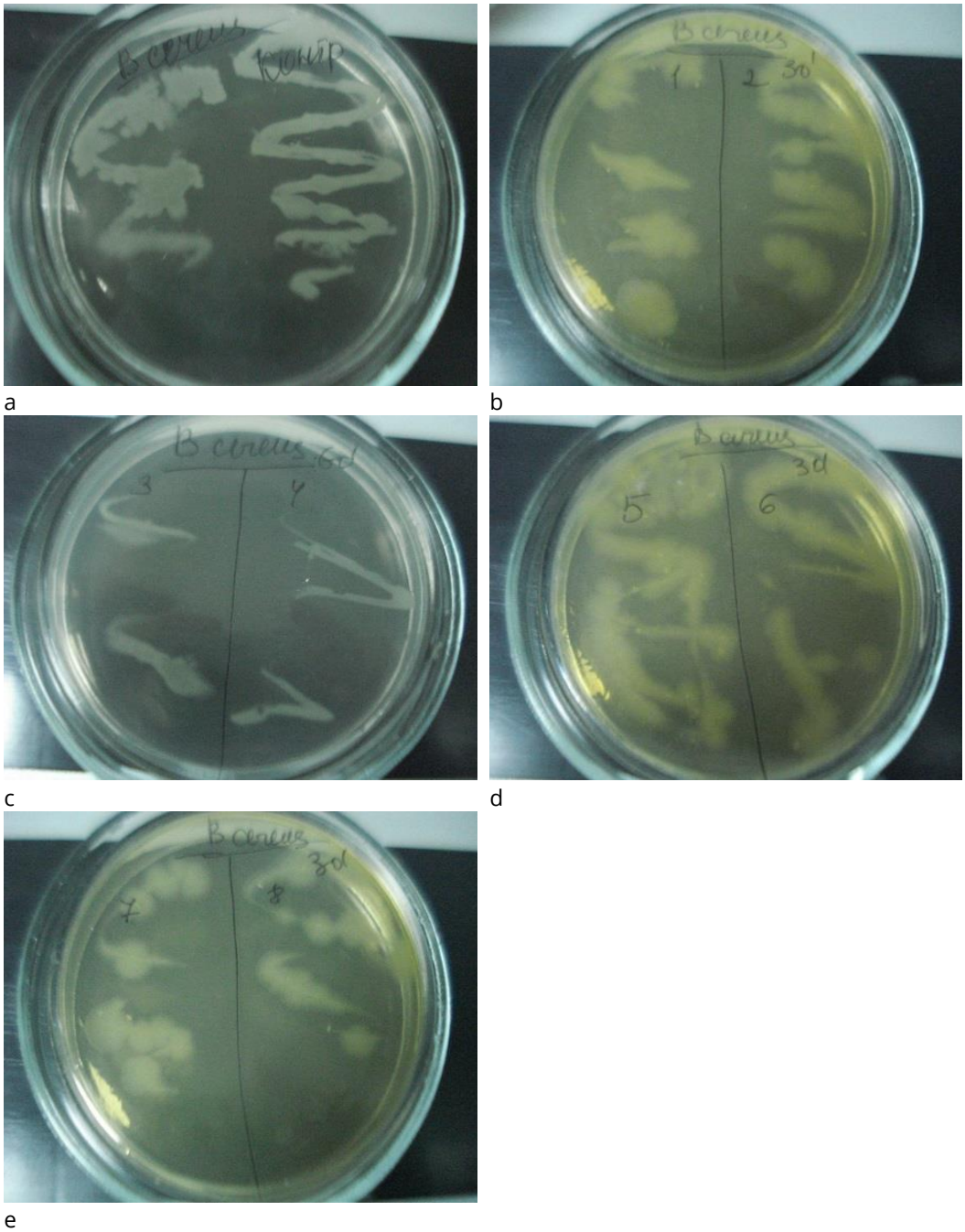

Fig. 1. Growth of a culture of *B. cereus* 8035 after exposure to disinfectants. a. Control. The presence of culture growth; b. The presence of growth of the strain *B. cereus* 8035 under the action of propolis, in the native and diluted state, for 30 min exposure; c. The presence of growth of the strain *B. cereus* 8035 under the action of chlorophyllipt, in the native and diluted state, for 60 min exposure; d. The presence of growth of the strain *B. cereus* 8035 under the action of sterility, in the native and diluted state for 30 min exposure; e. The presence of growth of the strain *B. cereus* 8035 under the action of ethanol 96%, in the native and diluted state for 30 min exposure.

