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DEVELOPMENT OF 'BONDARMINE' DISINFECTANT FORMULATION AND STUDY OF ITS TUBERCULOSIS EFFECT

Bondarchuk A. O. [1], Pavlichenko O. V. [1], Zavgorodniy A. I. [2], Paliy A. P. [2]

[1] Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine, e-mail: ancheystar777@ukr.net
[2] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: paliy.dok@gmail.com

Summary. The article presents the results of studying the bactericidal properties of the disinfectant 'Bondarmin', consisting of potassium peroxymonosulfate, sulfonol (surfactant), adipic acid (hexanedioic acid), and sodium chloride. The active component of the preparation is potassium peroxymonosulfate ($KHSO_5$), which being HSO_5 ion in solution, has an oxidizing effect on the cysteine moieties of microorganism proteins. The highest oxidation-reduction potential is achieved in an acidic medium (pH 2.0–2.3), which is provided by a buffer additive — adipic acid, as well as additionally the presence of sodium chloride in the solution, which creates a high ionic force of the solution, and therefore contributes to the high osmotic pressure of the solution — important factors of biocidal action. $KHSO_5$ slowly oxidizes chlorides to chlorine, which has an additional bactericidal effect (high availability of disinfectant to internal surfaces). Studies on the bactericidal properties of the 'Bondarmin' disinfectant were carried out in accordance with the methodological recommendations 'Determination of bactericidal properties of disinfectants, disinfection and control of its quality in tuberculosis of farm animals'. Experimental studies have established bactericidal effect of disinfectant 'Bondarmin' in concentration 1.0% per exposure of 3 hr in relation to atypical mycobacteria *M. fortuitum* and tuberculosis pathogens *M. bovis* and *M. avium*

Keywords: mycobacteria, test objects, exposure, concentration, bactericidal action

Introduction. Animal husbandry today is a branch of the industrial complex of Ukraine, which is characterized by high efficiency and technological efficiency and is able to provide the population with high-quality food products in a minimum period of time (Kovalenko et al., 2020).

Along with this, diseases of infectious etiology cause significant losses to farms, which negatively affects the epizootic situation and the economy of enterprises (Mustapha et al., 2018).

Along with this concentration of livestock in a limited area, it conditions favorable conditions for the emergence and spread of diseases of contagious etiology. The course of diseases in livestock leads to a decrease and loss of productivity, decrease in animal body weight gain, death, additional financial costs for therapeutic and preventive measures, deterioration in the safety and quality of livestock products (Bondarchuk, Paliy and Blazheyevskiy, 2019, Saccucci et al., 2018). Measures to combat infectious diseases can only be effective if the epizootic chain is broken, so the development and introduction of new disinfectants is an urgent task of veterinary science (Nechyporenko et al., 2019).

Disinfectants should have high biocidal capacity, prolonged action, reduced aggressiveness to equipment and treated surfaces, safety for personnel and the environment, ease of use, moderate price (Rutala and Weber, 2016). Over time, microorganisms develop resistance to disinfectants. Systematic rotation of desiccants based on various active substances can effectively prevent this phenomenon (Kanishchev and Eremeeva, 2016).

It should be noted that today a large number of biocides are used in veterinary practice, which have differences in the form of release and chemical components. The selection of disinfectants is carried out, taking into account the method of keeping animals and the type of livestock room, while the type of floor of the room is necessarily taken into account (Shkromada et al., 2019). Thus, based on the above-mentioned, the development and use of the latest disinfectants with an expanded spectrum of their antimicrobial activity by combining active components, their ability to prevent the occurrence of resistance in bacteria and viruses is a pressing issue (Paliy et al., 2020b, 2021). It should be emphasized that working solutions of new combinations of desiccants should have the property of use in the presence of animals. First of all, this is important because disinfecting into this method will reduce the total bacterial contamination of livestock premises and improve microclimate parameters and veterinary and sanitary conditions for animals and poultry in general (Paliy et al., 2020a).

