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A STUDY OF THE EFFICIENCY OF MODERN DOMESTIC DISINFECTANTS IN THE SYSTEM OF TB CONTROL ACTIVITIES

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Due to the absence of elaborated efficient means for specific prevention of bovine tuberculosis, it is extremely important to detect and eliminate the source of infection and to take veterinary and sanitary preventive measures. Here the critical role is attributed to disinfection, which breaks the epizootic chain due to the elimination of pathogenic microorganisms in the environment and involves the application of disinfectants of different chemical groups. **Aim.** To study the tuberculocidal properties of new disinfectants DZPT-2 and FAG against atypical mycobacteria *Mycobacterium fortitum* and a TB agent *Mycobacterium bovis.* **Methods.** The bacteriological and molecular-genetic methods were used. **Results.** It was determined that DZPT-2 preparation has bactericidal effect on *M. fortuitum* when used in the concentration of 2.0 % of the active ingredient (AI) when exposed for 5–24 h, while disinfectant FAG has a bactericidal effect in the concentration of 2.0 % when exposed for 24 h. Disinfectant DZPT-2 in the concentration of 2.0 % of the AI, when exposed for 5–24 h, and FAG preparation in the concentration of 2.0 %, when exposed for 24 h, and with the norm of consumption rate of 1 cubic decimeter per 1 square meter disinfect the test-objects (batiste, wood, glazed tile, metal, glass), contaminated with the TB agent *M. bovis*. **Conclusions.** Disinfecting preparations of DZPT-2 in the concentration of 2.0 % of AI when exposed for 5 h and FAG in the concentration of 2.0 % when exposed for 24 h may be used in the complex of veterinary and sanitary measures to prevent and control TB of farm animals. The possibility of using the polymerase chain reaction as an additional method of estimating tuberculocide activity of disinfectants was proven.

Key words: disinfectant, bactericidal activity, concentration, exposure, mycobacterium.

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INTRODUCTION

Over 100 years have passed since Robert Koch discovered the causative agent of tuberculosis (1882); at present this disease is wide-spread on all the continents of the Globe, causing considerable economic damage to many countries [1]. One third of the global population, approximately 1.9 billion of people, are infected with the TB agent, including about 60 million patients with TB [2]. In Ukraine the main reason of high TB morbidity rate and mortality due to it is the increase in the proportion of polyresistant strains of mycobacteria [3]. There is no connection between the indices of TB morbidity for cattle and humans [4].

Now bovine tuberculosis is widely yet unevenly spread in the world: on the continent Europe the number of adverse places regarding the global index is 70.98 %, and that of sick animals – 68.7 %; on the Asian continent – 1.21 and 10.6 % respectively; in Africa – 16.25 and 10.2 %; in America – 9.15 and 8.64 %; in Australia and Oceania – 2.41 and 3.22 % [5]. Annual detection of animals with changes, remarkable for TB, in TB-safe regions, areas, and republics testifies to the fact that the actual epizootic situation is somewhat underestimated compared to the official data [6].

Disinfection is given one of the most relevant places in the system of special veterinary and sanitary mea-

sures of controlling TB which are taken with the purpose of prophylaxis and improvement of animal farms. The aim of the system is to eliminate the causative agent in the environmental objects i. e. at the stage of infection transmission to the susceptible animal [7].

It was established that most contemporary disinfection regimes, indicated in instructions on using corresponding disinfectants, do not demonstrate any tuberculocidal activity [8, 9], while one of the issues, requiring urgent solutions, is the systematic sensitivity estimation of microorganism strains (both reference, and circulating strains) regarding disinfectants [10].

While contacting the disinfectants in bacteriostatic concentrations the microorganisms remain viable, but lose their remarkable biochemical properties, which testifies to mutagenic changes [11] and promotes the formation of acquired resistance to similar preparations [12]. It was established that microorganisms acquire their resistance in case of long-term application of disinfectants in the production without any rotation [13].

The causative agents of TB and atypical mycobacteria are characterized by high plasticity of adaptive properties to the effect of antibacterial preparations and are placed in the order of ascension: *Mycobacterium bovis, M. tuberculosis, M. avium, M. fortuitum*, and the acquirement of stable resistance by mycobacteria is accompanied with the change in some differentiated phenotype properties [14].

The assortment of disinfectants, currently used to eliminate TB agents in environment, is very limited [15]. Most disinfectants are toxic for animals and cannot be used in their presence. In many cases they cause the damage of farm equipment and machinery. Therefore, the search for and testing of new disinfectants, their introduction into production is a relevant task, ensuring the prevention and control of tuberculosis of animals is an important task. It is especially true for domestic disinfectants.

The aim of the work was to study bactericide properties of atypical mycobacteria and the causative agent of TB, *M. bovis*, regarding new disinfecting preparations DZPT-2 and FAG.

