Part 2. Biosafety and emergent diseases

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BACTERICIDAL PROPERTIES OF MYCOBACTERIA DISINFECTANTS

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Summary. Bactericide properties of disinfectant preparation 'Virosan' have been determined on *M. fortuitum* depending on the solution concentration, exposure. Also tuberculocidal effect on *M. bovis* was determined at different test-objects. Preparation 'Virosan' has the bactericide effect on tuberculosis agent of bovine species *M. bovis* in a 2.0% concentration with 24 hours' exposure and 3.0–4.0% concentrations with 5–24 hours' exposure.

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

Introduction. Tuberculosis — is an infectious disease of animals, humans, birds and sometimes cold-blooded animals which occurs in many cases 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) making the sources of re-infection (Zavhorodnii et al., 2007; Arkhipova and Bessonova, 2007).

Tuberculosis is one of the most distributed zoonotic diseases among farm animals that causes great economic losses for the animal husbandries consisted from the losses by the productivity decreasing, planned or unplanned animal slaughter, carcasses utility, losses of reproduction value and also putting into practice additional diagnostic testing and veterinary sanitation measures. Economic losses caused by tuberculosis depend on the spread of disease in groups and efficiency of health-improving method and antiepizootic measures (Oshchepkov and Arzhakov, 2002; Eremeeva, 2009).

Tuberculosis agents are resistant to the various environmental factors and chemical compounds used for the prophylactic and forced disinfection. So, mycobacteria *M. bovis* have been remained viable for 21 months, and in the non-sterile soil — 1–1.5 months in the sterile soil (Kolychev, 1984), on the pastures they survive during the summer period (Kolychev, 1984; Kolychev and Karimova, 1984; Kadochkin, 1990; Vysotskiy, 2000), in the manure — 475 days, in the river water — 2 months, in the swamp water — 12 months, in the sea water — 200 days (Kislenko and Shepmov, 1972), in frozen meat — 45 days, in butter — to 300 days, in cheese — 260 days (Kirilova and Merkulova, 1990) and in milk — 14–18 days (Klebanova, 1931; Abdulin et al., 1969). In addition there have been some reports to certify that *M. bovis* preserved virulent properties for 24–26 months on the soil surface in depth 5–10 cm (Zvozchyk, 1966; Kislenko and Shepmov, 1972), and in the permafrost soils of Yakut for 27–30 months. These mycobacteria species have been viable in the barley grain — 1,358 days, in peas — 976 days, in wheat — 972 days, in the mixed feed — 793 days and preserved pathogenic properties 1,388, 883, 879 and 700, and in the unchanged bedding — 9.5 years (Kaplun and Kolyvanova, 1988; Dzyombak, 2011).

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

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 desinfection and arthropods and rodents control) have been used (Bublii, 2000).

Chemical disinfectants (chlorine containing preparations, alkaline, aldehyde, derivatives of guanidine series etc.) have been widely used for the prophylactic and forced disinfection as the solutions for the surface irrigation or as aerosols in veterinary practice. However, the quantity of preparations has not been fully satisfied with modern requirements the market. Studies by Oshchepkov and Arzhakov (2002) revealed that out of 54 strains 7 mycobacteria species from which 9 museum and 45 epizootic demonstrated the high resistance to the preparations widely used in veterinary medicine. All the tested strains have been resistant to nirtan, amfolan and soda lye; only the strains *M.B.5, M. intracellularae, M. smegmatis, M. bovis 8, M. bovis* 14 have been sensitive to chlorine containing preparations (chloramine-b, neutral hypochlorite calcium); the rest ones have been even resistant to 5.0% solutions of these preparations. All the strains have been also resistant to a 3.0% formaldehyde solution and 1.0% glutaraldehyde solution, and only *M. phlei*, *M. fortinum* and field strains (epizootic)of *M. bovis* have been resistant to a 4.0% formaldehyde solution. The alkaline formaldehyde solution (3.0%) concentration had no effect on any tested strains and a 3.5% solution showed a harmful effect on such strains as *M.B-5, M. smegmatis, M. bovis, M. bovis* 14; 4.0% solution — on all the strains. The field culture isolates *M. phlei, M. fortuitum* and *M. bovis* have been resistant to 1.0% — solution of alkaline glutaraldehyde and all sensible tested cultures of mycobacteria have been resistant to a 4.0% solution (Dekanosidze, 1986).

