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STUDY OF THE BACTERICIDAL ACTIVITY OF THE 'DEZV ULTRA' DISINFECTANT AGAINST MYCOBACTERIA

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Summary. Timely and effective disinfection of livestock facilities and the environment is essential for maintaining bovine tuberculosis control. Currently, a significant number of disinfectants are used for disinfection, but not all of them have a bactericidal effect against tuberculosis pathogens and atypical mycobacteria at concentrations and exposures recommended by developers. The introduction of new disinfectants into veterinary practice is not possible without first determining their biocidal properties against zoonotic pathogens, including pathogenic and atypical mycobacteria, in the laboratory. The bactericidal activity of the disinfectant 'DezV Ultra' was determined using the suspension method and on test objects using test cultures of mycobacteria (*M. bovis, M. avium, M. fortuitum*). The quality of disinfection was tested on laboratory animals. The study was conducted at the Laboratory of Tuberculosis of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'. According to the results of the studies, the disinfectant 'DezV Ultra' was found to destroy the tuberculosis pathogens *M. bovis* and *M. avium* on test objects with a biological load at concentrations of 2.0% and 3.0% with exposures of 24 h and 3 h, respectively. The bactericidal effect of 'DezV Ultra' against *M. fortuitum* in a suspension application was observed at a concentration of 1.0% for 24 h, 2.0% for 5 h, and 3.0% for 3 h. Conclusions: The disinfectant 'DezV Ultra' is recommended for the preventive and mandatory disinfection of premises in agricultural enterprises, regardless of whether they are considered safe or high-risk for bovine or poultry tuberculosis

Keywords: *Mycobacterium*, disinfection, glutaraldehyde, test objects, guinea pigs

Introduction. Tuberculosis is an infectious disease that poses a particular danger to farm animals, wild animals, and humans. It is characterized by the formation of avascular nodules (tubercles) in various organs with caseous necrosis, and it usually progresses chronically.

Producing high-quality livestock products poses many challenges to veterinary medicine. One important challenge is developing modern methods to diagnose and prevent particularly dangerous diseases, such as tuberculosis (TB).

According to a 2024 report by the World Health Organization, ending the global tuberculosis epidemic remains a distant goal. The number of new tuberculosis cases continues to slowly grow worldwide. In 2020, 10.1 million people contracted tuberculosis; in 2021 — 10.4 million; and in 2023 — 10.8 million. In 2023, global TB deaths were estimated at 1.25 million, 1.09 million of which were among people without HIV and 161,000 of which were among people with HIV (WHO, 2024).

In recent years, Ukraine has experienced a severe tuberculosis epidemic. According to the WHO, the estimated incidence of TB (new cases per 100,000 people) increased by more than 5% in Ukraine between 2015 and 2023. In 2024, the Center for Public Health of the Ministry of Health of Ukraine reported that the number of newly registered TB cases, including relapses, was 18,140, or 44.2 per 100,000 people. Incidence rates were recorded in all 24 regions of Ukraine, ranging from 32.0 to 86.2 per 100,000 people. The highest number of patients was recorded in Poltava Region (61.7 people per 100,000), Zakarpattia Region (69.6 people per 100,000), Kirovohrad Region (75.4 people per 100,000), Odesa Region (79.7 people per 100,000), and Dnipro Region (86.2 people per 100,000) (WHO, 2024; PHCMOHU, 2025).

Regarding the epizootic situation of bovine tuberculosis (TB), more than 50 million cattle worldwide are infected, resulting in about $3 billion in economic losses per year. In some parts of Africa, the infection rate can be as high as 50%. The disease is endemic in Central and South America, where it is particularly prevalent in dairy cattle. Thanks to preventive and rehabilitative measures implemented in most European Union countries, cattle herds have recovered from this infection. However, sporadic cases of the disease in healthy herds and recurrence of the infection in previously recovered herds are still reported. Sporadic outbreaks of bovine tuberculosis in Europe, Canada, and the United States typically occur in areas where livestock come into contact with wildlife populations (WOAH, 2025; Milián-Suazo et al., 2022).