MATERIALS AND METHODS

The bactericidal activity of disinfecting preparations DZPT-2 and FAG, elaborated by the specialists of SSC "IECVM" against mycobacteria were studied. The experiments were conducted following the methodological recommendations on detecting the bactericide activity of disinfectants which may be used to eliminate tuberculosis agents in environment [16].

The following test-cultures of mycobacteria were used in the experiments: *M. fortuitum* (strain 122), obtained from A. A. Tarasevych State Institute of Standardization and Control of Biomedical Preparations (SSRI SCBP) in 1995, museum specimen, nonpathogenic for laboratory and farm animals; *M. bovis* (Vallee strain), obtained from SSRI SCBP in 1990, museum specimen, pathogenic for cattle and laboratory animals.

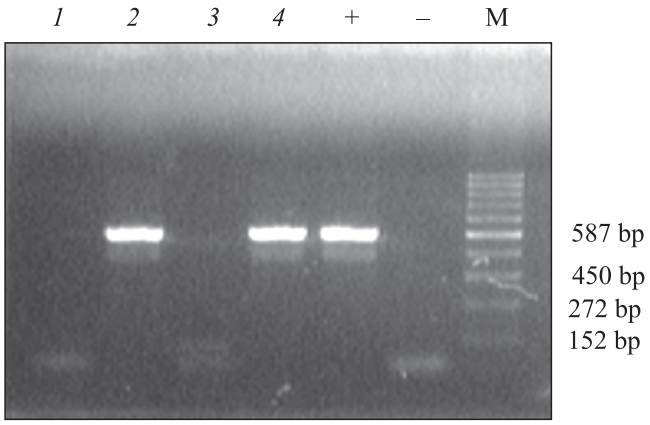
The culture of atypical mycobacteria was incubated for 14–21 days, and the culture of TB causative agent, *M. bovis,* – for 30–45 days on potato-glycerol Pawlowsky medium at 37.5 ± 0.5 °C.

Prior to the experiment using test-cultures of atypical mycobacteria *M. fortuitum* which grew on Pawlowsky medium, the suspension of 2 billion bacterial units in 1 cc of isotonic solution was prepared. For this purpose the bacterial mass of the mycobacteria test-culture was transferred via the bacteriological loop into sterile vials of 100–200 cc, previously weighed on the analytical scales, and the weight of mycobacteria therein was measured with subsequent introduction of the required volume of the sterile isotonic solution. The vials were shaken for 30 min on the reciprocating shaker to obtain homogeneous suspension of mycobacteria.

Then the working solutions of preparations were introduced in the vials of 20 cc, 10 cc per vial. Each experimental vial was introduced 0.2 cc of the suspension of atypical mycobacteria. The content of vials was thoroughly mixed, maintaining the required exposure of the disinfectant action. The vials with the suspension of mycobacteria test-culture, which were introduced 10 cc of sterile isotonic solution instead of disinfecting preparation solution, were used as a control of bactericidal action of the investigated preparation.

Then the samples of 10 cc were taken from the experiment and control vials, transferred into centrifugal tubes and centrifugated at 3.000 rpm for 15 min.

To terminate the action of the preparation in the experiment tubes, both the precipitate, formed after the centrifugation, and the control samples were washed twice with the sterile isotonic solution by centrifugation.



The electrophoregram of PCR products with primers JB21/22: 1 – DZPT-2 (2.0 % at AI – 24 h); 2 – DZPT-2 (1.0 % at AI – 24 h); 3 – FAG (2.0 % – 24 h); 4 – FAG (1.0 % – 24 h); "+" and "–" – positive and negative control samples respectively; M – molecular weight marker M100

Then the suspension of the precipitate was cultivated on the culture medium to cultivate mycobacteria. The tubes with the cultures were kept in the thermostat at 37 °C for 90 days with the registration of the culture growth each 3–5 days after the cultivation.

The determination of bactericidal properties of the preparations was also conducted using test-objects: wood, ceramic tile, batiste, glass, metal, using *Mycobacterium bovis* test-culture and the biological load (organic fertilizer).

Each test-object was applied the mixture, containing 1 cc of the suspension of the test-culture of tuberculosis agent and 0.5 cc of sterile organic fertilizer. Then the experimental test-objects were treated with the working disinfecting solution. Sterile isotonic solution was applied to the control test-objects instead of the disinfectant. Having kept the required exposure, the scrapes and smears were taken from each control and experiment test-object using the sterile isotonic solution and Petri dishes, the content of which was transferred to the centrifugal tubes to centrifuge for 30 min at 3.000 rpm. To neutralize the action of the preparations, the precipitate in the tubes was washed with the sterile isotonic solution via centrifugation. The precipitate, obtained from the experimental and control samples, was resuspended in 5 cc of the sterile isotonic solution; then it was transferred to the culture medium using the sterile pipette to cultivate mycobacteria and used to infect guinea pigs.

