https://doi.org/10.26898/0370-8799-2021-5-14
УДК: 573.6.086.83:615.371
Тип статьи: оригинальная
Type of article: original

КОНСТРУИРОВАНИЕ И ИЗУЧЕНИЕ ИММУНОГЕННОСТИ ВИРУС-ВАКЦИНЫ ПРОТИВ ВИРУСНЫХ ПНЕВМОЭНТЕРИТОВ ТЕЛЯТ

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Изучена на лабораторных животных иммуногенность вирус-вакцины против вирусных пневмоэнтеритов молодняка крупного рогатого скота. Представлены результаты подбора оптимальных вакцинных штаммов по изучению действия различных инактивантов на вирус инфекционного ринотрахеита, вирусной диареи, парагриппа-3, респираторно-синтициального вируса, ротавируса и коронавируса. Накопление авирулентных вакцинных штаммов вирусов проводили с использованием общепринятых вирусологических методов на перевиваемых культурах клеток МДБК (клеток почек теленка) и СПЭВ (клеток почки эмбриона поросенка). Для отработки режимов инактивации вакцинных штаммов – компонентов экспериментальной вакцины – в качестве инактивирующих веществ использовали теотропин и формалин. Изучены антигенная активность аттенуированных штаммов вирусов инфекционного ринотрахеита, вирусной диареи, парагриппа-3, респираторно-синтициального вируса, ротавируса и коронавируса на белых мышах и телятах и уровень специфических антител в сыворотках крови морских свинок, иммунизированных вакциной с использованием разных адъювантов. При конструировании новой вирус-вакцины против вирусных пневмоэнтеритов использованы авирулентные штаммы вирусов: инфекционного ринотрахеита (ИРТ-ВБФ-ВГАВМ № 404); диареи (ВД-ВБФ-ВГАВМ № 406); парагриппа-3 (ПГ-ВБФ-ВГАВМ № 403); респираторно-синцитиального вируса (РСВ-ВБФ-ВГАВМ № 405); ротавируса (РТВ-ВБФ-ВГАВМ № 401) и коронавируса (КВ-ВБФ-ВГАВМ № 407). Выбранные вакцинные штаммы нереактогенные, вызывают активную выработку противовирусных антител в достаточно высоких титрах как у лабораторных животных (белые мыши), так и у сельскохозяйственных (крупный рогатый скот). Наиболее эффективным инактивантивирующим веществом является 0,1%-й теотропин и 0,2%-й формалин. При подборе оптимальных адъювантов для конструирования вирус-вакцины использовали два вида масляных адъювантов – ИЗА 15 и ИЗА 25. Адъювант ИЗА 15 использован в количестве 15% от количества антигенов, ИЗА 25 – 25%. Оптимальным адъювантом при изготовлении экспериментальной вирус-вакцины против вирусных пневмоэнтеритов молодняка крупного рогатого скота является адъювант ИЗА 15 в 15%-й концентрации.

Ключевые слова: вакцина, инактивант, адъювант, культура клеток, пневмоэнтериты, телята

DESIGN AND STUDY OF IMMUNOGENICITY OF VIRUS VACCINE AGAINST VIRAL PNEUMOENTERITIS OF CALVES

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The immunogenicity of a virus vaccine against viral pneumoenteritis in young cattle was studied in laboratory animals. The results of the selection of optimal vaccine strains to study the effects of different inactivants on infectious rhinotracheitis virus, viral diarrhea, parainfluenza-3, respiratory