European countries use a full range of microbiological and serological tests, and also molecular genetic methods (PCR) to monitor animal welfare and conduct monitoring studies. These methods allow for the timely identification of pathogens and the source of infection (WOAH, 2020, 2023, 2024).

According to official data, Ukraine is free of bovine tuberculosis. However, the migration of people, wild animals, and domestic animals transported from occupied territories to other regions without prior examination or quarantine restrictions may complicate the epidemic and epizootic situation. Additionally, the failure to fully implement veterinary and sanitary measures may contribute to this complication.

One of the main factors in the transmission of tuberculosis pathogens is the environment, which allows them to remain viable and retain virulence. To break the transmission mechanism, all potentially contaminated environmental objects must undergo high-quality cleaning and disinfection.

The timely and effective disinfection of livestock premises and the environment is crucial to the system of measures for preventing and controlling tuberculosis in farm animals. Inadequate disinfection can cause new and recurring tuberculosis outbreaks.

The primary objective of disinfection is to destroy pathogens in the animal environment and prevent the occurrence and spread of the disease (Paliy et al., 2020; Zavgorodniy et al., 2013).

In animal husbandry, disinfectants containing phenols, chlorine, alkalis, aldehydes, and acids are used (Paliy, Stegniy and Vedmid, 2016).

The Ukrainian market currently offers a wide range of disinfectants. However, most of these disinfectants are intended for human medicine and are not effective when used by veterinary specialists. This is due to significant bacterial contamination of veterinary objects, a high biological load, and other factors. Using such preparations does not lead to the destruction of microorganisms. Bacteriostatic concentrations of disinfectants only slightly decrease their number for a short period (Paliy et al., 2020; Zavgorodniy et al., 2013; Paliy, Stegniy and Vedmid, 2016). Consequently, various countries around the world are searching for and developing new disinfectants with a broad spectrum of bactericidal action that comply with environmental safety requirements and are not excessively toxic.

The study **aimed** to examine the bactericidal activity of the disinfectant 'DezV Ultra' against *Mycobacterium fortuitum*, *M. bovis*, and *M. avium.*

Materials and methods. The study used the disinfectant 'DezV Ultra', which contains the active ingredient glutaraldehyde. The bactericidal activity of 'DezV Ultra' was tested on the tuberculosis pathogens *M. bovis* (strain Vallee), *M. avium* (strain IECVM UAAS), and atypical mycobacteria (*M. fortuitum* strain 122). Mycobacterial cultures were used that were stored in the museum collection of the Laboratory of Tuberculosis of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine). The mycobacterial cultures were grown on Pavlovsky potato medium for 30 days, 20 days, and 10 days, respectively, at a temperature of 37.5 ± 0.5°C. Experiments used bacterial mass from non-inactivated test cultures of mycobacteria with typical tinctorial, cultural, and biological properties. The bactericidal effect of 'DezV Ultra' on the tuberculosis pathogens *M. bovis, M. avium,* and *M. fortuitum* was determined by three methods: suspension, contact-suspension *in vitro*, and biological (*in vivo*) (Zavgorodniy et al., 2007).

In vitro studies. The initial bactericidal activity of the drug was determined using the suspension method with a fast-growing culture of atypical mycobacteria of the species *M. fortuitum.*

Working solutions of the disinfectant 'DezV Ultra' were prepared at concentrations of 0.5%, 1.0%, 2.0%, and 3.0%. 10 cm^3 of each solution was added to 20.0 cm^3 vials. Then, 0.2 cm^3 of *M. fortuitum* suspension was added to each bottle with the disinfectant using a sterile pipette. The suspension was added in concentrations of 2×10^9, 1×10^9, and 1×10^6 bacterial cells in 1.0 cm^3 of sterile 0.85% sodium chloride solution. The vial contents were thoroughly mixed and kept at the specified exposure times (1 h, 3 h, 5 h, and 24 h). After exposure, 10.0 cm^3 samples were transferred from the vials to centrifuge tubes and centrifuged at 3,000 rpm for 15 min. To control for the bactericidal action of the test preparation, a suspension of the test culture was used in which 10.0 cm^3 of sterile isotonic sodium chloride solution was added instead of the disinfectant solution. For the negative control, the test culture was treated with a 3.0% formaldehyde solution at the same concentrations.