The aim of the research was to develop a new disinfectant 'Bondarmin' for wet disinfection of livestock premises and veterinary facilities.

Material and methods. The study was carried out in accordance with the methodological recommendations 'Determination of bactericidal properties of disinfectants, disinfection and control of its quality in tuberculosis of

farm animals' (Zavhorodnii et al., 2007). At the beginning of the studies, the bactericidal effect of an aqueous solution of chemical substances on atypical mycobacteria of the species *M. fortuitum* was established.

The next step was establish the bactericidal action of the disinfectant on various test objects contaminated with 2 billion veils separately with *M. bovis* and *M. avium* mycobacteria. As test objects, batiste, unpainted wood, tile, metal, plastic, and glass were used. Sterile pus (as a biological defense against chemical factors) was also added to the dependence of mycobacteria at a rate of 2:1. The infective material was evenly distributed on the surface of each test and control object 'Bondarmin' disinfectant was used at a concentration of 1.0, 1.5, and 2.0% for expositions 1, 3, 5, and 12 hrs.

A final study of the bactericidal properties of the disinfectant against *M. fortuitum, M. bovis*, and *M. avium* was carried out by bioprobe. In order to carry out this study, we selected 30 guinea pigs with an average weight of 330 ± 20 g and 12 rabbits with an average weight of 2.5 ± 0.2 kg. All animals were tested using tuberculin (PPD) for mammals at a dose of 0.1 ml.

After a negative response was determined in animals to the introduction of the allergen, 7 groups (3 research and 4 control) were formed from them (each group had 6 animals):

I research group (guinea pigs) — animals were injected subcutaneously with flushes (1.0 cm^3) from test objects (composite sample) contaminated with the pathogen of tuberculosis *M. bovis* and treated with disinfectant 'Bondarmin' (1.0%, 3 hrs);

II research group (rabbits) — animals were injected subcutaneously with flushes (1.0 cm^3) from test objects (composite sample) contaminated with the pathogen of tuberculosis *M. avium* and treated with disinfectant 'Bondarmin' (1.0%, 3 hrs);

III research group (guinea pigs) — animals were injected subcutaneously (1.0 cm^3) with fast-growing atypical mycobacteria *M. fortuitum* and treated with disinfectant 'Bondarmin' (1.0%, 3 hrs);

IV control group (guinea pigs) — animals were injected subcutaneously with flushes (1.0 cm^3) from test objects (composite sample) contaminated with the pathogen of tuberculosis *M. bovis* and treated with sterile saline;

V control group (rabbits) — animals were injected subcutaneously with flushes (1.0 cm^3) from test objects (composite sample) contaminated with the pathogen of tuberculosis *M. avium* and treated with sterile saline;

VI control group (guinea pigs) — animals were injected subcutaneously (1.0 cm^3) with fast-growing atypical mycobacteria *M. fortuitum* and treated with sterile saline;

VII control group (guinea pigs) — intact animals.

Laboratory animals were observed for three months, once a month they were examined by an allergic method for tuberculosis, by using mycobacterial allergens. The animals of groups I, IV, VII were intracorally injected with tuberculin (PPD) for mammals at a dose of 0.1 cm^3; II, V — tuberculin (PPD) for poultry at a dose of 0.1 cm^3; III, VI, VII — allergen from atypical mycobacteria (AAM) at a dose of 0.1 cm^3.

Results and discussion. To create a new disinfectant, we studied and analyzed a number of chemical ingredients. At the same time, the most promising substances were identified in our opinion: allicin, cetylpyridinium chloride, potassium peroxymonosulfate, on the basis of which it is possible to create an innovative disinfectant.

In order to determine their potential for use as an active substance in a new disinfectant, we conducted studies to determine their bactericidal effect on the test culture of atypical mycobacteria *M. fortuitum* (strain No. 122). Experimental studies were carried out using the culture method of studies (Table 1).

