Part 2. Biosafety

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STUDY OF THE BACTERICIDAL PROPERTIES OF AN OXYGEN-CONTAINING DISINFECTANT AGAINST MYCOBACTERIA

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Summary. This study aimed to determine the bactericidal properties of an oxygen-based disinfectant against mycobacteria and evaluate its effectiveness in decontaminating production surfaces. The study material was the universal, oxygen-containing disinfectant 'Famidez Sanoksil 100', which contains hydrogen peroxide, silver nitrate, and phosphoric acid. The preparation's bactericidal activity was assessed against a *Mycobacterium phlei* test culture using the suspension method at concentrations of 0.5%, 1.0%, 2.0%, and 3.0%, under various exposure conditions. Additionally, tuberculocidal properties were determined on test objects (batiste, wood, and tile) contaminated with *M. bovis* culture. The decontamination effectiveness of the test objects was confirmed by a biological study on laboratory animals (guinea pigs), which included an intradermal tuberculin test as well as pathological and bacteriological examinations. The bactericidal activity of the disinfectant was found to be directly dependent on the concentration of the working solution and the exposure time. Destruction of the *M. phlei* test culture was observed after 48 h at a concentration of 1.0%, whereas a 2.0% solution of the disinfectant provided a tuberculocidal effect only after 24–48 h. The 3.0% solution was the most effective, ensuring complete inactivation of mycobacteria after 5 h, 24 h, and 48 h of contact. After treatment with a 3.0% solution of the preparation, no mycobacterial growth was detected on test objects contaminated with the *M. bovis* culture (after exposure for 5 h, 24 h, and 48 h). In the biological experiment, laboratory animals in the experimental groups did not react to the tuberculin injection, and no mycobacterial cultures were isolated from their biomaterial. These results indicate the disinfectant's pronounced tuberculocidal properties and confirm its effectiveness in decontaminating mycobacterium-contaminated objects

Keywords: disinfection, *Mycobacterium bovis*, atypical mecobacteria, test objects, guinea pigs

Introduction. Structural transformations in Ukraine's economy have exacerbated the agricultural sector crisis and significantly altered domestic and international operating conditions for livestock enterprises. Currently, livestock farming is one of the key components of Ukraine's national economy and positions the country among the leading exporters of certain types of animal products. However, the sector is currently in a difficult state due to prolonged military hostilities, the consequences of the pandemic, and general economic instability (Ushkalov, 2023; Bozhyday and Kralia, 2026).

Under these conditions, food producers must ensure product quality, increase export potential, and promptly identify and resolve issues related to maintaining facilities and equipment in accordance with established hygiene requirements (Betoret, Betoret and Glicerina, 2024).

The approach to ensuring proper sanitary conditions at facilities — including those involved in primary production — has changed significantly: it is no longer limited to measures taken after the fact to mitigate the consequences of adverse impacts. Instead, it involves a systematic set of preventive measures regulated by production control programs that enable the timely resolution of hygiene and anti-epizootic issues (Awuchi, 2023; Ushkalov et al., 2025).

Within the framework of veterinary and sanitary measures aimed at maintaining proper sanitary conditions in livestock facilities and improving the safety of raw materials, feed, and animal-derived products, disinfection plays a leading role (Zavgorodniy et al., 2013; Paliy, 2018).

The rational selection of disinfectants is of fundamental importance in practical veterinary medicine, as such products must combine high biological efficacy, environmental safety, and cost-effectiveness (Rodionova, Paliy and Khimych, 2021).

The main goal of disinfection is to interrupt the epizootic process by targeting the key link in pathogen transmission. This factor facilitates its movement from the source of infection to a susceptible organism and to contaminated environmental objects, raw materials, or food products. Mycobacteria, particularly the causative agents of animal tuberculosis, require special attention in this context, as they are characterized by increased resistance to physical and chemical factors due to the specific structure of their cell wall, enriched with mycolic acids. Their ability to persist for long periods in the environment and on production infrastructure surfaces

necessitates the use of highly effective disinfectants with proven mycobactericidal activity, which is a crucial component of the veterinary and sanitary measures system (Garkavenko and Kovalenko, 2017; Wales et al., 2021; Pedreira, Taşkın and García, 2021).

The problem of tuberculosis in cattle remains a pressing issue for many countries around the world, as the pathogens of the *Mycobacterium tuberculosis* complex, particularly *M. bovis*, are highly resistant to environmental factors and can persist for long periods in soil, water, and on surfaces in production facilities. Under current conditions, disease control requires a comprehensive approach that combines diagnosis, isolation of infected animals, and the implementation of systematic veterinary and sanitary measures, including cleaning and disinfection (Paliy et al., 2024; Korniienko et al., 2021).

