Rehydration mechanism of prokaryotic cells of the genus Salmonella by physiologically optimal diluent

Elena Valentinovna Svetlakova[1], Anatolii Nikolaevich Kononov[2], Marina Nikolaevna Verevkina[3], Nadegda Arkadevna Ogeredova[4], Alexsandr Nikolaevich Simonov[5]

FSBEI of HPE "Stavropol State Agrarian University" Zootechnicheski per., 12, Stavropol, 355017, Russia
[1]Stavropol State Agrarian University Mimoz Str., 22a, Stavropol, Russia
[2]Stavropol State Agrarian University Ynosti Prospekt, 9, Stavropol, Russia
[3] Stavropol State Agrarian University Gragdanskay Str.,16, Stavropol, Russia
[4]Stavropol State Agrarian University Pushkina Street, 20, Stavropol, Russia
[5]Stavropol State Agrarian University Prigorodnay Str., 213/3, Stavropol, Russia
e-mail: alenka6121970@mail.ru

Abstract. Reactivation of microbial cells is disrupted when water or saline solution are used as diluents of dry bacterial vaccines for homoiothermal animals. Physiologically optimal diluents are recommended to be used in such cases, i.e. isotonic diluents for microbial solutions with a concentration of sodium chloride of 0.08 mol/L with phosphate buffer basis for Salmonella vaccines. Furthermore, for better reactivation of microbial vaccines in dry vaccines we recommend to add 8-10% of meat-peptone broth to diluents.
[Svetlakova E.V.,Kononov A.N.,Verevkina M.N.,Ogeredova N.A., Simonov A.N. **Rehydration mechanism of prokaryotic cells of the genus Salmonella by physiologically optimal diluents**. *Life Sci J* 2014;12(12s):1008-1011] (ISSN:1097-8135). http://www.lifesciencesite.com. 218

Keywords: biological preparations, freeze-drying, rehydration, reactivation, diluent, physiologically optimal solution, distilled water, salmonella, sodium chloride, vaccines, phosphate-buffered saline, meat-peptone broth, beef extract agar.

Introduction

Sterile distilled water and cooled boiled water or saline are used in biotechnological industry for dissolving lyophilized vaccines and preparing dry biological preparations. The main disadvantage of such diluents is that they serve as hypotonic solution for living bacteria in which a part of bacteria die upon rehydration and reactivation due to excessive addition of large quantities of water (condition called plasmoptisis) and so preparations become to be less active [1, 2, 3].

Main part

It is known that sodium chloride solution at the concentration of 0.15 mol/L is used as diluent for dry living biological preparations (vaccines) (Instruction on the use of dry live vaccines swine erysipelas, strain BP-2; swine salmonellosis of the suppressing reverant Salmonella choleraesuis No. 9; against listeriosis of farm animals, AUF strain) [4, 5]. It was found that the main disadvantage of these methods is that that 0.15 mol/L concentration of sodium chloride solution is isotonic to homoiothermal animals. At the same time this solution is hypertonic for the majority of living microbial cells contained in dry antibacterial vaccines. In such sodium chloride concentration some of the bacteria are killed due to the outflow of free water from the cytoplasm to the external environment, i.e. in the direction of higher salt concentration (condition called plasmolysis). This makes preparations from living microorganisms less active.

In 1984 F. Gerhard proposed to use phosphate-saline solution comprising 8.5 g of NaCl, 0.3 g of anhydrous KH_2PO_4, 0.6 g of anhydrous Na_2HPO_4, 0.1 g of gelatin and distilled water to a volume of 1 liter as a diluent of dry live vaccines. After performing analysis it was found that the disadvantage of this method is that the sodium chloride concentration of 0.15 mol/L can be considered as hypertonic medium for most bacteria, causing partial loss of these microbial cells. Thus this can reduce the activity of biological preparations containing living microorganisms. This also doesn't provide for reactivation of living microbial cells from anabiotic state after freeze-drying of preparation.

The main purpose of our research was to obtain physiologically optimal diluent of dry live bacterial vaccines, with maximum preservation of living microbial cells and their reactivation upon resuspension in such a diluent.

In order to achieve this goal, we made a phosphate saline diluent containing physiologically optimal amount of ingredients for microorganisms. For preparation of such diluent of dry live bacterial vaccines we used 1.8 g of anhydrous KH_2PO_4, 9.5 g of anhydrous Na_2HPO_4 and 100 ml of meat-peptone

broth containing 200 mg/kg of amine oxide, 1.6% of peptone, and 70-100mg/kg tryptophan, and distilled water up to the volume of 1 liter. To this mixture we added sodium chloride in the concentration of 0.08 mol/L. In the resulting diluent we adjusted pH to 7.2-7.4 followed by sterilization at 120 °C for 40 minutes. After sterilization we took the sample of diluent and defined the value of its pH, the amount of amine nitrogen, peptone and sodium chloride.