Table 1 — Bactericidal properties of chemicals to *M. fortuitum* (strain No. 122)

Application mode, %/hrs	Results	
	experience	control
Potassium peroxymonosulfate		
0.5/3	+	+
0.5/5	+	+
0.5/24	+	+
1.0/3	–	+
1.0/5	–	+
1.0/24	–	+
1.5/3	–	+
1.5/5	–	+
1.5/24	–	+
2.0/3	–	+
2.0/5	–	+
2.0/24	–	+
Cetylpyridinium chloride		
0.5/3	+	+
0.5/5	+	+
0.5/24	+	+
1.0/3	+	+
1.0/5	+	+
1.0/24	+	+
1.5/3	+	+
1.5/5	+	+
1.5/24	+	+
2.0/3	+	+
2.0/5	+	+
2.0/24	+	+
Allicin		
0.5/3	+	+
0.5/5	+	+
0.5/24	+	+
2.0/3	+	+
2.0/5	+	+
2.0/24	+	+

Notes: '–' — there is no growth of mycobacteria, '+' — the existing growth of mycobacteria.

From the results of Table 1, it can be seen that potassium peroxymonosulfate has bactericidal properties with respect to *M. fortuitum* at a concentration of 1.0% per exposure of 3 to 24 hrs. In addition, the test powders, such as cetylpyridinium chloride and allicin did not show bactericidal effect to the mycobacteria test culture when tested at a concentration of up to 2.0% per action for 24 hrs, and therefore their further use as active substances is not appropriate.

According to the results of the studies, potassium peroxymonosulfate turned out to be an effective bactericide in the test culture of mycobacteria. This substance has been identified by us as the active ingredient for the further design of the new disinfectant.

In order to improve the physicochemical properties of this compound and reduce its content in the preparation, the adjuvants shown in Table 2 were added to the formulation of the new agent.

Table 2 — Disinfectant research formulations (working solutions)

No.	Composition	Content
1	Potassium peroxymonosulfate	1.0%
	Sulfonol	0.1%
	Adipic acid	0.5%
	Sodium chloride	0.4%
	Water	to 100%
2	Potassium peroxymonosulfate	2.0%
	Sulfonol	0.2%
	Adipic acid	1.0%
	Sodium chloride	0.8%
	Water	to 100%

The presence of bactericidal action compositions of chemical compounds (Table 2) was determined in tests with *M. fortuitum* test culture (No. 122). The results of the experiment are shown in Table 3.

Table 3 — Bactericidal action of chemical compounds to *M. fortuitum*

Chemical compounds	Exposition	Results	
		experience	control
No. 1	10 min	+	+
	30 min	+	+
	1 hr	+	+
	2 hr	+	+
	3 hr	–	+
	4 hr	–	+
	5 hr	–	+
No. 2	10 min	+	+
	30 min	+	+
	1 hr	+	+
	2 hr	+	+
	3 hr	–	+
	4 hr	–	+
	5 hr	–	+

Notes: '–' — there is no growth of mycobacteria, '+' — the existing growth of mycobacteria.

From the results shown in Table 3 it can be seen that these chemical compounds, when combined in these ratios, exhibit a bactericidal effect on *M. fortuitum* mycobacteria starting from exposure of 3 hrs or more.

So, after determining the compatibility of these chemical compounds, the most optimal ratio of their contents in the new disinfectant was calculated.

Thus, the new disinfectant consists of: potassium peroxymonosulfate — 50.0%, sulfonol — 5.0%, adipic acid — 25.0%, sodium chloride — 20.0%. This composition was called 'Bondarmin' by us and taken for further experimental studies.

The study of the bactericidal properties the disinfectant 'Bondarmin' was carried out using a suspension method using a test culture of *M. fortuitum* mycobacteria (strain No. 122). Working solutions of the disinfectant were prepared just before the start of the studies in sterile vials. Disinfectant 'Bondarmin' was examined at a concentration of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% for the drug when exposed to 1, 3, 5, and 24 hrs. The results of the studies are shown in Table 4.