As can be seen in Figure 1b, anthrax-like microbes of strain *B. cereus* 8035 showed active growth for 30 min of propolis exposure in the native and diluted state after 24 hours of cultivation. The disinfectant effect of propolis was also absent after continuation of incubation for up to 48 hours. Under the action of chlorophyllipt, in the native and diluted state, active growth of strain *B. cereus* 8035 was observed for 60 min of exposure. Disinfecting effect of chlorophyllipt after prolongation of incubation for up to 48 hours was not observed. Strelium showed no disinfectant effect on of the anthrax-like microbes strain *B. cereus* 8035 for 30 min exposure. Even after 48 hours of incubation, no inhibition of bacterial growth was detected. Under the action of ethanol 96% for 30 min exposure was recorded growth of strain *B. cereus* 8035. Under the action of propolis for 30 min of exposure, in the native state, after 24 hours of incubation, the microbes of strain *B. anthracis Sterne*

$34F_2$ did not show active growth. In the diluted state under the action of propolis exposure for 30 min was registered growth of microbes (Figure 2).

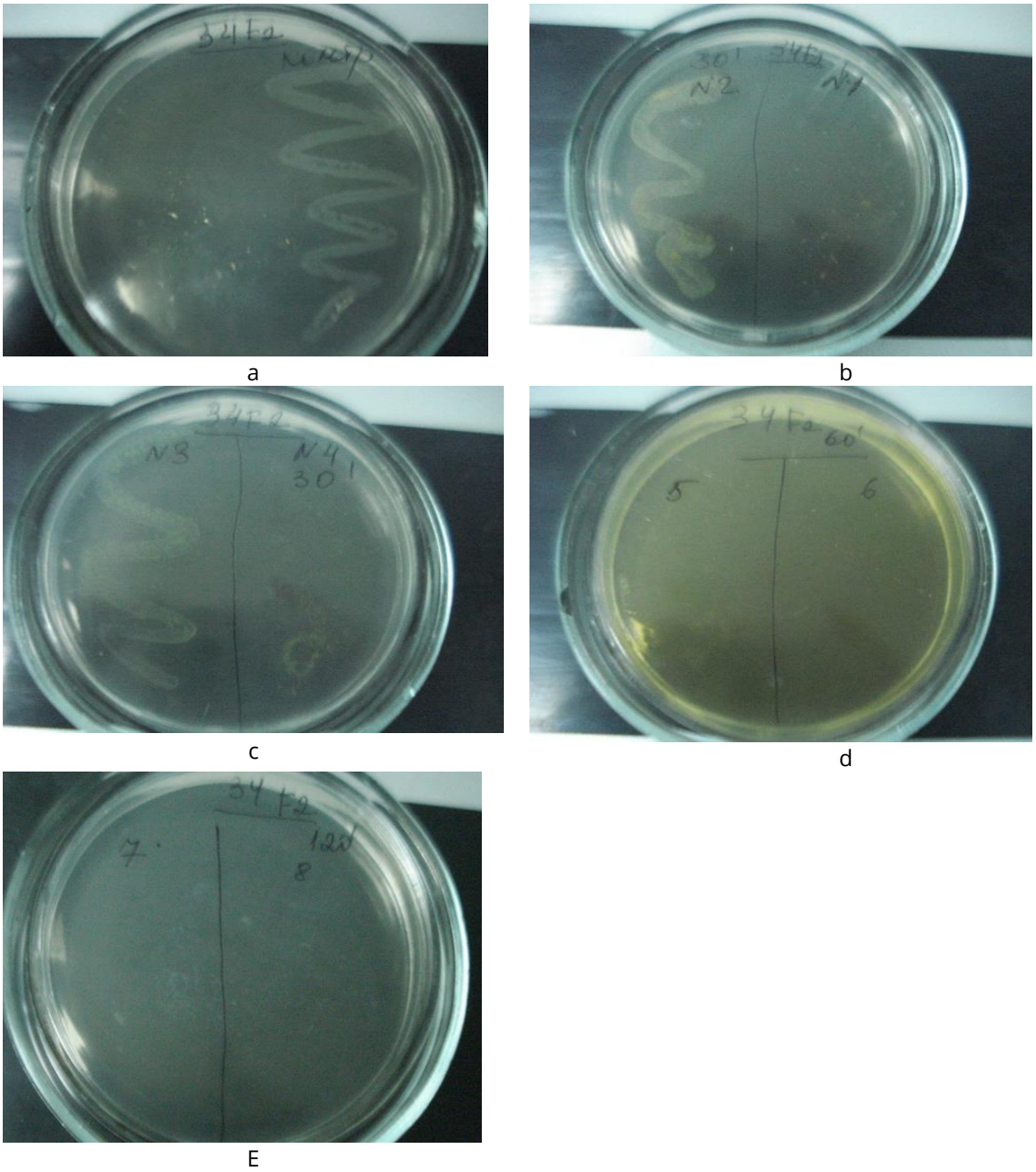

Fig. 2. Growth of *B. anthracis* Sterne 34F2 culture after exposure to disinfectants. a. Control. The presence of growth culture of *B. anthracis* 34F2 Sterne; b. The effect of propolis, in the native and diluted state on the culture, for 30 minutes of exposure; c. The effect of chlorophyllipt, in the native and diluted state, for 30 minutes of exposure; d. The effect of sterilium on the culture for 60 minutes expositions; e. No growth of *B. anthracis* 34F2 Sterne culture under the action of ethanol 96%, for 120 minutes exposure.

