

# **STUDY EFFECT OF METABOLITES VACCINE STRAIN E. COLI IBM-1 ON LABORATORY ANIMALS**

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The results of the study of the influence of metabolites vaccine strain of E. coli IBM-1-white mice. Found that E. metabolites IBM-1 coli are harmless to the health of laboratory animals in the vaccine Metakol potentiate the development of DTH, indicates the presence of vaccine-specific immune stimulating properties.

***Keywords:** E. coli IBM-1 metabolite, laboratory animals, safety, delayed-type hypersensitivity.*

The development of animal husbandry in Ukraine in the current environment requires effective interventions for the prevention of diseases, provide timely evidence-based care [1, 7, 12].

In the farms of Ukraine with the onset of winter-spring period, gastro-intestinal diseases of calves become widespread. In this period of massive calving, low nutritional value of diets of pregnant cows, inadequate sanitation facilities and maternity dispensaries contribute to the emergence of gastrointestinal diseases recorded in 80-100% of newborn calves are killed 25-30% [2, 4, 5].

Timely preventive vaccination specific means of protection sets the stage for successful breeding. The development of efficient, friendly means is relevant in the current economic conditions.

Based on strains of E. coli end owed adhesin and enterotoxin past decade to intensive research to develop vaccines against colibacillosis the main colonizing factor of K-99 [3]. The advantage of the efficiency of recombinant vaccines over the natural noted by many researchers. Designing vaccines based on adhesin and

enterotoxin is a promising direction. Such vaccines are highly immunogenic, provide stable protection of calves derived from immunized mothers against pathogen infection by E.coli [6, 7, 8, 11].

**The purpose of the study** – the study of the influence of metabolites vaccine strain of E. coli IBM-1 organisms in laboratory animals – white mice.

**Materials and methods.** Investigated the microbial cell vaccine strain of E. coli IBM-1 and the product of their life - exotoxin. Vaccine strain of the pathogen E. colibacteriosis coli IBM-1 was obtained from the collection of microorganisms SSI "National Center for Biotechnology Innovation", works for which are performed since 1989 under the leadership of AI UAAN A.I. Zaviriuha. Exotoxin strain of E. coli IBM-1 obtained by filtration through a bacterial filter F grade 5.

Exotoxin microbe received during its cultivation in a liquid culture medium for 48 hours. After filtration through a bacterial candle brand F5 and check for contamination of fungal and bacterial microflora, determined its safety. The filtrate was injected into white mice subcutaneously at a dose of 0.2 cm<sup>3</sup>. For the delayed type hypersensitivity reaction, a preparation comprising Metakol dose of 0.4 cm<sup>3</sup> were administered vnutricherevno white mice.

The presence of specific properties was studied using delayed-type hypersensitivity (DTH) which occurs some time after repeated administration of the antigen. This reaction occurs only to the antigen that triggered its development [8, 9, 10]. Senior filtrate obtained by filtration through a bacterial filter. The sterility of the filtrate was determined DSTU 4483:2005. The study harmlessness - tests on laboratory animals, conducted by the GATS 46.024-2002.

**The results of research.** The experiments began working on obtaining pure cultures of the vaccine strain E. coli IBM-1. Strain culture were plated on medium Endo (№ 4). Pure culture was isolated by the method Drigalskogo.

The prepared culture was plated on a Petri dish with medium Endo (№ 4) was incubated in a thermostat at a temperature of  $37 \pm 1$  ° C for 48 hours. The resulting culture was rinsed in saline prepared sterile containers were counted number of microbial cells per 1 cm<sup>3</sup>. The total number of them amounted to 850 million microbial cells.

The resulting microbial cell suspension was seeded in a liquid culture medium (nutrient broth), which was incubated for 48 hours in a thermostat at a temperature of  $37 \pm 1$  ° C. There after exotoxin obtained by freezing. Number 1022362. The USSR, which was tested for sterility according to GOST 4483-2002. With a sample of exotoxin ( $50 \text{ cm}^3$ ) were collected in  $0.1 \text{ cm}^3$ , were seeded into 10 tubes with CBC with resazurin for cultivation at  $-35$  ° C and 10 vials of TGS with resazurin for cultivation at  $-25$  ° C. Before using tubes TGS resazurin incubated at  $35$  ° C - 10 days for the control of a suitable medium. Ten tubes CBC used to control with the same mode of cultivation. Crops were incubated in 14 days.

If visual examination of crops in the 1 -, 4 - and 14 - day observation resazurin color has not changed. Wednesday was no flakes and turbidity.

The presence of irritating properties of the vaccine strain of metabolite E.coli IBM-1 is determined by the inflammatory swelling after injection. The sterile filtrate of culture liquid was injected in a volume of  $0.2 \text{ cm}^3$  white mice weighing 18-20g was administered to the tail root.

To experience formed the experimental and control groups of 10 animals each. Mice in the control group was administered the same dose of study sterile saline under aseptic and antiseptic. At the site of metabolites observed reactions (swelling, redness), fever. Within 10 days of observation all experimental and control animals survived. Exotoxin vaccine strain of E. coli IBM-1 was harmless to laboratory animals.