Table 4 — Bactericidal properties of 'Bondarmin' disinfectant in solution to *M. fortuitum* (n = 5)

Concent-ration, %	Exposition, hrs					Control	
	1	3	5	12	24	negative	positive
0.5	+	+	+	+	+	–	+
1.0	+	–	–	–	–	–	+
1.5	+	–	–	–	–	–	+
2.0	–	–	–	–	–	–	+
2.5	–	–	–	–	–	–	+
3.0	–	–	–	–	–	–	+

Notes: '–' — there is no growth of mycobacteria, '+' — the existing growth of mycobacteria.

From the results given in Table 4, it can be seen that the disinfectant 'Bondarmin' in the concentration 0.5% by exposure from 1 to 24 hrs does not bactericidally affect the test culture of *M. fortuitum*, while an increase in the concentration of the agent up to 1.0–1.5% per action from 3 hrs predetermines the death of mycobacteria. High bactericidal properties with respect to atypical fast-growing mycobacteria, the test agent exhibits for actions in concentration 2.0–3.0% and exposure from 1 to 24 hrs.

The positive results obtained give us reason to continue studying the bactericidal properties of the developed disinfectant directly with the pathogens of tuberculosis of animals and birds.

The next stage of our research was the study of the bactericidal properties of disinfectant on test objects with *M. bovis* and *M. avium* cultures. It is necessary to conduct studies directly with tuberculosis pathogens, bringing the experimental conditions closer to production conditions. To this end, we have determined the physical nature of the materials used in animal husbandry. The results of the studies are presented in Table 5.

Table 5 — Bactericidal properties of 'Bondarmin' disinfectant to test objects with cultures *M. bovis* (n = 3) and *M. avium* (n = 3)

Test culture	Application mode, %/hrs	Test objects						Control	
		B	W	T	M	P	G	negative	positive
Mycobacterium bovis (Vallee strain)	1.0/1	+	+	+	+	+	+	–	+
	1.0/3	–	–	–	–	–	–	–	+
	1.0/5	–	–	–	–	–	–	–	+
	1.0/12	–	–	–	–	–	–	–	+
	1.5/1	+	+	+	+	+	+	–	+
	1.5/3	–	–	–	–	–	–	–	+
	1.5/5	–	–	–	–	–	–	–	+
	1.5/12	–	–	–	–	–	–	–	+
	2.0/1	–	–	–	–	–	–	–	+
	2.0/3	–	–	–	–	–	–	–	+
	2.0/5	–	–	–	–	–	–	–	+
	2.0/12	–	–	–	–	–	–	–	+
Mycobacterium avium (IECVM UAAS strain)	1.0/1	+	+	+	+	+	+	–	+
	1.0/3	–	–	–	–	–	–	–	+
	1.0/5	–	–	–	–	–	–	–	+
	1.0/12	–	–	–	–	–	–	–	+
	1.5/1	+	+	+	+	+	+	–	+
	1.5/3	–	–	–	–	–	–	–	+
	1.5/5	–	–	–	–	–	–	–	+
	1.5/12	–	–	–	–	–	–	–	+
	2.0/1	–	–	–	–	–	–	–	+
	2.0/3	–	–	–	–	–	–	–	+
	2.0/5	–	–	–	–	–	–	–	+
	2.0/12	–	–	–	–	–	–	–	+

Notes: '–' — there is no growth of mycobacteria, '+' — the existing growth of mycobacteria; B — batiste, W — unpainted wood, T — tile, M — metal, P — plastic, G — glass.

Statistical processing of the obtained 'Bondarmin' disinfectant regimens was performed using non-parametric statistical criteria (Z mark). To this end, the results of studies of a single experiment with *M. bovis* culture, performed in triplicate, in which the mode of use of the disinfectant was 1.0% per exposure of 3 hrs, were taken into account. The results of the studies are shown in Table 6.