The tubes with the cultures were kept in the thermostat at 37 °C for three months with the registration of the culture growth each 3–5 days after the cultivation.

The samples of the cultural filtrate were studied for the presence of genetic material of *M. bovis* using the method of polymerase chain reaction (PCR) [17]. The total DNA was isolated using the commercial set for nucleic acid extraction DNA-sorb-B, produced by the Central Scientific Research Institute of Epidemiology (Russian Federation). The amplification reaction was

Table 1. The bactericide properties of disinfectants DZPT-2 (by AI) and FAG regarding *M. fortuitum* (M ± *m*, *n* = 4)

Regime of disinfectant application		Number of CFU of *M. fortuitum*			
		Disinfectant		Control	
Concentration used, %	Exposure, h	DZPT-2	FAG	Negative	Positive (average at the exposure)
0.5	1	145.2 ± 5.5	149.0 ± 1.2	–	149.0 ± 2.0 (1 h)
	5	116.8 ± 2.1*	125.3 ± 2.1*	–	
	24	110.2 ± 8.8*	120.1 ± 3.5*	–	
1.0	1	90.7 ± 0.9*	95.5 ± 3.7*	–	146.7 ± 1.0 (5 h)
	5	78.2 ± 7.7*	90.5 ± 2.0*	–	
	24	67.8 ± 2.4*	69.4 ± 8.4*	–	
1.5	1	34.4 ± 7.2*	39.4 ± 9.8*	–	148.8 ± 2.1 (24 h)
	5	13.5 ± 0.9*	19.1 ± 3.6*	–	
	24	8.4 ± 2.1*	8.9 ± 0.8*	–	
2.0	1	8.8 ± 1.2*	10.9 ± 2.7*	–	
	5	–	4.0 ± 0.3*	–	
	24	–	–	–	

Note. "–" – No mycobacteria growth, *p < 0.001.

Table 2. The bactericide properties of disinfectants regarding tuberculosis agent *M. bovis* ($n = 3$)

Preparation	Application regime, % (h)	Test-object					Control
		Batiste	Wood	Glazed tile	Metal	Glass	
DZPT-2	2.0 (5)	–	–	–	–	–	
	2.0 (24)	–	–	–	–	–	+
FAG	2.0 (5)	+	+	–	–	–	+
	2.0 (24)	–	–	–	–	–	+
							+

Note. "–" – No growth of colonies; "+" – growth of colonies.

conducted using the commercial set PCR-Core, produced by IsoGene (Russian Federation) and the systems of primers JB21/22 (to identify the genetic material of *M. bovis*). The reference culture *M. bovis* (Vallee strain) and tris-EDTA-buffer were used as a positive and negative control, respectively. The electrophoretic analysis was conducted using the electrophoresis set, produced by SPO Narvac (RF). The agarose concentration in the gel was 1.5 % at 120 V.

RESULTS AND DISCUSSION

The results of studies in detecting bactericide properties of disinfectants DZPT-2 and FAG regarding atypical mycobacteria *M. fortuitum* are presented in Table 1.

It was determined that DZPT-2 preparation has bactericide effect on *M. fortuitum* when used in the concentration of 2.0 % of the active ingredient (AI) when exposed for 5–24 h, while disinfectant FAG has a bactericide effect in the concentration of 2.0 % when exposed for 24 h. The experiments with the cultures of tuberculosis causative agents *M. bovis* were conducted on the test-objects (batiste, wood, glazed tile, metal, glass) taking the bioburden into consideration (Table 2).

The results of the studies testify that the elaborated disinfectants possess tuberculocide properties. The results of statistical processing of the results demonstrate that DZPT-2 in the concentration of 2.0 % of AI when exposed for 5 h and FAG in the concentration of 2.0 % when exposed for 24 h disinfect all the test-objects, contaminated with *M. bovis*, with 99 % probability. The results of the cultural studies were confirmed by PCR (Figure).

Figure presents the stripes with the wavelength from 152 bp to 587 bp. It was established that after the action of disinfectants DZPT-2 and FAG in the concentration of 2.0 % there was no genetic material of the causative agent of tuberculosis *M. bovis*, detected in the experimental samples.

Summarizing the results obtained, it is possible to state that the scientists of SSC "IECVM" elaborated new highly efficient disinfecting preparations, the approbation of which demonstrated the presence of high bactericide properties regarding the atypical mycobacteria and the causative agent of tuberculosis *M. bovis.* Compared to their current analogues, these disinfecting agents have a great advantage due to their high tuberculocide properties and are a promising tool to be introduced into agriculture.

