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Research Article

Efficiency of Disinfectant Agent and Probiotic Preparation against Pathogen Microorganisms during Cleaning of Animal Living Houses

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ABSTRACT

This study evaluates the effectiveness of disinfectant preparation "Kristall-900"and probiotic preparation which used during the cleaning of animal living boxes. It was found that the disinfectant during surface treatment had a significant effect on the reduction of total number of microorganisms. While using the probiotic preparation the total number of microorganisms decreased, the greatest seeding was detected in washings from wall surfaces, and the smallest number of microorganisms was found in washings from drinking bowls.

Key words: Disinfection, Bacteria, Probiotic Preparation, Enterobacteria

INTRODUCTION

Well-being and quality of products depends largely on the hygienic condition of the premises of the enterprise, and one of the important elements of quality maintenance is the sanitation aspect, which is of a dual nature and is mainly expressed in savings. It is known that microorganisms are the object of disinfection, which lead to deterioration of the quality of the product and, eventually, to its deterioration (Tretyak *et al.*, 2017). In the modern world for the sanitary treatment of animal containment facilities, a huge amount of various kinds of disinfectants have been developed, each of which has both advantages and disadvantages. The disadvantage of all disinfectants is the nonspecific action of chemicals that kill both good and harmful microorganisms, as a result, a clean surface is created on which a rapid re-contamination (re-colonization) of pathogenic bacteria occurs (Tarabukina 2000; Aswathar 2008).

Disinfection gives a fast, but short and unstable period of reducing the number of microorganisms. In this case, bacteria, especially their pathogenic species, show a strong tendency to resistance to any substances that can damage or destroy them (Shkarin and Blagonravova, 2010).

In connection with the problems of resistance of pathogens to disinfectants that have arisen at the present time, their concentration and the frequency of processing are continuously increasing, which has a harmful effect on man and the environment due to harmful chemical ingredients in their composition (Illarionov *et al.*, 2010; Miroshnikova *et al.*, 2016).

The main advantage of using probiotics is that with their help, a stable solution to the problems of fighting pathogenic and opportunistic microorganisms was found, and the issues of sustainability in general were removed from the agenda.

MATERIALS AND METHODS

The Kristall-900 is a highly concentrated antiseptic agent with a wide range of action. It used for disinfection, cleaning and deodorizing, including disinfection of veterinary objects and prevention of infectious diseases of animals, as well as an effective cleaning agent for premises and equipment. The presence of aldehyde provides a deodorizing effect, removes the smell of urine and ammonia from the animal stalls and compartments. The Kristall-900 is effective against gram positive and gram negative bacteria (including vegetative and spore forms), viruses (both shell and non-envelope), fungi, yeast, molds and algae. It is not territogenic, carcinogenic, mutagenic and embryotoxic (Vysotsky, 2005). In working concentrations can be used to disinfect the room volume in the presence of animals. The concentration of the working solution of the agent depends on the method of application from 0.25% to 2%.

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Cultures were cultured for 16, 24 and 36 hours in a thermostat at 300°C, 370°C, and 420°C. On Petri dishes with selective medium grew colonies of white, beige, yellow, orange and green; with smooth and wavy edges; shine, dull and mealy colonies were revealed by gloss and transparency. Some colonies had antagonistic activity, which was evident from the lysis zone, which in some strains was 0.5 to 2 mm. If necessary, the cultures were further purified by the Gold method by seeding on agarized nutrient media. A total of 93 isolates were selected in the first stage and 137 with repeated screenings.

Statistical analyses

Statistical analysis was performed using Statistica 12.0 (STATISTICA, 2014; StatSoft Inc., Tulsa, OK, USA). The differences between samples were evaluated using ANOVA method. The differences were considered to be statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

At the first stage, we conducted experiments to test the effectiveness of the Kristall-900 disinfectant. Before the beginning of the experiment, the qualitative microflora of the tested surface was analyzed. For this purpose, flushes were taken from the objects that were plated on the plates with meat infusion agar medium, MRS-4. Species composition of isolated microorganisms from household items of livestock houses before treatment with a disinfectant is presented in Table 1.