Effects of chlorophyllipt in a native and diluted state; after 30 min, the growth of *B. anthracis Sterne* $34F_2$ culture was observed (Fig. 2b). Active disinfecting effect on microbes *B. anthracis Sterne* $34F_2$ for 60 minutes exposure revealed sterilium (Fig. 2d). Microbial growth after incubation at 37 °C for 24 hours was absent. Under the action of ethanol 96%, during 120 min of exposure, the growth of strain *B. anthracis* 34 F_2 *Sterne* was not observed (Figure 2e).

Figure 3b shows that the effect of propolis, in the native and diluted state on the culture of *B. anthracis K-79Z,* for 30 min of exposure is not effective and there was growth of the strain. There is also no effect of chlorophyll on *B. anthracis K-79Z*. Chlorphyllipt in the native and diluted state did not show a disinfectant effect on *B. anthracis K-79Z* and after 30 minutes of exposure to this drug – the growth of the experimental culture is registered (Fig. 3c). Exposure to 30 minutes of sterilium was not effective against *B. anthracis K-79Z*, recorded growth of bacilli (Figure 3d). After incubation at 37°C for 24 hours, the growth of the strain was active. Study of the effect of ethanol 96%, after 30 min. exposure also showed the absence of disinfecting effects. After 24 hours of incubation of the inoculations under the influence of ethanol 96%, the growth of *B. anthracis K-79Z* culture was established (Fig. 3e).