It was found that the prepared diluent of dry living bacterial vaccine was transparent standard with the following content: amine nitrogen 200-220 mg/kg; peptone 0.024 mol/kg; tryptophan 100-120 mg/kg; sodium chloride 0.05 mol/L and pH 7.0-7.2.

During preparation of a diluent for dry live bacterial vaccines, rehydration and reactivating of microbial cells and standardization of preparations we carried out comparative experimental studies. We found that the use of distilled water or sodium chloride at the concentration of 0.15 mol/L as a diluent for live dry vaccines decreases the viability of bacteria. At the same time this solution is isotonic for homoiothermal animals.

Experiments to test various diluents for dry monovalent vaccines made from the following serovars of Salmonella: Salmonella typhimurium, S. dublin, S. choleraesuis and bivalent vaccine made from serovars Salmonella typhimurium and S. choleraesuis (in equal proportions) were performed in triplicate. Traditionally recommended diluents of the vaccines were tested primarily, i.e. mentioned vaccines were diluted in sterile distilled water and sodium chloride at the concentration of 0.15 mol/L. Research results were compared with the data on the persistence of viable cells after dilution of vaccines with sodium chloride solution isotonic for microbial cells in distilled water with its concentration of 0.08 mol/L.

Experiments were performed on the same series of vaccines using appropriate diluents. To do this we made decimal dilutions of preparations 10^{-1} to 10^{-8}. After 30-40 minutes of exposure from dilutions 10^{-7} - 10^{-8} we performed inoculations on the meat-peptone broth (MPA) in Petri dishes (five cups for each dilution). Incubation was performed in a thermostat at the temperature 37 °C. After appearance of microbial growth, colony counting was carried out in 24 hours, and then we determined the number of viable microbial cells (billion/ml) by conventional microbiological methods. The results of the studies are presented in Table 1.

Table 1. Number of living Salmonella upon their rehydration from the dried vaccines with various diluents

	Vaccines comprising serovariants							
	S.typhimurium		S. dublin		S. choleraesuis		S. choleraesuis S.typhimurium	
	milliard/ml	%	milliard/ml	%	milliard/ml	%	milliard/ml	%
Conventional diluents								
Distilled water	5.1 ± 0.03 *	91.5 ± 2.1 *	3.32 ± 0.02 *	91.2 ± 1.4 *	6.5 ± 0.3 *	76.2 ± 1.4 *	3.83 ± 0.04 *	90.5 ± 3.2 *
NaCl solution at the concentration of 0.15 mol/L	5.35 ± 0.03	96.5 ± 3.6	3.48 ± 0.03 *	95.4 ± 0.2 *	6.85 ± 0.2 *	79.9 ± 0.4 *	4.03 ± 0.1 *	95.3 ± 0.7 *
Isotonic solution for microbes (control)								
NaCl solution at the concentration of 0.08 mol/L	5.57 ± 0.1 *	100 ± 0.01 *	3.64 ± 0.04 *	100 ± 0.01 *	8.57 ± 0.18 *	± 100 0.01 *	4.23 ± 0.05 *	100 ± 0.01 *

Note: * - p <0.05.

Table 1 shows that in our experiments sodium chloride solution with sodium chloride concentration of 0.08 mol/l can be considered as isotonic solution for microbes. In this solution the preservation of Salmonella is better as compared to distilled water and sodium chloride at the concentration of 0.15 mol/L. Thus, viability of Salmonella typhimurium, S. dublin, and S. choleraesuis upon the dilution of monovalent vaccine with distilled water as compared with a diluent of sodium chloride (control) at the concentration of 0.08 mol/L was below 8.5; 8.8 and 23.8%, respectively. Upon the dilution of monovalent vaccines with sodium chloride at the concentration of 0.15 mol/L the content of viable Salmonella was somewhat higher than that in the previous version, but lower than that in the control by 3.5; 4.4 and 20.1%. In the bivalent vaccine consisting of S. typhimurium and S. choleraesuis preservation of the viable microbial cells was lower as compared to the control upon the dilution with distilled water by 9.5% and dilution with sodium chloride at the concentration of 0.15 mol/L by 4.7 %.

Convinced in the probability of theoretical and methodological approach to the studied problem, we have found it necessary to develop such diluent for microorganisms on the basis of an isotonic solution. This diluent will ensure better preservation and reactivation of living microbial cells and would be universal for most living dry biological preparations. For this purpose in order to achieve better reactivation of the microbial cells we added meat-peptone broth (BCH) at the concentration of 8, 10 and 12% to isotonic solution of sodium chloride at the concentration of 0.08 mol/L. Results of the study are shown in the Table 2.