The results of the analysis showed that the microflora of the surface under investigation before disinfection is mostly presented by gram positive and gram negative microorganisms, the species composition of microflora isolated from various surfaces in the hospital was as follows: *Bacillus subtilis, Proteus mirabilis, Proteus vulgaris, Bacillus coagulans, Enterococcus faecalis, Bacillus mycoides, Enterobacter sp, Klebsiella pneumonia, Bacillus thuringiensis, Escherichia coli Pantoeaagglomerans, Pseudomonas stutzeri, Pseudomonas stutzeri, Mycoides odoratus, Pseudomonascomposti,, Pseudomonas mendocina, Lactobacillus sp.* Species composition of isolated microorganisms from household items of cattle-breeding houses after treatment with disinfectant with sustained temporary exposure is presented in Table 2.

According to the data indicated in Table 2, it follows that, at 30 minute exposure, bactericidal activity against such microorganisms as *Enterobacter, E. coli, Pseudomonas stutzeri, Pseudomonas mendocina,* at 2 hours exposure*Ent. Faecalis, Enterobacter, E. coli, Pr. mirabilis, Klebsiella pneumonia,Pantoea agglomerans, Pseudomonas stutzeri, Myroides odoratus, Pseudomonas mendocina, Lactobacillus* is exhibited, at 24 hours exposure – *Ent. Faecalis, Enterobacter, E. coli, Pr. mirabilis, Klebsiella pneumonia,Pantoea agglomerans, Pseudomonas stutzeri, Myroides odoratus, Pseudomonas mendocina, Lactobacillus.*

In the course of the research, it was found that the disinfectant under investigation during surface treatment had a significant effect on the total number of microorganisms. At the second stage, we conducted experiments to test the effectiveness of the probiotic preparation Pip House Cleaner. Species composition of isolated microorganisms from household items of livestock houses after treatment with a probiotic detergent with a sustained time exposure is presented in Table 3.

As can be seen from the table, the total number of microorganisms decreased, the greatest seeding was detected in washings from wall surfaces, and the smallest number of microorganisms was found in washings from drinking bowls. In Table 3 we see the efficacy of Pip House Cleaner in 0.5 and 1% concentration. The data in Table 3 show that, at 30 minutes exposure, Pip House Cleaner exhibits efficacy against such microorganisms as *Myroidesodoratus*, at 2 hours – *Myroidesodoratus, Ent. Faecalis*, at 24 hours – *Myroidesodoratus, Ent. Faecali, Pr. Mirabilis, Pr. Vulgaris, Enterobacter, Pseudomonas composti.*

According to the data indicated in Table 3, it follows that, at 30 minutes exposure, it is effective against such microorganisms as *Pr. Mirabilis, Myroidesodoratus,* during 2 hourexposure*Pr. Mirabilis, Myroidesodoratus , Ent. Faecalis, E. coli,* 24 hour exposure*Pr. Mirabilis, Pr. Vulgaris, Ent. Faecalis, B. coagulans, B. mycoides, Enterobacter, Kliebcilla pneumonia, E. coli, Pantoeaagglomerans, Pseudomonas stutzeri, Mycoides*

Table 1: Qualitative composition of the microflora of the surfaces under investigation

Names of defined microorganisms	Name of objects of veterinary supervision				
	Floor	Feeding box	drinking bowl	wall	Metal partition
Bacillus subtilis	+	+	+	+	+
Proteus mirabilis	+	-	+	-	+
Proteus vulgaris	-	-	-	+	+
Bacillus coagulans	+	-	-	-	-
Enterococcus faecalis	+	-	-	+	+
Bacillus mycoides	+	-	-	+	+
Enterobacter sp	-	+	+	+	+
Klebsiella pneumonia	+	-	+	+	+
Bacillus thuringiensis	+	-	-	+	+
Escherichia coli	-	-	+	-	+
Pantoeaagglomerans	+	-	-	+	+
Pseudomonas stutzeri	-	-	+	+	+
Mycoides odoratus	-	-	-	-	+
Pseudomonascomposti	+	+	-	+	+
Pseudomonas mendocina	+	-	+-	+	+
Lactobacillus sp	+	+	-	+	+

