Part 3. Biosafety

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ESTIMATION OF *MYCOBACTERIA* RESISTANCE TO DISINFECTING PREPARATION

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Summary. Disinfection is the important measure in tuberculosis control systems. It allows preventing further environmental *Mycobacteria* distribution. The aim of work was to determine the bactericide properties of new disinfectant 'Virosan Max' on *M. fortuitum* depending on the solution concentration, time exposure, and bactericidal effect on *M. bovis* at different test objects. The study was carried out using classical microbiological techniques, reference, and field *Mycobacteria* strains. Bactericidal properties of disinfectant preparation 'Virosan Max' was studied on *M. fortuitum* depending its solution concentration and exposure. In addition, tuberculocidal effect on *M. bovis* at different test objects determined. Bactericidal properties of disinfectant preparation with the test culture of atypical *Mycobacterium* of type *M. fortuitum* determined a suspension method with application 1.0% and 3.0% water solution for displays 3, 5, and 24 hours. Tuberculocidal property disinfectant preparation with the test culture of *M. bovis* conducted on contamination objects of (glass, ceramic tile, and wood) tests with the biological loading and application of in 3.0% and 4.0% concentrations of solution for displays 5–24 hours. The bactericidal effect disinfectant preparation 'Virosan Max' was determined at presence height colonies *Mycobacterium* on a dense egg nourishing environment in control test tubes and absence of height initial cultures *M. fortuitum*, *M. bovis* in experience test tubes. The 'Virosan Max' disinfectant shows bacteriostatic properties only against atypical mycobacteria *M. fortuitum* in 1.0% concentrations under 3–24 hours of exposure, and in concentration 3.0% under 3–5 hours of exposure. Preparation 'Virosan Max' demonstrates the bactericide effect on tuberculosis agent of bovine species *M. bovis* in a 3.0% concentration with 24 hours' exposure and 4.0% concentrations with 5–24 hours' exposure.

Keywords: bactericidal properties, disinfectants, *Mycobacterium bovis*, *Mycobacterium fortuitum*, test objects

Introduction. Disinfection of animal facilities is the one of methods for tuberculosis-prevention countermeasures system in the complex of veterinary activities tended to one of the units in epizootology — factors of agent transmission from the sick animal to the susceptible (healthy) animal and the agent destruction in the environment (Bezrukava, Nalyvaiko and Nalyvaiko, 2008).

Tuberculosis — is an infectious disease of animals, humans, birds, and sometimes cold-blooded animals. The disease occurs mostly in the chronic form and characterized by formation of non-vascular nodes (tubercules) in various organs and tissues subjected to the serous disintegration.

Mycobacterium bovis, *Mycobacterium tuberculosis*, *Mycobacterium avium* have been the causative agents of tuberculosis in humans and farm animals.

The basic source of tuberculosis agents are the sick animals which release mycobacteria with their secrets and excretes and infect the susceptible animals through the factors of transmission (manure, soil, water, feed-stuff, equipment, contaminated buildings for animals, personnel uniforms) producing the sources of TB re-infection (Zavhorodnii et al., 2007; Arkhipova and Bessonova, 2007).

The causative agent of tuberculosis in farm animals is *Mycobacterium bovis*, *Mycobacterium tuberculosis*, *Mycobacterium avium*. The main source of the TB pathogens is the infected animals that secrete *Mycobacteria* with secrets and excreta. Due to the transfer factors (manure, soil, remains of food, water, pastures, equipment contaminated by the causative agent for animals, overalls of service personnel), contamination of susceptible animals occurs and creates secondary sources of infection.

Various methods of disinfection are described: physical (high temperature, exposure, ultrasound, and high frequency current), chemical (chemical disinfectants in liquid, aerosol and gas states to be used both for the immediate treatment and in the special devices) and biological (microorganisms or their metabolic products to be used for biologic manure disinfection and arthropods and rodents control) have been used (Bublii, 2000).

The concentration and exposure of disinfectant effect are the basic conditions to influence on disinfection efficiency. The determination of its minimal concentration specifies microorganisms' devitalization and exposure directly depends on its concentration and bactericidal properties (Skrypnyk, 2007).

The result of bacterium cell contact with disinfectant agents depends not only from the structure of microorganisms and their resistance to the chemical factors but also from the capability of chemical preparation to reveal bactericidal effect from the one hand and bacteriostatic properties in another case from the other hand (Shishkov and Urban, 1991).

There are no drugs that would meet all the requirements for disinfectants. It is proved that a universal drug cannot be fully effective, safe and environmentally friendly for present time. Disinfectants based on only one of the available chemical groups have prospects for wide practical application. Proceeding from this, the question becomes the development of new drugs that are antitoxic, with a wide range of antimicrobial effects and do not have corrosive properties (Skrypnyk, 2007).

Therefore, before disinfection, it is necessary to select the appropriate disinfectant taking into account the type of disinfection, the object that will be disinfected and the presence of certain disinfectants on the farm.

Due to the fact that the disinfection regimes provided by the instructions and guidance do not always have a positive effect.

The 'Virosan Max' preparation is generally not recommended for disinfection of animal facilities suspected with Mycobacterial contamination.

Therefore, the purpose of our study was to determine the bactericidal properties of this disinfectant in relation to *M. fortuitum*, depending on the concentration of the solution and exposure action.

Tuberculocide action against *M. bovis*, *M. avium*, *M. scrofulaceum*, *M. smegmatis*, *M. phlei* on various test objects was compared with reference disinfectants previously studied by us.

Disinfection conditions provided by instructions and manuals are not always effective (Arzhakov and Arzhakov, 2009; Katoch, 2004) that's why the goal of our research to determine bactericidal properties of the new preparation 'Virosan Max' on *M. fortuitum* according to the solution concentration, exposure effect and tuberculocide effect on *M. bovis* at different test objects.