To stop the action of the disinfectant in the test vials, the sediment formed after centrifugation, as well as the control samples, were washed twice with a sterile isotonic solution by repeated centrifugation. The sediment was resuspended by adding 5.0 cm^3 of a 0.85% sodium chloride solution. After that, the resulting sediment suspension was inoculated onto a nutrient medium for the cultivation of mycobacteria (n = 5). The tubes with inoculations were cultivated in a thermostat at a temperature of 37.5 ± 0.5°C for 90 days, and the growth of cultures was recorded every 5–7 days.

Based on the results obtained for *M. fortuitum*, the parameters (concentration and exposure) of the disinfectant's disinfecting action were further determined by the contact-suspension method. This method used *M. bovis* and *M. avium* at a concentration of 2×10^9 bacterial cells in 1.0 cm^3 of 0.85% sodium chloride solution on test objects (batiste, wood, and ceramics) with a biological load (manure). A mixture containing 1.0 cm^3 of a suspension of test cultures of the tuberculosis pathogens *M. bovis* and *M. avium* and 0.5 cm^3 of sterile manure was applied to each test object for this purpose. After 12 h, the test objects were treated with working solutions of the disinfectant. For the positive control, the test objects were treated with a sterile isotonic sodium chloride solution instead of the disinfectant. For the negative control, the test objects were treated with a 3% formaldehyde solution.

After the specified exposure period, scrapings were taken from each of the experimental and control test objects. The scrapings were washed with a sterile isotonic solution in Petri dishes. The contents of the dishes were then transferred to centrifuge tubes and centrifuged at 3,000 rpm for 15 min. To neutralize the drug's effect, the sediment was washed twice with sterile isotonic solution by centrifugation. The sediment from the experimental and control samples was then resuspended in 5.0 cm^3 of sterile isotonic solution and inoculated onto a dense nutrient medium to cultivate mycobacteria.

The test tubes containing the cultures were stored in a thermostat at a temperature of 37.5 ± 0.5°C for 90 days. Growth was recorded every five to seven days.

In vivo studies. The biological study was conducted on six clinically healthy guinea pigs (three in the experimental group and three in the control group), as well as two experimental rabbits and two control rabbits. None of the animals reacted to the tuberculin (PPD) test before the experiment. The laboratory animals were injected with a decontaminated suspension of sediment obtained after treating the experimental and control test objects with 2.0% 'DezV Ultra' for 24 h. The guinea pigs were inoculated subcutaneously in the groin with a 1.0 cm^3 dose of the decontaminated *M. bovis* test culture suspension. The rabbits were administered a 1.0 cm^3 dose of the *M. avium* test culture suspension intravenously.

The laboratory animals were observed for 90 days. During this time, the animals underwent an intradermal tuberculin test once every 30 days (three times total). Animals that died during the experiment or were euthanized after its completion were examined for tuberculosis using pathological and cultural methods.

Statistical analysis was performed by counting colony-forming units (CFUs). The results were processed by methods of variation statistics. To compare mean values Student's *t*-test was used (Van Emden, 2019).

Results. The results of a preliminary study of the bactericidal activity of 'DezV Ultra' against atypical mycobacteria (*M. fortuitum*) using the suspension method are shown in Table 1.