Table 2: Results of the study of bactericidal effectiveness of Kristall-900 1% concentration

Nameof isolated microorganisms	Before treatment	After treatment		
		Time of exposure		
		30 minutes	2 hours	24 hours
B. subtilis	+	+	+	+
Pr. Mirabilis	+	+	-	-
Pr. Vulgaris	+	+	+	+
B. coagulans	+	+	+	+
Ent. Faecalis	+	+	-	-
B. mycoides	+	+	+	+
Enterobacter	+	-	-	-
Klebsiella pneumonia	+	+	-	-
Bacillus thuringiensis	+	+	+	+
E. coli	+	-	-	-
Pantoeaagglomerans	+	+	-	-
Pseudomonas stutzeri	+	-	-	-
Myroidesodoratus	+	+	-	-
Pseudomonas composti	+	+	+	+
Pseudomonas mendocina	+	-	-	-
Lactobacillus.	+	+	-	-

Table 3: Results of efficacy of 1% concentration of Pip House Cleaner

Nameof isolated microorganisms	Before treatment	After treatment		
		Time of exposure		
		30 minutes	2 hours	24 hours
B. subtilis	+	+	+	+
Pr. Mirabilis	+	-	-	-
Pr. Vulgaris	+	+	+	-
B. coagulans	+	+	+	-
Ent. Faecalis	+	+	-	-
B. mycoides	+	+	+	-
Enterobacter	+	+	+	-
Klebsiella pneumonia	+	+	+	-
Bacillus thuringiensis	+	+	+	+
E. coli	+	+	-	-
Pantoeaagglomerans	+	+	+	-
Pseudomonas stutzeri	+	+	+	-
Myroidesodoratus	+	-	-	-
Pseudomonas composti	+	+	-	+
Pseudomonas mendocina	+	+	+	-
Lactobacillus.	+	+	+	+

Table 4: Cultural and morphological properties of lactobacilli

	Growth on MRSC-1	Growth on MRS-4	Microscopy
Group 1	- uniform turbidity along the column, precipitate - opacity of the medium with a transparent ring from above,precipitate - side-wall growth is formed, precipitate - medium remains clear, precipitate	Colony diameter 0.1-0.2 cm, white, gray, smooth edges	Gr+ short, long rods, sometimes with rounded ends, coccoid, arranged differently
Group 2	- opacity of the medium with a transparent ring from above, precipitate - medium remains clear, precipitate - uniform turbidity along the column, precipitate	Diameter of the colony is 0.1 cm, gray, the edges are uneven, sometimes the center is expressed	Gr + large rods, arranged with chains, bend, curve

Table 5: Bacterial contamination of sick and healthy animals

Bacteria	Sick animal			Healthy animal		
	06.06.2015	04.07.2015	14.08.2015	06.06.2015	04.07.2015	14.08.2015
E.coli	8.39±0.26	6.14±0.22	5.46±0.24	8.3±0.23	4.39±0.22	3.76±0.26
Enterococcus	5.00±0.20	4.96±0.23	4.84±0.21	4.9±0.20	4.3±0.21	3.21±0.19
Bacillus	2.3±0.12	3.7±0.17	2.7±0.12	3.6±0.12	6.43±0.16	9.21±0.15
Lactobacillus	6.98±0.16	7.62±0.17	7.24±0.24	6.7±0.14	9.7±0.17	8.63±0.23
Bifidobacteria	5.22±0.22	6.65±0.16	8.12±0.15	6.32±0.22	8.42±0.16	8.73±0.15
Staphylococcus	1.79±0.22	1.53±0.25	1.26±0.17	1.2±0.21	1.3±0.23	1.2±0.17
Proteus	1.56±0.26	1.72±0.22	1.32±0.18	1.32±0.23	1.21±0.21	1.1±0.22

odoratus, Pseudomonas mendocina. To isolate probiotic cultures and to create a probiotic preparation, the work was carried out to single out isolates from the obtained samples. To obtain microorganisms of the genus *Lactobacillus*, there were selected isolates which were Gram-positive, non-spore immovable rods with rounded ends, showed active growth on medium MRS-1, lack of growth on meat-peptone agar, and were catalase negative.