syncytial virus, rotavirus and coronavirus are presented. The accumulation of avirulent vaccine virus strains was carried out using established virological methods on transplanted cell cultures of MDBC (calf kidney cells) and SPEV (fetal pig kidney cells). Theotropine and formalin were used as inactivating agents to develop inactivation regimes for vaccine strains - components of the experimental vaccine. The antigenic activity of attenuated strains of infectious rhinotracheitis virus, viral diarrhea, parainfluenza-3, respiratory syncytial virus, rotavirus and coronavirus on white mice and calves and the level of specific antibodies in the sera of guinea pigs immunized with vaccines using different adjuvants were studied. Avirulent strains of viruses were used in the design of a new virus vaccine against viral pneumoenteritis: infectious rhinotracheitis (IBR-VBF-VSAVM No. 404); diarrhea (VD-VBF-VSAVM No. 406); parainfluenza-3 (PG-VBF-VSAVM No. 403); respiratory syncytial virus (RSV-VBF-VSAVM No. 405); rotavirus (RTV-VBF-VSAVM No. 401) and coronavirus (CV-VBF-VSAVM No. 407). The selected vaccine strains are non-reactive and induce active production of antiviral antibodies in sufficiently high titres in both laboratory animals (white mice) and farm animals (cattle). The most effective inactivating agents are 0.1% theotropine and 0.2% formalin. Two types of oil-based adjuvants, ISA 15 and ISA 25, were used to select the optimal adjuvants for the design of the virus vaccine. Adjuvant ISA 15 was used at 15% of the antigen quantity, ISA 25 at 25%. The adjuvant IZA 15 at a concentration of 15% is the optimum adjuvant for the preparation of an experimental virus vaccine against viral pneumoenteritis in young cattle.

Keywords: vaccine, inactivant, adjuvant, cell culture, pneumoenteritis, calves

Для цитирования: *Красочко П.А., Понаськов М.А.* Конструирование и изучение иммуногенности вирус-вакцины против вирусных пневмоэнтеритов телят // Сибирский вестник сельскохозяйственной науки. 2021. Т. 51. № 5. С. 117–123. https://doi.org/10.26898/0370-8799-2021-5-14

For citation: Krasochko P.A., Ponaskov M.A. Design and study of immunogenicity of virus vaccine against viral pneumoenteritis of calves. *Sibirskii vestnik sel'skokhozyaistvennoi nauki = Siberian Herald of Agricultural* Science, 2021, vol. 51, no. 5, pp. 117–123. https://doi.org/10.26898/0370-8799-2021-5-14

Конфликт интересов
Авторы заявляют об отсутствии конфликта интересов.
Conflict of interest
The authors declare no conflict of interest.

INTRODUCTION

Currently, pneumoenteritis of young cattle of viral etiology is widespread in livestock complexes and farms [1, 2]. According to N.A. Kovalev et al. and E.V. Sussky et al., the incidence of pneumoenteritis in newborn calves reaches from 214 to 260% of the number of births. Respiratory diseases of viral etiology account for 33 to 60% and gastrointestinal diseases for 55-70% of all cases in calves. Lethality from these diseases is high and varies from 45 to 70%[1] [3–5].

However, these infections most often develop in association form, where two or more infectious pathogens are involved in the infection process, causing a more severe course of the disease with a high mortality rate. The most frequently diagnosed pathogen associations are IBR + VD (infectious rhinotracheitis + viral diarrhoea); IBR + PI-3 (parainfluenza-3); IBR + PI-3 + VD; IBR + VD + RS (respiratory syncytial virus); rota + corona virus + VD [4, 5].

Timely diagnosis to assess the etiological structure of the infectious agents[2] (see footnote 1) plays an important role in anti-epizootic measures. [1].

In industrial livestock farming, the main effective way to prevent further spread of viral infections in calves is specific prevention which is based on the use of virus vaccines and hyperimmune sera or globulins [4-7].

[1]Krasochko P.A., Krasochko I.A. Diagnostics, prevention and therapy of respiratory gastrointestinal diseases of young animals. Problems of pathology, sanitation and infertility in animal husbandry: materials of international scientific and practical conference devoted to the 100th anniversary of H.S. Goregliad and M.K. Yuskovets. Vitebsk, 1998. pp. 15-18.

[2]Gromov I.N., Prudnikov V.S., Krasochko P.A., Motuzko N.S., Zhurov D.O. Sampling for laboratory diagnosis of bacterial and viral diseases of animals: guidance manual. Vitebsk, 2020.