According to the results of the data given in Table 6, it can be stated that the disinfectant 'Bondarmin' inactivates the test culture of the tuberculosis pathogen *M. bovis* when used at a concentration of 1.0% per exposure for 3 hrs, regardless of the physical nature of the test object, with a probability of 99%, which indicates the prospect of using this drug as a tuberculosis disinfectant.

Table 6 — Statistical processing of data on studies of bactericidal action of disinfectant 'Bondarmin' (1.0%, 3 hrs)

Test object, quantity		Growth of mycobacteria colonies on nutrient medium				Criterion Z
		to action (add)	after action (add)	control		
				negative	positive	
1	B	+	–	–	+	positive
2		+	–	–	+	positive
3		+	–	–	+	positive
1	W	+	–	–	+	positive
2		+	–	–	+	positive
3		+	–	–	+	positive
1	T	+	–	–	+	positive
2		+	–	–	+	positive
3		+	–	–	+	positive
1	M	+	–	–	+	positive
2		+	–	–	+	positive
3		+	–	–	+	positive
1	P	+	–	–	+	positive
2		+	–	–	+	positive
3		+	–	–	+	positive
1	G	+	–	–	+	positive
2		+	–	–	+	positive
3		+	–	–	+	positive

Notes: '–' — there is no growth of mycobacteria, '+' — the existing growth of mycobacteria; B — batiste, W — unpainted wood, T — tile, M — metal, P — plastic, G — glass; criterion Z — a positive effect of the action.

The biological test in determining the tuberculosis regime of the disinfectant is a necessary final step in determining the final mode of disinfectant use in laboratory conditions. The results of the studies are shown in Table 7.

Table 7 — Biological study of tuberculosis action of disinfectant 'Bondarmin' (1.0%, 3 hrs) on guinea pigs and rabbits

Group	Day of research	Laboratory animal number					
		1	2	3	4	5	6
I	30	–	–	–	–	–	–
	60	–	–	–	–	–	–
	90	–	–	–	–	–	–
II	30	–	–	–	–	–	–
	60	–	–	–	–	–	–
	90	–	–	–	–	–	–
III	30	–	–	–	–	–	–
	60	–	–	–	–	–	–
	90	–	–	–	–	–	–
IV	30	+	+	dead	+	+	dead
	60	dead	+	~	dead	dead	~
	90	~	dead	~	~	~	~
V	30	dead	dead	dead	dead	dead	dead
	60	~	~	~	~	~	~
	90	~	~	~	~	~	~

Continuation of Table 7

Group	Day of research	Laboratory animal number					
		1	2	3	4	5	6
VI	30	+	+	+	+	+	+
	60	+	+	+	–	+	+
	90	+	–	–	–	–	–
VII	30	–	–	–	–	–	–
	60	–	–	–	–	–	–
	90	–	–	–	–	–	–

Notes: '–' — there is no reaction to the administration of allergens; '+' — reaction to the administration of allergens is present; '~' — the absence of an animal in the experience.

By analyzing the results of the bioprobe in laboratory animals, determined that the experimental animals, which were administered flushes from test objects after their treatment with the disinfectant 'Bondarmin' in no case reacted to the intracutaneous administration of the corresponding allergens, which indicates the absence of infection of these animals with mycobacteria. Therefore, it is confirmed that these microorganisms are completely inactivated by the disinfectant under investigation.

In addition, in laboratory animals of control groups (IV–VI), the manifestation of allergic reactions of varying intensity to the administration of mycobacterial allergens was noted. Rabbits died from a septic form of tuberculosis.

In control laboratory animals, lesions characteristic of tuberculosis were found at the autopsy. In research laboratory animals, no characteristic tuberculosis lesions were found during autopsy.

Conclusions. A new disinfectant 'Bondarmin' was developed, which included potassium peroxymonosulfate — 50%, sulfanol — 5.0%, adipic acid — 25%, sodium chloride — 20%. Disinfectant 'Bondarmin' exhibits bactericidal properties at a concentration of 1.0% per exposure of 3 hours relative to *M. fortuitum*, *M. bovis*, *M. avium.*

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