Table 2. Number of live Salmonella cells upon their rehydration from the dried vaccines with diluents based on sodium chloride in the concentration of 0.08 mol/L depending on the content of meat-peptone broth.

Contents of meat-peptone broth %	Vaccines comprising serovariants							
	S. typhimurium		S. dublin		S.choleraesuis		S.choleraesuis S. typhimurium	
	milliard/ml	%	milliard/ml	%	milliard/ml	%	milliard/ml	%
8	6.68 ± 0.01 *	97.8 ± 0.16 *	4.47 ± 0.02	98.7 ± 0.08	9.78 ± 0.08 *	98.9 ± 0.08 *	4.79 ± 0.008 *	98.2 ± 0.14 *
10	6.83 ± 0.01	100.0 ± 0.001 *	4.48 ± 0.01	100 ± 0.00 1	9.89 ± 0.01 *	100 ± 0.001 *	4.82 ± 0.006 *	100 ± 0.00 1 *
12	6.74 ± 0.004 *	98.7 ± 0.08 *	4.40 ± 0.004 *	98.2 ± 0.01 *	9.8 ± 0.1 *	99.1 ± 0.08 *	4.82 ± 0.006 *	98.8 ± 0.34 *

Note: * - p <0.05

These tables show that there's no considerable difference in the quality of diluents consisting of sodium chloride at the concentration of 0.08 mol/L, 8, 10, 12% meat-peptone broth is not available. However, 10% concentration of meat-peptone broth considered as a control by the results of the second experiment was the most optimal solution for each version of the vaccines. The number of viable cells was higher by 0.9-1.8% as compared to rehydrated vaccine by diluents with the same basis, with a content of meat-peptone broth of 8% and 12% (P <0.001).

The selected diluent for rehydratation of salmonella from the dried vaccine and consisting of 0.5% sodium chloride solution in distilled water with the inclusion of 10% meat-peptone broth is the most optimal and versatile for many dry bacterial vaccines.

Determination of the viability of Salmonella was carried out in the same series of vaccines according to the procedure described in the first experiment. The obtained results are presented in the Table 3.

Table 3. Number of live Salmonella upon rehydration from the dry live vaccines with diluents based on sodium chloride at the concentration of 0.08 mol/L and 10% of the meat-peptone broth.

Diluents	Vaccines comprising serovariants							
	S.typhimurium		S.dublin		S.choleraesuis		S.choleraesuis S.typhimurium	
	milliard/ml	%	milliard/ml	%	milliard/ml	%	milliard/ml	%
NaCl solution at the concentration of 0.08 mol/L (control)	5.6 ± 0.4	100 ± 0.01	3.55 ± 0.2 *	100 ± 0.01	8.57 ± 0.18 *	± 100 0.01	2.3 ± 0.2	± 100 0.01
NaCl solution at the concentration of 0.08 mol/l +10% of meat-peptone broth	6.65 ± 0.2 *	120.2 ± 0.1 *	4.4 ± 0.2 *	120.6 ± 1.8 *	9.76 ± 0.18 *	113.8 ± 1.14 *	2.73 ± 0.28 *	118.4 ± 2.4 *

Note: * - p <0.05

Analysis of the data from the Table 3 demonstrates that on addition of 10% meat-peptone broth to sodium chloride at the concentration of 0.08 mol/L the number of live Salmonella serovars increases as compared to the control by 14-20%. All the obtained results of the experiments, except for the bivalent vaccine are statistically significant.

In medical and veterinary industry there's a large amount of dry bacterial preparations from pure cultures containing live microbial cells and viruses. This is mainly dry bacterial vaccines consisting of attenuated strains of microorganisms (anthrax vaccine from the strain "55 VNIIVViM", vaccine against swine erysipelas from the strain BP-2; vaccine against listeriosis from the strain "AUF"; brucellosis vaccine from the strains "19", "82" and others) and probiotics, which are pure cultures of lactic acid bacteria (including Bifidobacteria). The former ones are currently widely used in medical and veterinary practice for the treatment of animals and humans suffering from dysbacterioses and other diseases [6, 7, 8].