Currently, the biological industry of the Republic of Belarus produces only two associated vaccines - multivalent inactivated viral vaccine against infectious rhinotracheitis, viral diarrhoea, rota-, coronavirus infection of cattle "Tetravak" and viral vaccine live culture against infectious rhinotracheitis, viral diarrhoea, parainfluenza-3. "Kombovak" vaccine (vaccine against infectious rhinotracheitis, parainfluenza-3, viral diarrhoea and respiratory syncytial, rota- and coronavirus infection in cattle) produced by SPU "Vetbiokhim" (Russia) is widely used in the market of biological preparations used in animal husbandry.

Modern agriculture of the republic needs biopreparations with a wider range of pathogens, which also include parainfluenza-3 and respiratory syncytial virus. In this regard, the design of domestic virus-vaccine against infectious rhinotracheitis, viral diarrhea, parainfluenza-3, respiratory syncytial, rota-, coronavirus infection in cattle is an urgent task [1, 8, 9].

The use of highly immunogenic virus strains, the development of optimal means of virus inactivation and the use of adjuvant immunostimulants to improve the efficiency of the immune response are important in vaccine design [3, 5, 7, 8, 10, 11].

In the process of work, we developed polyvalent inactivated viral vaccine against infectious rhinotracheitis, viral diarrhoea, parainfluenza-3, respiratory syncytial, rota and coronavirus infection in cattle "Bolshevak", studied its effect on immunity and metabolic processes in animals[3] (see footnote 2) [2, 5, 9-11].

The aim of the study was to investigate the immunogenicity of the viral vaccine against viral pneumoenteritis in young cattle in laboratory animals.

MATERIAL AND METHODS

Studies were conducted in the conditions of the Department of Epizootology and Infectious Diseases, vivarium, as well as in the branch laboratory of veterinary biotechnology and contagious diseases of animals of VSAVM, Research Institute of Applied Veterinary Medicine and Biotechnology of the Vitebsk State Academy and in livestock farms of Vitebsk region (Republic of Belarus).

A comparative study of the infectious activity of the following avirulent vaccine virus strains was carried out in the design of the viral vaccine against viral pneumoenteritis: infectious rhinotracheitis (IBR-VBF-VSAVM No. 404 and KMIEV-6), diarrhoea (VD-VBF-VSAVM No. 406 and KMIEV-7), parainfluenza-3 (PI-VBF-VSAVM No. 403 and KMIEV-8), respiratory syncytial virus (RSV-VBF-VSAVM No. 405 and RSV), rotavirus (RTV-VBF-VSAVM No. 401 and KMIEV No. 3) coronavirus (CV-VBF-VSAVM No. 407 and KMIEV No. 1)

The accumulation of avirulent vaccine virus strains was carried out using common virological methods on transplanted cell cultures of MDBC (calf kidney cells) and SPEV (fetal pig kidney cells).

Theotropin and formalin were used as inactivating agents to develop modes of inactivation of vaccine strains, the components of the experimental vaccine. For this purpose, inactivating agents in various concentrations (from 0.1 to 0.5%) were added to the pre-titrated virus-containing liquid.

The exposure of vaccine strains to inactivant was 12 and 24 hours. After 6, 12 and 24 hours, samples of virus-containing material were taken and the completeness of inactivation in cell cultures was studied in two to three passages. An indicator of complete inactivation was the presence of CPD (cytopathic action - the occurrence of degenerative changes in cell cultures) after the virus came into contact with the inactivant.

Two types of oil-based adjuvants, ISA 15 and ISA 25 (Montide, Seppic, France), were used to select the optimal adjuvants for the design of the virus vaccine. The adjuvant ISA 15 was used in an amount of 15% of the number of antigens, and ISA 25 in an amount of 25%.