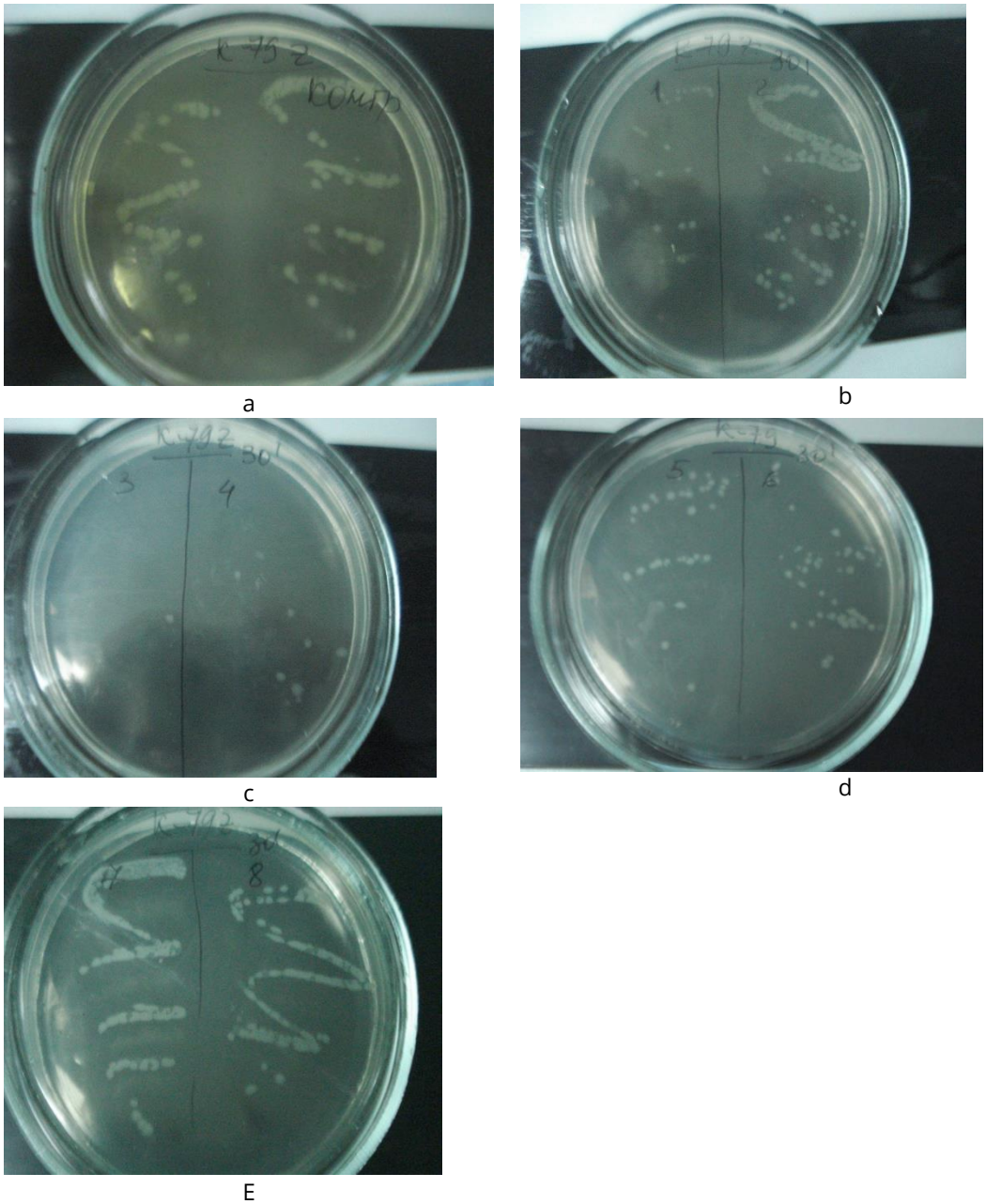

Fig. 3. Growth of *B. anthracis* K-79Z after exposure to disinfectants. a. Control. The presence of growth culture of *B. anthracis* K-79Z; b. The effect of propolis, in the native and diluted state on the culture, for 30 minutes of exposure. Registration of culture growth; c. The effect of chlorophyllipt, in the native and diluted state, for 30 minutes of exposure. Registration of culture growth; d. The effect of sterilium of the strain *B. anthracis* K- 9Z under the action of sterile, for 30 min exposure; e. The presence of growth of the culture of *B. anthracis* K-79Z under the action of ethanol 96%, for 30 minutes exposure.

When using a solution of chlorophyllipt and propolis at exposures of 30, 60, 120 minutes and 24 hours, there was a clear growth of all experimental cultures (Table 1).

Table 1. The results of the study of the bactericidal action of disinfectants of plant origin on spore cultures.

The name of the strain	Concentration	Disinfectants							
		chlorophyllipt				propolis			
		30 min	60 min	120 min	24 hours	30 min	60 min	120 min	24 hours
B. anthracis K-79Z	Native	+	+	+	+	+	+	+	+
	1:1	+	+	+	+	+	+	+	+
B. anthracis Sterne 34F₂	Native	+	+	+	+	+	+	+	+
	1:1	+	+	+	+	+	+	+	+
B. cereus 8035	Native	+	+	+	+	+	+	+	+
	1:1	+	+	+	+	+	+	+	+

(+) - The presence of growth; (-) - Lack of growth.

After application of ethanol 96% and sterilium in the native and diluted state relative to the strain *B. anthracis Sterne* $34F_2$, even at minimal exposure, the growth of the culture was not observed, which indicates the bactericidal effect of the

disinfectant (Table 2).

Table 2. The results of the study of the bactericidal action of disinfectants of synthetic origin on spore cultures.

The name of the strain	Concentration	Disinfectants							
		Ethanol 96%				Sterilium			
		30 min	60 min	120 min	24 hours	30 min	60 min	120 min	24 hours
B. anthracis K-79Z	Native	+	+	+	+	+	+	+	+
	1:1	+	+	+	+	+	+	+	+
B. anthracis Sterne 34F₂	Native	-	-	-	-	+	-	-	-
	1:1	+	-	-	-	+	-	-	-
B.cereus 8035	Native	+	+	+	+	+	+	+	+
	1:1	+	+	+	+	+	+	+	+

(+) - The presence of growth; (-) - Lack of growth.