The efficiency of disinfectants against the on causative agent depends on many reasons and foremost on the bactericidal properties of these, concentration of solution and its temperature, characteristics and environment temperature in which the agent contacts with the disinfectant. The disinfectant has to be used in a proper way (Dekanosidze, 1986).

It has been obvious that the knowledge about agent's resistance in the environment is necessary for the correct using of various disinfectants to annihilate them (Subbotina, 1991). So, the data testify that the tuberculosis agent can be preserved in the environment with its virulent properties and stipulate tuberculosis in the susceptible animals. Continuous preservation of mycobacteria in the environment is always a threat for the new recurrence of tuberculosis infection. Hence, it has been necessary to carry on thorough disinfection of animal facilities and objects to control the tuberculosis agent (Oshchepkov and Arzhakov, 2002; Haverkort, 2003).

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

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

The aim of the work. Was to determine the bactericide properties of new disinfectant 'Virosan' on *M. fortuitum* depending on the solution concentration, time exposure, and anti-tuberculosis 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 0.5, 1.0, 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 1.0, 2.0, 3.0, and 4.0% concentrations of disinfectant under 5–24 hours of exposure. Bactericidal effect of disinfectant 'Virosan' 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.

The primary growth of 3–5 colonies *M. fortuitum* on the culture medium has been noticed on the 5th day of cultivation with after treatment with disinfectant 'Virosan' in 0.5 and 1.0% concentrations and 3 hours' exposure. 30–50 colonies have been noticed on the 8th day in the test tubes on the surface of medium.

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' in 0.5 and 1.0% solutions and 5 hours' exposure. The growth of colonies on the culture medium surface increased and calculated more than 50 colonies in 30 days after cultivation of bacteria material treated with the preparation. The growth of mycobacteria colonies has not been found in any cases on the surface of culture medium with the preparation use in 0.5 and 1.0% concentrations and 24 hours' exposure. 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' and 3 hours' exposure.

Table 1 — Bactericide properties of disinfectant 'Virosan' 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
0.5	3	+	+++	#	#	#	++	#	#	#	#
	5	–	+	+++	+++	#	++	#	#	#	#
	24	–	–	–	–	–	++	#	#	#	#
1.0	3	+	+++	#	#	#	+++	#	#	#	#
	5	–	+	+++	#	#	++	#	#	#	#
	24	–	–	–	–	–	++	#	#	#	#
3.0	3	–	++	#	#	#	++	#	#	#	#
	5	–	–	–	–	–	+++	#	#	#	#
	24	–	–	–	–	–	++	#	#	#	#

Notes: '–' — 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 growth of colonies *M. fortuitum* hasn't been recorded in the result of 'Virosan' interaction in concentration of 3.0%, with 5 and 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' in 0.5 and 1.0% concentrations with 3–5 hours' exposure and in a 3.0% concentration with 3 hours' exposure has only bacteriostatic properties against atypical mycobacteria species (*M. fortuitum*). The bactericidal properties of disinfectant 'Virosan' on *M. fortuitum* have been shown in 0.5 and 1.0% concentration with 24 hours' exposure and in a 3.0% solution with 5–24 hours' exposure.

Further, the final determination of bactericidal properties of disinfectant 'Virosan' on tuberculosis agent *M. bovis* (*Vallee* strain) in the experiments at the test objects in 1.0, 2.0, 3.0, and 4.0% concentration with 5 and 24 hours' exposure has been carried out.

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

Disinfecting preparation 'Virosan' in a 1.0% concentration with 5–24 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 has not been noticed on the test-objects under the effect of 2.0% preparation with 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 13th–15th days after cultivation, and on the 30th 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 action of desinfectant 'Virosan' on *M.bovis* at the test objects

The regime of use		The name of test object			Control
Solution concentration, %	Exposure, hours	Glass	Ceramic tile	Wood	
1.0	5	–	–	++	#
	24	–	–	++	#
2.0	5	–	–	+	#
	24	–	–	–	#
3.0	5	–	–	–	#
	24	–	–	–	#
4.0	5	–	–	–	#
	24	–	–	–	#

Notes: '–' — 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' hills the tuberculosis agent *M. bovis* in a 2.0% concentration of aqueous solution with 24 hours' exposure and 3.0 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 1000 cm^3/m^3.

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