To evaluate the efficacy of the adjuvants, studies were carried out on guinea pigs. For this purpose, three groups of guinea pigs of

[3]Krasochko P.A. Mono- and associative viral respiratory infections in cattle (immunological diagnosis, prevention and therapy): thesis of the Doctor of Science in Veterinary Medicine. Minsk, 1997. 45 p.

five animals each were formed according to the paired-analysis principle. Guinea pigs of experimental group 1 received intramuscularly 0.5 cm^3 of the developed vaccine with ISA 15 adjuvant two times at 21-day intervals into the inner thigh surface, those of experimental group 2 received 0.5 cm^3 of the developed vaccine with ISA 25 adjuvant, and those of control group 3 received a placebo. Blood serum samples were taken from guinea pigs of all groups 21 days after re-injection. The titer of antiviral antibodies in serum samples was determined by RIPR.

RESULTS AND DISCUSSION

The results of the comparative study of the selected vaccine strains infectivity are presented in Table 1.

The following avirulent virus strains should be used in the design of vaccines against viral pneumoenteritis in young cattle: Infectious rhinotracheitis (IBR-VBF-VSAVM No. 404); diarrhoea (VD-VBF-VSAVM No. 406); parainfluenza-3 (PI-VBF-VSAVM No. 403); respiratory syncytial virus (RSV-VBF-VSAVM No. 405); rotavirus (RTV-VBF-VSAVM No. 401); coronavirus (CV-VBF-VSAVM No. 407).

The results of the tests to determine the completeness of virus inactivation are shown in Table 2.

In studies of the effect of inactivating agents on ECD cell culture, it was determined that the use of formalin in concentrations above 0.3% and theotropin above 0.1% caused degeneration of the monolayer.

The use of the studied inactivating agents in low concentrations (0.1% tetropin, 0.2% formalin) was found to inactivate viruses of infectious rhinotracheitis, diarrhoea, parainfluenza-3, respiratory syncytial virus, bovine rotavirus and coronavirus.

The results of the antiviral antibody titres after administration of the developed virus vaccine with different adjuvants to guinea pigs are shown in the figure.

Immunization of guinea pigs with test samples of vaccine with different oil-based adjuvants induces the production of specific antibodies in high titres. The use of the adjuvant ISA 15 promoted an increase in the titre of antibodies in guinea pig sera against the test viruses in the range 3.6 ± 0.3 to 5.8 ± 0.3 log^2, the adjuvant ISA 25 in the range 3.0 ± 0.3 to $5.0 + 0.3$ log^2.

CONCLUSIONS

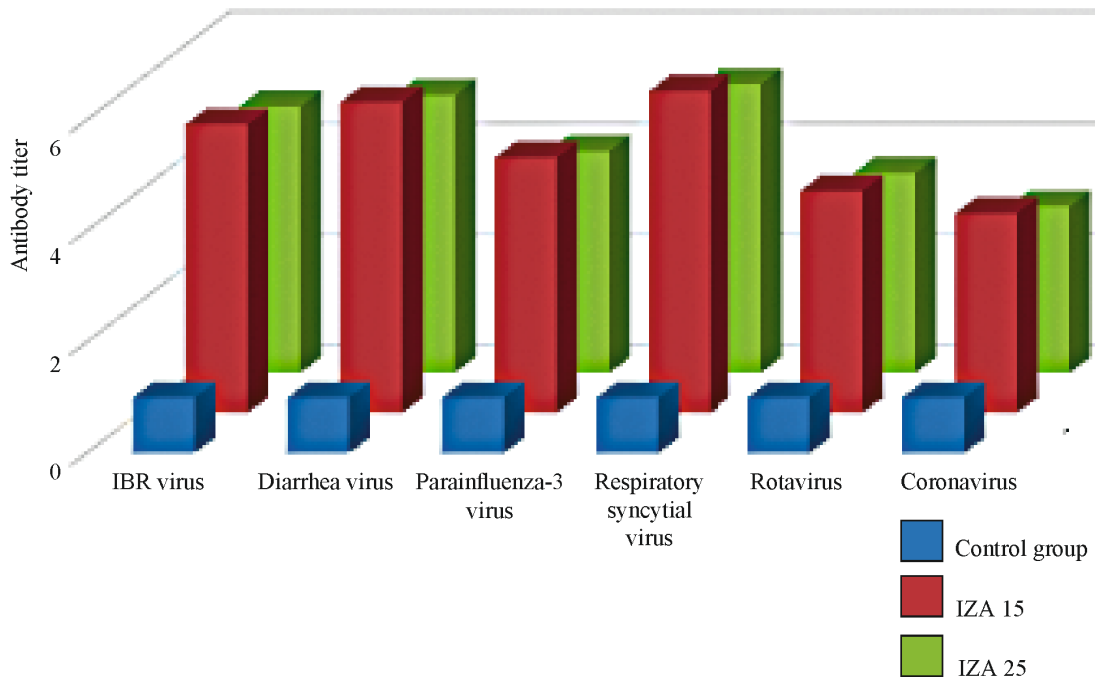
1. When designing an experimental vaccine against viral pneumoenteritis of young cattle, the following avirulent virus strains should be used: Infectious rhinotracheitis (IBR-VBF-VSAVM No 404); diarrhoea (VD-VBF-VSAVM No 406); parainfluenza-3 (PI-VBF-VSAVM No 4 03); respiratory syncytial virus (RSV-VBF-VSAVM No 405); rotavirus (RTV-VBF-VSAVM No 401); coronavirus (CV-VBF-VSAVM No 407).