Before using such biological products according to the current instruction, one needs to dilute the contents of the vial or ampoule with cooled boiled, distilled water or sterile sodium chloride at the concentration of 0.15 mol/L. However, many veterinarians named these liquids as solvents of probiotic vaccines and probiotics. However, it is proved that one can't solve bacterial cells in these preparations with boiled or distilled with water as they may die. All meaning of using dry biological preparations is to keep contained microbial cells *in vivo* and thus preserve their living condition. Therefore, water or sodium chloride solutions should not be regarded as solvents of vaccines and probiotics. On the contrary, these substances should be considered as diluents, providing primarily rehydration and reactivation of living microbial cells in biological preparations being in the state of anabiosis after freeze-drying. In these biological preparations the recommended liquids dissolve only a filler of bacterial mass. The former substance is added to the biomass prior to freeze-drying of the preparation in the form of so-called protective medium.

Our point of view on the application of different liquids for the dilution of bacterial preparation is that on this step no physiological properties of the microorganisms are taken into account. We have found that the isotonic (physiologically optimal) solution of sodium chloride for the majority of bacteria can be considered at the concentration of 0.08 mol/L. Therefore, when developing new kinds of medium for microbes, NaCl

is added to its composition in indicated concentrations.

However, excluding physiological needs of microorganisms in the quality of rehidrantes it is recommended to use boiled tap water as well as sodium chloride solution at the concentration of 0.15 mol/L. A significant shortcomings of these rehidrantes is that boiled tap water, and especially the distilled one, is a hypotonic media in which most of the bacteria can't survive. For this reason ready biological preparation considerably loses its activity due to plasmoptis of the microbial cells [9, 10].

Physiological saline solution in a concentration of 0.15 mol/L, which is recommended for homoiothermal animals for diluting dry live vaccines, is a hypotonic solution for live microbial cells. Due to this many cells also die on rehydratation (50%) because of the outflow of water from the cytoplasm to the outside environment (the condition called plasmolisis), which also leads to a considerable loss of drug activity [11, 12].

Conclusion

Thus the sodium chloride solution with its concentration of 0.08 mol/liter is physiologically optimal solution for dilution of dry live vaccines against salmonellosis in farm animals. On addition of meat-peptone broth of different concentrations to these solutions cell viability increases and the recommended diluents become more effective and physiologically more optimal for dry living biologal preparations. They're enriched with nutrients (proteins, amino acids, etc.), which are beneficial not only to rehydrate the cells of microorganisms, but also to promote their reactivation.

Corresponding Author:
Dr.Svetlakova Elena Valentinovna
FSBEI of HPE "Stavropol State Agrarian University"
Zootechnicheski per., 12, Stavropol, 355017, Russia
e-mail: alenka6121970@mail.ru

References

1. Patent "Concentrated diluent for virus vaccines" of 10.02.2002 Volume 2179035 RF, A61K39/12.
2. Patent "Artificial physiological saline and its production method" of 10.04.2008 (date of application transfer 27.09.2006) Volume 2352342.
3. Pavlenko, I.V., 2003. Development of the main technological processes in industrial production of dry vaccine against listeriosis of farm animals, thesis of Candidate of Biological Sciences, Moscow, pp: 123.
4. Glazer, A.N., and H. Nikaido, 2007. Microbial Biotechnology: Fundamentals of Applied Microbiology, Second Edition. Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo: Cambridge University Press, pp: 576.
5. Smith, J.E., 2009. Biotechnology, Fifth Edition. Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo, Delhi: Cambridge University Press, pp: 280.
6. Gusev, V.V., 2005. The development of technology for production of a vaccine against salmonellosis of pigeons, thesis of Candidate Biological Sciences, Obolensk, pp: 117.
7. Solovyov, B.V., 2001. Technology of industrial production of culture vaccines against Marek's disease and infectious bursal disease, thesis of PhD of Biological Sciences, Moscow, pp: 325.
8. Potemkin, A.A., T.A. Avdeeva, T.B. Slepenko and E.F. Tokarick. Patent "Diluent for the dry virus vaccine against Marek's disease" Volume SU 563175 - A61K39/12C12K5. Medicinal preparations containing antigens or antibodies (materials for immunoassay G01N 33/53)
9. Sidorov, Y.I., 2012. Industrial freeze-drying devices of the periodical action in biotechnology. Biotechnology, Vol.5., 6: 39-46.
10. Avni, M. and A.M. Avunduk, et al, 2003. The Comparison of Efficacies of Topical Corticosteriods and Nonsteroidal Anti-inflammatory Drops on Dry Eye Patients: A. Clinical and Immunocytochemical Study. American Journal of Ophthalmology, Elsevier Science, 136(4): 593-602.
11. Svetlakova, E.V., 2003. Improving ways for increasing the survival rate of microbial cells in dry live vaccines and probiotics, thesis of Candidate of Biological Sciences, Krasnodar, pp: 20.
12. Stability Testing of New Drug Substances and products. Q1A (R2). ICH Harmonised Tripartite Guideline, 2003. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), pp: 22.

8/28/2014