Conclusion

Studies have shown that bacteria of the strain *B. cereus* 8035 were resistant to disinfectants of synthetic origin in the native and diluted state at exposures of 30, 60, 120 minutes and 24 hours of incubation, as evidenced by the intensive growth of the culture on MPA. In the study of the disinfecting effect of bactericidal agents of ethanol 96% and sterillium on the *B. anthracis K-79Z*, it was found that after exposure of 30, 60, 120 minutes and 24 hours, growth was noted when using the native and diluted agent. Strains *B. anthracis K-79Z* and *B. cereus* 8035 were found to be more resistant to disinfectants of plant and non-plant origin. In the future, the continuation of studies of strains of the causative agent of anthrax and anthrax-like bacilli on sensitivity to disinfectants and the relationship between the toxigenic activity of strains.

References

Cavallo, J.D., Ramisse, F., Girardet, M., Vaissaire, J., Mock, M., & Hernandez, E. (2002). Antibiotic susceptibilities of 96 isolates of *Bacillus anthracis* isolated in France between 1994 and 2000. Antimicrob Agents Chemother, 46(7), 2307-2309.

Chekman, I.S., Bielenichev I.F., Horchakova N.O., Lukianchuk V.D., Bukhtiiarova N.V., & Morhuntsova S.A. (2016). Zahalna farmakolohiia. Zaporizhzhia [Western Pharmacology]. Kyiv. (in Ukrainian).

Cizauskas, C.A., Bellan, S.E., Turner, W.C., Vance, R.E., & Getz, W.M. (2014). Frequent and seasonally variable sublethal anthrax infections are accompanied by short-lived immunity in an endemic system. J. Anim Ecol, 83(5), 1078-1090.

Counotte, M.J., Minbaeva, G., Usubalieva, J., Abdykerimov, K., & Torgerson, P.R. (2016). The burden of zoonoses in Kyrgyzstan: a systematic review. PLoS Negl Trop Dis, 10 (7):e0004831.

Danyleichenko, V.V., Klymniuk, S. I., Korniichuk, O., Kutsyk, R.V., Loban, H. A., Minukhin, V.V. et al. (2017). Mikrobiolohiia, virusolohiia, imunolohiia [Microbiology, virusolohiia, immunologia]. Vinnytsia: Nova Knyha. (in Ukrainian).

Dixon, T.C., Meselson, M., Guillemin, J., & Hanna, P.C. (1999). Anthrax. New England Journal of Medicine, 341(11), 815-826.

Furtat, I.M., Nivievska, T.V., Horbatko, L.,O. & Mykhalskyi, L.O. (2004). Dezynfikuiucha diia perekysu vodniu ta lizoforminu na hramnehatyvni bakterii, yaki kontaminuiut vyrobnytstva kharchovoi promyslovosti [Disinfecting effect of hydrogen peroxide and lysoformin on gram-negative bacteria that contaminate food industry production]. Magisterium. Pryrodnychi nauky – Magisterium. Natural sciences. National University Kyiv-Mohyla Academy, 16, 29-35.

Gunar, O.V., Sakhno, N.G., Grigoreva, V.E., & Bulgakova, G.M. (2015). Antimikrobnaya aktivnost preparatov propolisa pri provedenii ekspertizy kachestva lekarstvennykh sredstv. [Antimicrobial activity of propolis preparations during the examination of the quality of medicines]. Vedomosti Nauchnogo czentra ekspertizy sredstv mediczinskogo primeneniya. – Vedomosti Scientific center for the examination of medical devices, 2, 17-19. (in Russian).

Hanna, P. (1998). Anthrax pathogenesis and host response. Curr Top Microbiol Immunol, 225, 13-35.

Hashemi, S.A., Azimian, A., Nojumi, S., Garivani, T., Safamanesh, S., & Ghafouri, M.A. (2010). Case of Fatal Gastrointestinal Anthrax in North Eastern Iran Arch Iran Med, 13(2), 156-9.

Hatami, H., Ramazankhani, A., & Mansoori, F. (2010). Two cases of gastrointestinal anthrax with an unusual presentation from Kermanshah (Western Iran). Arch Iran Med, 13(2), 156-9.

Horzheiev, V.M. (2013). Porivnialna kharakterystyka dezinfikuiuchykh zasobiv [Comparative characteristics of disinfectants]. Veterynarna medytsyna – Veterinary medicine, 97, 180-181. (in Ukrainian).