Already from the results of the first studies it was obvious that the cultures we collected have rod-shaped lactic acid bacteria of the species *Lactobacillus spp*. Further, microscopy of the obtained isolates was carried out. The investigated lactic cultures were represented by rods, cocci, differing in length, thickness, and nature of location. Morphological and cultural features of lactobacilli were studied. By the nature of growth on solid nutrient media, all the cultures of lactobacilli studied were divided into 2 groups:

The first group was characterized by growth on the MRS-4 agar medium in the form of superficial round colonies with distinct edges, white or gray, ranging in size from petty to small. The second group, which was in the percentage ratio of less than 20% of the studied cultures, formed colonies with uneven edges, gray, often with a densified center. Growth in a liquid nutrient medium was characterized by a turbidity of the medium or a lack of formation of turbidity. The second group is predominantly represented by long, thick rods arranged singly, sometimes in short chains.

Thus, according to the morphological and cultural properties of lactobacillus, it can be conditionally divided into two main groups, differing in the nature of growth on dense and liquid nutrient media, and also in the microscopic picture. As a result, according to the phenotypic characteristics, 24 isolates of cultures were assigned to the genus Lactobacillus.

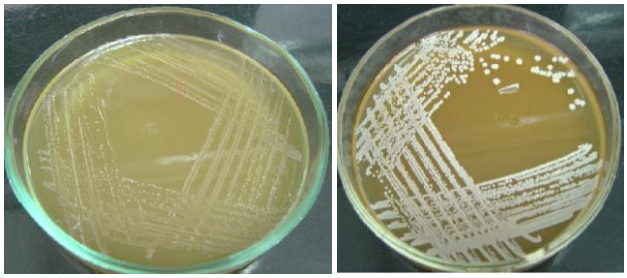

Fig. 1: Growth of colonies of isolated lactobacilli on medium MRS-4.

Spore-forming cultures were presumably born by the genus Bacillus. On meat-peptone agar, after 24 hours of incubation in a thermostat at 37°C, they formed flat, dry colonies of dense consistency with a characteristic white granular coating easily removed from the agar, the diameter of the colonies being 2.5 mm. The edges are almost slightly cut, which corresponds to the literary data. Also, cultures were isolated that, when grown on meat-peptone broth, gave abundant growth, forming a thin, leathery white film that grew on the walls of the tube. On the agarized wort the colonies are dirty white, round, with an uneven edge.

As a result, according to phenotypic traits, 19 isolates of cultures were assigned to the genus Bacillus. Characterization of the level of bacterial contamination of groups in the analysis of "Sick" and "Healthy" groups of animals showed differences in microbial composition (Table 5).

Conclusion

In the course of the studies, a lack of bactericidal activity was noted for sporeforming microorganisms of *B. subtilis, B. coagulans, B. mycoides, Pr. vulgaris, B. Thuringiensis,* whereas the bactericidal activity is high for enterobacteria.On the basis of the data obtained, the efficiency of the Kristall-900 disinfectant in a 0.5% concentration was 37%, with a 1% concentration of 62%. Thus, it can be concluded that the use of the proposed disinfectant of chemical nature does not fully ensure high-quality and safe disinfection of the treated surfaces. In the course of the research it was established that the qualitative composition of the microbiocenosis is represented by 7 families (*E.coli, Enterococcus, Bacillusspp., Lactobacilluspp., Bifidobacteriaspp., Staphylococcuspus., Proteusspp*).

Based on the data obtained, the dependence of the composition of various groups of the microorganism in the safe groups of animals over the disadvantaged was revealed. Thus, it was found that in the healthy group, the level of the representatives of the group of lactic acid bacteria was significantly higher, whereas in the group with sick animals the indices of such species as proteia and bacteria of the E. coli group were increased.

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