An analysis of the epizootic situation regarding bovine tuberculosis in Ukraine indicates that the effectiveness of anti-epizootic measures largely depends on strict adherence to sanitary requirements on farms. Proper organization of cleaning and disinfecting premises, equipment, and vehicles, alongside allergic testing and diagnostic slaughter of reactive animals, plays a crucial role in reducing the risk of pathogen persistence and circulation within herds (Korniienko et al., 2021).

International studies also confirm the need for an integrated approach to tuberculosis control at the farm level. Thus, the development and implementation of protocols to minimize the risk of tuberculosis spread on farms involves not only testing animals but also a set of biosecurity measures, including the disinfection of equipment, animal housing areas, and zones of potential contact with wild reservoirs of infection (Wales et al., 2021). The authors emphasize that underestimating the role of environmental sanitation can contribute to the reintroduction or continued circulation of the pathogen.

The importance of controlling environmental factors in the spread of mycobacteria is also confirmed within the framework of the 'One Health' concept. A review by Zhang et al. (2022) emphasizes that environmental contamination is a significant link in the transmission of the *Mycobacterium tuberculosis* complex between animals, humans, and the environment. Systematic cleaning and disinfection of premises, instruments, vehicles, and water supply systems are considered critical measures aimed at breaking the epizootic chain.

Additionally, it has been established that farms with substandard veterinary, sanitary, and hygiene control measures are at a higher risk of introducing *M. bovis* into their herds. A study by Weldegebriel et al. (2025) identified several key risk factors, including noncompliance with biosecurity requirements, inadequate sanitation of facilities, and the uncontrolled introduction of new animals into the herd. These findings suggest that disinfection should be considered a strategic component, rather than an auxiliary measure, in tuberculosis prevention and control programs for livestock operations.

Therefore, current scientific data confirm that effectively controlling tuberculosis in livestock farming is impossible without systematically implementing cleaning and disinfection measures that inactivate mycobacteria in the production environment and minimize the risk of further transmission.

The current market offers a wide variety of disinfectants for use on livestock farms. These disinfectants come in various combinations of active ingredients, dosage forms, and from different manufacturers. However, not all of them have proven mycobactericidal properties or meet their claimed specifications under real-world production conditions. This creates a need for targeted research, the results of which would provide a well-founded assessment of the bactericidal activity of individual agents, particularly oxygen-based disinfectants, against mycobacteria. Furthermore, the use of the same disinfectants may lead to the emergence of resistant mycobacterial forms, which would contribute to their prolonged persistence in the environment.

The study **aimed** to determine the optimal concentrations of working solutions and exposure times for the bactericidal activity of the oxygen-based disinfectant 'Famidez Sanoksil 100' against *M. bovis* and atypical mycobacteria.

Materials and methods. The study utilized cultures of *M. phlei* (accession No. 23) and *M. bovis* (Vallee strain) from the collection housed at the Laboratory of Tuberculosis of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

The bacterial cultures of *M. phlei* and *M. bovis* were grown on Pavlovsky's nutrient medium for 14–21 days, and *M. bovis* for 30–45 days at a temperature of 37.5 ± 0.5 °C.

The preparation 'Famidez Sanoksil 100' (DezoMark LLC, Ukraine), a disinfectant widely used in human medicine, was used as a model oxygen-containing disinfectant in the study. 'Famidez Sanoksil 100' contains hydrogen peroxide (50.0 g/100 g), silver nitrate, phosphoric acid, and water; it was studied at concentrations of 0.5%, 1.0%, 2.0%, and 3.0%.

The bactericidal properties of the disinfectant 'Famidez Sanoksil 100' were determined using the suspension method, test objects, and biological testing in accordance with the methodological guidelines 'Determination of the Bactericidal Properties of Disinfectants, Disinfection Procedures, and Quality Control in the Context of Tuberculosis in Farm Animals' (Zavgorodniy et al., 2007).

The biological study was conducted on six clinically healthy guinea pigs (three experimental and three control) weighing 300–350 g, none of which had reacted to mammalian tuberculin (MTB) before the start of the experiment. The experimental group received a subcutaneous injection of *M. bovis* suspension obtained after treating the test objects with a 3.0% solution of 'Famidez Sanoksil 100' (24 h exposure), while the control

group received an untreated bacterial mass at a dose of 1 cm^3. The animals were observed for 90 days and underwent tuberculin skin testing during this period. Upon completion of the experiment or in the event of an animal's death, postmortem and bacteriological examinations were performed.

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

Results and discussion. The results of determining the bactericidal activity of the disinfectant 'Famidez Sanoksil 100' on a test culture of the atypical mycobacterium *M. phlei* using the suspension method are presented in Table 1.