Karomatov, I. D. (2014). Propolis: ispolzovanie v mediczine [Propolis: use in medicine]. Molodoj uchenyj. – Young scientist, 3 (62), 183–199.

Keim, P., Price, L.B., Klevytska, A.M., Smith, K.L., Schupp, J.M., Okinaka, R., et al. (2000). Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. J Bacteriol, 182, 2928-2936.

Khadzhyeva, Z.D., Teunova E.A. & Krakhmalev Y.S. (2010). Yzuchenye antymykrobnoi aktyvnosty lekarstvennыkh preparatov s fytoэkstraktom. [The study of the antimicrobial activity of drugs with phytoextract]. Fundamentalnыe yssledovanyia. – Basic research, 11, 152-154.

Olefir, Y.V., Lutseva, A.I., Gunar, O.V., Sakhno, N.G., & Grigorieva, V.E. (2015). Experimental evaluation of the methods for

determining the antimicrobial activity of chlorophyllipt. The Bulletin of the Scientific Centre for Expert Evaluation of Medicinal Products, 4), 47-50. (in Russian).

Owen, J.L., Yang, T., & Mohamadzadeh, M. (2015). New insights into gastrointestinal anthrax infection. Trends Mol Med, 21(3), 154-63.

Prokopishyn, V.K. (2015). Shho treba znaty pro sybirku [What you need to know about Anthrax] Veterynarna medycyna Ukrayiny – Veterinary Medicine of Ukraine, 2 (228). 35-37.

Salmanov, A. H., & Mariievskyi, F.M. (2011). Problema antybiotykorezystentnosti ta shliakhy yikh vyrishennia v Ukraini. [The problem of antibiotic resistance and ways of their solution in Ukraine]. Intra-hospital infections and mechanisms of resistance of their pathogens to antimicrobials: materials inter. Vnutrishno likarniani infektsii ta mekhanizmy rezystentnosti yikh zbudnykiv do antymikrobnykh preparativ. Proceed. Int. Conf. Kyiv, 7-11. (in Ukrainian).

Shadomy, S., El Idrissi, A., Raizman, E., Bruni, M., Palamara, E., Pittiglio, C. (2016). Anthrax outbreaks: a warning for improved prevention, control and heightened awareness. EMPRES Watch, 37, FAO.

Swartz, M.N. (2002). Recognition and management of anthrax—an update. N Engl J Med, 346(8):634.

Yakubchak, O.M., Kovalenko, V.L., Khomenko, V.I., Denysiuk, H.M., Bondar, T.O., & Midyk, S.V. (2005). Rekomendatsii shchodo sanitarno-mikrobiolohichnoho doslidzhennia zmyviv z poverkhon test-obiektiv ta obiektiv veterynarnoho nahliadu i kontroliu: Metodychni rekomendatsii. [Recommendations for sanitary and microbiological examination of washes from surfaces of test objects and objects of veterinary supervision and control: Methodical recommendations]. Kyiv: NAU (in Ukrainian).

Yashchenko, M.F., Zaviriukha, A.I., Kovalenko, V.L., Chekhun, A.I., Bobylov V.M., & Shlapak Yu. V. (2009). Vplyv dezinfikuiuchykh zasobiv na aerobnu sporoutvoriuiuchu mikrofloru. [Influence of disinfectants on aerobic microflora]. Veterynarna biotekhnolohiia – Veterinary biotechnology, 14, 1-4.

Zatylnikova, O.O., Osolodchenko, T.P., & Kovalov, V.M. (2010). Antymikrobna aktyvnist ekstraktiv Iris pseudacorus L. [Antimicrobial activity of Iris pseudacorus L. extracts]. Annals of Mechnikov Institute. 4. 43-47. (in Ukrainian).

Zylfykarov Y.N. (2008). Novye podkhody v razrabotke y standartyzatsyy fytopreparatov yz эfyrnomaslychnoho syria. [New approaches in the development and standardization of herbal preparations from essential oil raw materials]. Doctor's thesis. Pyatigorsk. (in Russian).

Citation:

Zaviryukha, H.A., Yanenko, U.M., Vasylieva, T.B., Kosyanchuk, N.I., Sorokina, N.H., Kozlovska, H. V., Kravtsova, O.L., Marchuk, O.O., Muzykina, L.M. (2020). Influence of Antibacterial Agents on Vaccine Strains of Anthrax. *Ukrainian Journal of Ecology, 10*(5), 29-35.