The aim of the work was to determine the bactericide properties of new disinfectant 'Virosan Max' on *M. fortuitum* depending on the solution concentration, time exposure, and bactericide effect on *M. bovis* at different test objects.

Materials and methods. The bactericide properties of disinfectant and test culture of atypical mycobacteria species *M. fortuitum* were studied by suspension method using 1.0% and 3.0% aqueous solutions under exposure for 3, 5, and 24 hours. Tuberculocide properties of disinfectant and test culture of atypical mycobacteria species and *M. bovis* were tested on contaminated test objects (glass, ceramic tile and wood) under bioburden conditions and by using 3.0% and 4.0% concentrations of disinfectant under 5–24 hours of exposure. Bactericidal effect of disinfectant 'Virosan Max' was conducted by presence of mycobacteria colonies growth on egg cultural media in control tubes and by absence of *M. fortuitum* and *M. bovis* growth in tested treated tubes (Zavhorodnii et al., 2007).

Results. The results of conducted experiments for the determination of bactericide properties of disinfectant 'Virosan' on *M. fortuitum* with the suspension method have been presented in Table 1.

Table 1 — Bactericidal properties of disinfectant 'Virosan Max' on *M. fortuitum*

Regime of use		Growth of colonies, days									
Solution concentration, %	Exposure, hours	Experiment					Control				
		5	8	21	28	30	5	8	21	28	30
1.0	3	+	+++	#	#	#	+++	#	#	#	#
	5	–	+	+++	#	#	++	#	#	#	#
	24	+	++	+++	#	#	++	#	#	#	#
3.0	3	–	++	#	#	#	++	#	#	#	#
	5	–	+	++	+++	#	+++	#	#	#	#
	24	–	–	–	–	–	++	#	#	#	#

Remark: '–' — the growth of colonies is absent, '+' — the growth to 10 colonies, '++' — the growth from 11 to 20 colonies, '+++' — the growth from 21 to 50 colonies, '#' — the growth more than 50 colonies of mycobacteria.

The primary growth of 3–5 colonies *M. fortuitum* on the culture medium has been noticed on the 5th day of incubation after treatment with disinfectant 'Virosan Max' in 1.0% concentrations and 3 hours' exposure. 30–50 colonies have been noticed on the 8th day in the test tubes on the medium surface. The primary growth of colonies from 3 to 8 has been found on the 8th day of cultivation, and the growth intensity increased from 25 to 50 colonies on the 21st day after contact with 'Virosan Max' in 1.0% solutions and 5 hours exposure. The growth of colonies on the culture medium surface increased and calculated more than 50 colonies in 28 days after cultivation of bacteria material treated with the preparation. The growth of 12–15 colonies *M. fortuitum* has been noticed on the 8th day after cultivation and their intensity increased to 50 colonies with a 3.0% solution 'Virosan Max' and 3 hours' exposure.

The growth of colonies *M. fortuitum* was not occurred in the result of 'Virosan Max' interaction in concentration of 3.0% with 24 hours exposure. The growth from 15 to 20 colonies *M. fortuitum* has been noticed in control test tubes on the culture medium on the 5th day and their amount counted from 50 colonies and more in 8 days after cultivation.

The results of conducted experiments testify that preparation 'Virosan Max' in 1.0% concentrations with 3–24 hours exposure and in a 3.0% concentration with 3–5 hours exposure has only bacteriostatic properties against atypical mycobacteria species (*M. fortuitum*). The bactericidal properties of disinfectant 'Virosan Max' on *M. fortuitum* have been shown in a 3.0% solution with 24 hours exposure. Further, the final determination of bactericidal properties of disinfectant 'Virosan Max' against tuberculosis agent *M. bovis* (*Vallee* strain) in the experiments at the test objects in 3.0% and 4.0% concentration with 5 and 24 hours exposure has been carried out.

The results to determine the bactericidal action of disinfectant 'Virosan Max' on *M. bovis* at the test objects have been presented in Table 2.

It was detected, that disinfecting preparation 'Virosan Max' in a 2.0% concentration with 5 hours exposure destroyed *M. bovis* on the contaminated test objects: glass and ceramic tile, whereas the growth of mycobacteria colonies on the culture medium has been noted from the scrapes on wooden test objects. The growth of colonies was not noticed on the test objects under the effect of 3.0% and 4.0% preparation with 5–24 hours exposure.

In the control samples taken from the scrapers of glass, ceramic tile and wood the growth of colonies has been observed on the 13^{th}–15^{th} days after cultivation, and on the 30^{th} day their intensity calculated more than 50 colonies; in the microscopy of smears taken from grown cultures stained by Ziehl-Neelsen's method there have been visible short red rods with turned edges.

Table 2 — Bactericidal activity of disinfectant 'Virosan Max' on *M. bovis* at the test objects

The regime of use		The name of test object			Control
Solution concentration, %	Exposure, hour	Glass	Ceramic tile	Wood	
3.0	5	–	+	+++	#
	24	–	–	–	#
4.0	5	–	–	–	#
	24	–	–	–	#

Remark: '–' — the growth of colonies is absent, '+' — the growth to 10 colonies, '++' — the growth from 11 to 20 colonies, '+++' — the growth from 21 to 50 colonies, '#' — the growth more than 50 colonies of mycobacteria.

Conclusion. The disinfectant 'Virosan Max' hills the tuberculosis agent *M. bovis* in a 3.0% concentration of aqueous solution with 24 hours' exposure and 4.0% concentrations with 5–24 hours exposure can be used for the preventive and forced disinfection of livestock buildings at a rate of 1,000 cm^3/m^3.

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