Table 1 — Bactericidal properties of the drug 'Famidez Sanoksil 100' by the suspension method regarding *M. phlei*

Culture	Concentration of disinfectant solution, %	Exposure, h	Experiment	Control	
				Negative	Positive
M. phlei	0.5	5	+++	–	++++
		24	++	–	++++
		48	+	–	++++
	1.0	5	++	–	++++
		24	+	–	++++
		48	–	–	++++
	2.0	5	+	–	++++
		24	–	–	++++
		48	–	–	++++
	3.0	5	–	–	++++
		24	–	–	++++
		48	–	–	++++

Notes: '++++' — intense colony growth; '+++' — growth of 50 colonies; '++' — growth of 20 colonies; '+' — growth of up to 10 colonies; '–' — no growth.

As shown in Table 1, the bactericidal activity of the 'Famidez Sanoksil 100' preparation against the *M. phlei* test culture was directly dependent on the concentration of the working solution and exposure time. At a 0.5% concentration of the disinfectant solution and exposure times of 5 h, 24 h, and 48 h, the drug exhibited only bacteriostatic properties against *M. phlei*.

Increasing the disinfectant solution concentration to 1.0% with exposure of 5 h and 24 h resulted in the growth of isolated *M. phlei* colonies. However, with a 48 h exposure, no colony growth was observed on the culture medium, indicating the culture's inactivation. No growth of *M. phlei* colonies was observed at a disinfectant solution concentration of 2.0% with exposure times of 24 h and 48 h or at a concentration of 3.0% with exposure times of 5 h, 24 h, and 48 h, indicating the bactericidal effect of the preparation.

No mycobacterial growth was observed in the negative control samples, whereas colony growth was recorded in the positive control, confirming the correctness of the experimental setup and the reliability of the results obtained.

The next stage involved conducting studies with a culture of the tuberculosis pathogen *M. bovis* on test objects (batiste, wood, tile), taking into account the biological load. The results of determining the bactericidal activity of the drug 'Famidez Sanoksil 100' against *M. bovis* on the test objects are presented in Table 2.

As shown in Table 2, the disinfectant 'Famidez Sanoksil 100' at a concentration of 3.0% exhibits pronounced bactericidal properties against *M. bovis* cultures on various test objects after 5 h, 24 h, and 48 h in the presence of a biological load. Meanwhile, the positive control exhibited mycobacterial colony growth (*M. bovis*), while the negative control showed no growth, confirming the experiment's validity. These results indicate the tested preparation's high tuberculocidal activity against the tuberculosis pathogen on various types of surfaces that may be contaminated under industrial conditions.

To confirm the results of the cultural study on the bactericidal activity of the disinfectant under investigation, a biological study was conducted on laboratory animals.

According to the results of the intradermal tuberculin test using a mammalian PPD, a positive reaction was recorded only in animals of the control group, while a postmortem examination of guinea pigs infected with swabs from test objects (positive control) revealed lesions characteristic of tuberculosis in their internal organs.

Table 2 — Bactericidal properties of the drug 'Famidez Sanoksil 100' against *M. bovis* on test objects

Culture	Concentration, %	Exposure, h	Test object	Experiment	Control	
					Negative	Positive
M. bovis	3.0	5	Batiste	–	–	++++
			Wood	–	–	++++
			Tile	–	–	++++
		24	Batiste	–	–	++++
			Wood	–	–	++++
			Tile	–	–	++++
		48	Batiste	–	–	++++
			Wood	–	–	++++
			Tile	–	–	++++

Notes: '++++' — intense colony growth; '+++' — growth of 50 colonies; '++' — growth of 20 colonies; '+' — growth of up to 10 colonies; '–' — no growth.

Cultural studies of pathological material collected from experimental and control animals allowed for the isolation of *M. bovis* cultures exclusively from animals in the control group. In the experimental animals, no reactions to intradermal tuberculin injections or pathological lesions were observed, and after the completion of the experiment, no mycobacterial cultures were isolated from the biological material.

Conclusions. It has been established that the bactericidal effect of an oxygen-containing disinfectant depends on the concentration of the working solution and the duration of exposure.

Complete inactivation of the *M. phlei* test culture was achieved with a 1.0% solution after 48 h, while a 2.0% solution provided a tuberculocidal effect as early as 24 h and 48 h.

The 3.0% solution was the most effective, ensuring a complete absence of mycobacterial growth after 5 h, 24 h, and 48 h of contact, as well as the effective decontamination of objects contaminated with the *M. bovis* culture.

Biological tests on laboratory animals confirmed the absence of viable mycobacteria after treatment with the disinfectant, indicating its high tuberculocidal activity.

Based on the results of the study on the bactericidal efficacy of the disinfectant 'Famidez Sanoksil 100', its use is justified for preventive and emergency disinfection of agricultural enterprise premises, including tuberculosis foci in cattle and poultry. The optimal application method is a 2.0% aqueous solution with a 24 h exposure time, or a 3.0% solution with exposure times of 5 h, 24 h, and 48 h. The consumption rate is 1,000.0 cm^3 per 1 m^2 of treated surface area.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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