Analysis of the results shows that the drug 'DezV Ultra' exhibited a bacteriostatic effect against *M. fortuitum* at a concentration of 1×10^6 cells when a 0.5% solution was used for 3–24 h, a 1.0% solution for 1–3 h, and a 2.0% solution for 1 h. Regarding *M. fortuitum* with initial concentrations of 1×10^9 and 2×10^9 cells, 'DezV Ultra' had only bacteriostatic properties when used at a concentration of 0.5% for exposure times of 3–24 h, 1.0% for 1–5 h, 2.0% for 1–3 h, and 3.0% for 1 h.

Table 1 — Results of cultural studies of the bactericidal activity of 'DezV Ultra' against *M. fortuitum*, M ± m, n = 5

Mode of application		Concentration, CFU/cm^3				
		Experiment			Control	
Concentration of disinfectant solution, %	Exposure, h	1×10^6	1×10^9	2×10^9	Positive control	Negative control
0.5	1	90.2 ± 3.6	102.8 ± 5.2	118.6 ± 4.8	92.3 ± 2.8/ 1×10^6 106.8 ± 1.8/ 1×10^9 119.6 ± 2.2/ 2×10^9	–
	3	56.8 ± 4.8*	96.8 ± 5.5*	100.4 ± 6.3*		–
	5	26.8 ± 2.4*	72.2 ± 4.8*	88.2 ± 5.6*		–
	24	19.8 ± 6.0*	46.0 ± 3.2*	48.0 ± 4.4*		–
1.0	1	12.6 ± 2.4*	52.6 ± 4.8*	58.8 ± 4.2*		–
	3	8.5 ± 1.4*	36.2 ± 2.2*	46.2 ± 3.6*		–
	5	–	8.4 ± 1.4*	9.6 ± 1.6*		–
	24	–	–	–		–
2.0	1	6.5 ± 0.5*	28.8 ± 1.8*	38.4 ± 2.4*		–
	3	–	15.6 ± 1.9*	17.6 ± 0.9*		–
	5	–	–	–		–
	24	–	–	–		–
3.0	1	–	4.0 ± 0.7	4.8 ± 1.0		–
	3	–	–	–		–
	5	–	–	–		–
	24	–	–	–		–

Notes: * — $p < 0.05$ relative to control; '–' — no growth.

The bacteriostatic effect of the drug was manifested by a decrease in the number of colonies and their diameter, a delay in the appearance of primary colonies by 1–2 days, and at a 3% concentration of the drug by up to 4 days, compared with the positive control. It should be noted that there was almost no difference in the rate and intensity of colony growth after exposure to a 0.5% solution of 'DezV Ultra' for 1 h and the growth of *M. fortuitum* in control tubes that were not exposed to the drug. The results of the experiment show that the number of colonies that grew after exposure to the disinfectant depended on the initial concentration of mycobacteria. Under conditions of equal concentration and exposure to the preparation, a greater number of mycobacteria remained viable at a higher initial concentration of bacterial cells, which was reflected in the number of colonies that grew. Thus, when using an initial concentration of test cultures of 1×10^6 cells, compared to an initial concentration of 2×10^9 cells, the difference in colonies that grew after the drug was applied ranged from 1.3 (0.5% — 1 h) to 5.4 (1% — 3 h) times, and as the concentration and exposure of the drug increased, the difference in the number of colonies also increased.

When used as a suspension, 'DezV Ultra' exhibited bactericidal properties against *M. fortuitum* at an initial concentration of 1 million cells at concentrations of 1% for exposures of 5–24 h, 2% for exposures of 3–24 h, and 3% for exposures of 1–24 h. Regarding concentrations of *M. fortuitum* at 1 and 2 billion cells, the drug exhibited bactericidal activity at 1.0% for a 24-hour exposure; 2.0% for a 5- or 24-hour exposure; and 3.0% for a 3-, 5-, or 24-hour exposure.

Following positive preliminary study results, the final determination of the disinfecting action of the 'DezV Ultra' product was conducted on tuberculosis pathogens (*M. bovis* and *M. avium*) using test objects, including wood, ceramic tiles, and batiste.