CONCLUSIONS

It was established that disinfecting preparations of DZPT-2 in the concentration of 2.0 % at AI when exposed for 5 h and FAG in the concentration of 2.0 % when exposed for 24 h are remarkable for high bactericide properties regarding mycobacteria. They are promising agents in the veterinary medicine and control of bovine tuberculosis. The possibility of using the PCR as an additional method of estimating tuberculocide activity of disinfectants was proven.

Вивчення ефективності сучасних вітчизняних дезінфектантів у системі протитуберкульозних заходів

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У зв'язку з тим, що для специфічної профілактики туберкульозу великої рогатої худоби не розроблено ефективних засобів, провідного значення набувають ви-

явлення та ліквідація джерела збудника інфекції і проведення ветеринарно-санітарної профілактики. Важливу роль тут відіграє дезінфекція, яка забезпечує розрив епізоотичного ланцюга за рахунок знищення патогенних мікроорганізмів на об'єктах навколишнього середовища та передбачає застосування деззасобів з різних хімічних груп. **Мета.** Вивчення туберкулоцидних властивостей нових дезінфікуючих препаратів ДЗПТ-2 і ФАГ щодо атипових мікобактерій та збудника туберкульозу *Micobacterium bovis*. **Методи.** Використано бактеріологічний і молекулярно-генетичний методи. **Результати.** Встановлено, що препарат ДЗПТ-2 діє бактерицидно на *M. fortuitum* при застосуванні у концентрації 2,0 % за діючою речовиною (ДР) при експозиції протягом 5–24 год, а дезінфектант ФАГ чинить бактерицидну дію у концентрації 2,0 % за експозиції упродовж 24 год. Дезінфектант ДЗПТ-2 у концентрації 2,0 % за ДР за експозиції 5–24 год і препарат ФАГ у концентрації 2,0 % за експозиції 24 год і нормі витрат 1 дм^3/м^2 знезаражують тест-об'єкти (батист, дерево, кахель, метал, скло), контаміновані збудником туберкульозу *M. bovis*. **Висновки.** Встановлено, що дезінфікуючі препарати ДЗПТ-2 у концентрації 2,0 % за ДР при експозиції протягом 5 год та ФАГ у концентрації 2,0 % за експозиції 24 год можна застосовувати у комплексі ветеринарно-санітарних заходів при профілактиці і боротьбі з туберкульозом сільськогосподарських тварин. Доведено можливість використання полімеразної ланцюгової реакції як додаткового методу для оцінки туберкулоцидної активності деззасобів.

Ключові слова: дезінфектант, бактерицидні властивості, концентрація, експозиція, мікобактерії.

Изучение эффективности современных отечественных дезинфектантов в системе противотуберкулезных мероприятий

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В связи с тем, что для специфической профилактики туберкулеза крупного рогатого скота не разработано эффективных средств, ведущее значение приобретают выявление и ликвидация источника возбудителя инфекции и проведение ветеринарно-санитарной профилактики. Важная роль здесь принадлежит дезинфицирующим мероприятиям, обеспечивающим разрыв эпизоотической цепи за счет уничтожения патогенных микроорганизмов на объектах окружающей среды и предусматривающим применение дезсредств из разных химических групп. **Цель.** Изучение туберкулоцидных свойств новых дезинфицирующих препаратов ДЗПТ-2 и ФАГ относительно атипичных микобактерий и возбудителя туберкулеза *Micobacterium bovis*. **Методы.** Использованы бактериологический и молекулярно-генетический методы. **Результаты**. Установлено, что препарат ДЗПТ-2 действует бактерицидно на *M. fortuitum* при применении в концентрации 2,0 % по действующему веществу (ДВ) при экспозиции в течение 5–24 ч, а дезинфектант ФАГ проявляет бактерицидное действие в концентрации 2,0 % при экспозиции на протяжении 24 ч. Дезинфектант ДЗПТ-2 в концентрации 2,0 % по ДВ при экспозиции 5–24 ч и препарат ФАГ в концентрации 2,0 % при экспозиции 24 ч и норме расхода 1 дм^3/м^2 обеззараживают тест-объекты (батист, дерево, кафель, метал, стекло), контаминированные возбудителем туберкулеза *M. bovis*. **Выводы**. Установлено, что дезинфицирующие препараты ДЗПТ-2 в концентрации 2,0 % по ДВ при экспозиции 5 ч и ФАГ в концентрации 2,0 % при экспозиции 24 ч можно применять в комплексе ветеринарно-санитарных мероприятий при профилактике и борьбе с туберкулезом сельскохозяйственных животных. Доказана возможность использования полимеразной цепной реакции как дополнительного метода для оценки туберкулоцидной активности дезсредств.

Ключевые слова: дезинфектант, бактерицидные свойства, концентрация, экспозиция, микобактерии.

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