Табл. 1. Сравнительная инфекционная активность вакцинных штаммов
Table 1. Comparative infectious activity of vaccine strains

Virus	Strain	Virus titer	Strain	Virus titer
IBR	KMIEV – 6	6,5 lg TCD/50	IBR- VBF - VSAVM № 404	7,5 lg TCD /50
Diarrhoea	KMIEV – 7	7,0 lg TCD/50	VD- VBF - VSAVM № 406	7,8 lg TCD /50
Parainfluenza-3	KMIEV – 8	6,3 lg TCD /50	PI- VBF - VSAVM № 403	7,0 lg TCD /50
Rotavirus	KMIEV – 3	7,0 lg TCD /50	RTV- VBF - VSAVM 401	8,0 lg TCD /50
Coronavirus	KMIEV – 1	5,0 lg TCD /50	CV- VBF - VSAVM № 407	6,6 lg TCD /50
Respiratory syncytial virus	RSV	3,5 lg TCD /50	RSV- VBF - VSAVM 405	4,8 lg TCD 50

Табл. 2. Результаты изучения действия различных инактивантов на вакцинные штаммы
Table 2. Results of the study of the effect of various inactivants on vaccine strains

Type and strain of virus	Inactivating agent substance	Inactivation mode	
		Inactivant concentration, %	Exposure, h
IBR virus (IBR- VBF - VSAVM № 404)	Formalin	0,2	24
	Theotropin	0,1	24
Diarrhoea virus (VD- VBF - VSAVM № 406)	Formalin	0,2	12
	Theotropin	0,1	24
Parainfluenza-3 virus (PI- VBF - VSAVM № 403)	Formalin	0,2	12
	Theotropin	0,1	24
Rotavirus (RTV- VBF - VSAVM 401)	Formalin	0,2	12
	Theotropin	0,1	24
Coronavirus (CV- VBF - VSAVM № 407)	Formalin	0,2	24
	Theotropin	0,1	24
Respiratory syncytial virus (RSV- VBF - VSAVM 405)	Formalin	0,2	12
	Theotropin	0,1	24



Уровень специфических антител в сыворотках крови морских свинок, иммунизированных опытной вакциной с различными адъювантами, log^2

The level of specific antibodies in the blood sera of guinea pigs immunized with an experimental vaccine with various adjuvants, log^2

2. The selected vaccine strains are non-reactive and induce active production of antiviral antibodies in sufficiently high titres in both laboratory animals (white mice) and farm animals (cattle).

3. The most effective inactivating agents are 0.1% theotropine and 0.2% formalin.

4. The optimal adjuvant for the preparation of an experimental virus vaccine against viral pneumoenteritis in young cattle is the adjuvant ISA 15 at a concentration of 15%.

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Дата поступления статьи /Received by the editors 30.07.2021
Дата принятия к публикации /Accepted for publication 29.10.2021
Дата публикации/ Published 25.11.2021