Manure was used as a biological load for this purpose. Due to the risk of high concentrations of mycobacteria or tuberculosis pathogens accumulating in farm buildings and on premises, the maximum concentrations of *M. bovis* and *M. avium* (2×10^9 in 1.0 cm^3) were used to study the product's bactericidal activity on the test objects. The results of these studies are presented in Table 2.

Table 2 — Results of determining the bactericidal effect of the 'DezV Ultra' drug on *M. bovis* and *M. avium* cultures on test objects

Mode of application		Test-object			Control	
Concentration of disinfectant solution, %	**Exposure, h**	**wood**	**batiste**	**ceramic tiles**	**Positive control**	**Negative control**
M. bovis						
1	5	9.8 ± 1.2*	8.5 ± 0.9*	–	78.9 ± 4.5/wood 92.5 ± 6.2/batiste 86.8 ± 7.4/ceramics	–
	24	7.6 ± 2.4*	–	–		–
2	5	6.8 ± 0.8*	–	–		–
	24	–	–	–		–
3	3	–	–	–		–
	5	–	–	–		–
	24	–	–	–		–
M. avium						
1	5	9.4 ± 2.4*	8.8 ± 2.6*	–	88.2 ± 6.8/wood 104.5 ± 8.4/batiste 106.8 ± 8.6/ceramics	–
	24	8.0 ± 1.6*	–	–		–
2	5	7.6 ± 0.9*	–	–		–
	24	–	–	–		–
3	3	–	–	–		–
	5	–	–	–		–
	24	–	–	–		–

Notes: * — $p < 0.05$ relative to control; '–' — no growth.

The data in Table 2 show that the disinfectant 'DezV Ultra' at a concentration of 1.0% with an exposure time of 5–24 h and at a concentration of 2.0% with an exposure time of 5 h does not disinfect wood contaminated with *M. bovis* and *M. avium* pathogens. As for contaminated batiste, a 1% solution of the product also failed to inactivate the pathogens after 5 h of exposure. However, at all concentrations and exposure times, this product exhibited bactericidal activity on ceramic tiles contaminated with *M. bovis* and *M. avium*. This difference in the results of decontamination of wood and ceramics is due to the structure of the test objects, namely their porosity. The more pores there are in a test object, the more difficult it is to decontaminate it. When the preparation was used at a concentration of 2.0% for 24 h of exposure and at a concentration of 3.0% for 3–24 h of exposure, all test objects were decontaminated.

Biological testing confirmed the bactericidal properties of the experimental disinfectant at a concentration of 2.0% against *M. bovis* and *M. avium* pathogens after 24 h of exposure. To determine the quality of decontamination, guinea pigs and rabbits were injected with swabs from test objects. Only laboratory animals from the control groups reacted positively to intradermal administration of tuberculin (PPD) for mammals and birds. During the pathological examination of guinea pigs infected with wood swabs (positive control), lesions characteristic of tuberculosis were found, and in rabbits, the course of the infectious process was of the Yersin type. Cultural studies of pathological material taken from experimental and control animals isolated *M. bovis* and *M. avium* cultures only from animals in the control groups. The experimental animals did not respond to intradermal administration of tuberculins, and no mycobacterial cultures were isolated from the biomaterial after the experiment was completed.

Conclusions. 1. Bacteriological and biological studies of the bactericidal properties of 'DezV Ultra' concerning mycobacteria have established that this disinfectant destroys the tuberculosis pathogens *M. bovis* and *M. avium* at a concentration of 2.0% after 24 h of

exposure and at a concentration of 3.0% after 3–24 h of exposure.

2. 'DezV Ultra' can be used to preventively and obligatorily disinfect premises in agricultural enterprises with or without tuberculosis in cattle or poultry. It can be used in the form of a 2.0% aqueous solution for 24 h or a 3.0% solution for 3–24 h, at a rate of 1,000.0 cm^3 per 1.0 m^2.

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