To study the properties of specific metabolites vaccine strain E. coli IBM-1 vaccine composition used Metakol DTH reaction (delayed type hypersensitivity). For this group of mice formed weighing 16-18g, 10 animals in each. White mice in the right foot pad injected with  $0.2 \text{ cm}^3$  of 10% mixture of washed sheep red blood cells. Simultaneously each mouse was injected intraperitoneally Metakol vaccine dose of  $0.4 \text{ cm}^3$ . On the 5th day in the left foot pad was injected with 10% mixture of sheep erythrocytes. After 24 hours post-injection keeping the reaction was carried out by the difference in thickness of the legs (mm). The results of observation are shown in Tabl. Animals of the control and experimental groups revealed significant statistically significant difference in the intensity of the DTH reaction (control - 0,28

$\pm 0,08$ , experience -  $0,74 \pm 0,076$ ). The re-introduction into the left paw of mice suspension of sheep red blood cells after 24 h. after immunization with the vaccine caused them to develop DTH reaction.

**Results of the delayed-type hypersensitivity in mice to the test antigen  
(sheep erythrocytes) influenced vaccine Metakol**

Number of animals	Control group			Experimental group		
	the thickness of the right foot, mm	the thickness of the left foot, mm	the intensity of the reaction mm	the thickness of the right foot, mm	the thickness of the left foot, mm	the intensity of the reaction mm
1	1,6	1,9	0,3	2,0	2,9	0,9
2	1,6	1,9	0,3	1,8	2,9	1,1
3	1,6	1,9	0,3	2,0	2,5	0,5
4	1,5	1,7	0,2	1,9	2,9	1,0
5	1,5	1,8	0,3	2,0	2,5	0,5
6	1,8	2,0	0,2	2,0	2,9	0,9
7	1,6	1,9	0,3	1,9	2,8	1,9
8	1,7	2,0	0,3	1,9	2,5	0,6
9	1,6	1,9	0,3	2,0	2,5	0,5
10	1,6	1,9	0,3	2,0	2,5	0,5
M±m			0,28±0,08			0,74±0,076
= p>				0,01		

The greatest difference between the thickness of both paws of the experimental group was 1.9 mm, and a control - 0.3 mm. So, after the introduction of the vaccine into mice Metakol, potentiated the development of DTH, indicates the presence of vaccine-specific immune stimulating properties. Conducting the reaction is a convenient test to study the effect of the vaccine on cellular immunity factor.

## CONCLUSIONS

1. Metabolites of the vaccine strain E. coli IBM-1 are harmless to laboratory mice. After the introduction of exotoxin all animals remain alive at the injection site no swelling, redness and increased temperature.
2. The resulting exotoxin corresponds requirements DSTU 4483:2005.
3. Metabolites of the vaccine strain E. coli IBM-1 vaccine composition Metakol induce delayed-type hypersensitivity.

## REFERENCES

1. Бабинін О. Визначення економічних збитків при загибелі тварин / О. Бабинін, О. Астахова // Ветеринарна медицина України. – 2004. – №1. – С. 22.
2. Влияние сезонности на естественную резистентность организма коров-матерей и приплода / [В.Н. Денисенко, О.Н. Грызлова, Г.Н. Печников, П.А. Емельяненко] // Ветеринария. – 1987. – № 12. – С. 53 – 56.
3. Головки А.Н. Оценка иммунитета у коров после применения вакцины содержащей адгезивный антиген E. coli K 99 / А.Н. Головки, Г.В. Гнатенко // Ветеринария. – К.: Урожай, 1989. – Вып. 64. – С. 7 – 10.
4. Зароза В.Г. Колибактериоз новорожденных телят: Обзорная информация / В.Г. Зароза. – М.: НИИТЭИагропро, 1995. – 56 с.
5. Зароза В.Г. Профилактика и лечение желудочно-кишечных болезней новорожденных телят / В.Г. Зароза. – М.: ВНИИТЭИагропром. – 1989. – 57 с.
6. Зароза В.Г. Эшерихиоз телят / В.Г. Зароза. – Агропомиздат, 1991. – 238 с.
7. Клестова З. Нові проблеми сучасного свинарства / З. Клестова // Ветеринарна медицина України. – 2000. – №8. – С.27.
8. Коротяев А.И. Медицинская микробиология, иммунология и вирусология: учебник [для медицинских вузов] / А.И. Коротяев, С.А. Бабичев. – Санкт-Петербург: Специальная литература, 1999. – 580 с.
9. Клиническая иммунология и аллергология / [под ред. Г. Лолора-младшего, Т. Фишера, Д. Адельмана]. – М.: Практика, – 2000. – 806 с.

10. Спивак Н.Я. Интерферонпродуцирующая способность лимфоидных клеток свиней. / Н.Я. Спивак //.- К.: Наукова думка.– 1980.– С. 236-242.

11. Федоров Ю.Н. Факторы иммунологической защиты у новорожденных животных / Ю.Н. Федоров // Бюл. ВИЭВ.– 1982.– Т. 47. – С. 60 – 65.

12. Щербаков П.Н. Профилактика и лечение при желудочно-кишечных и респираторных болезнях телят / П.Н. Щербаков, А.Г. Гусев // Ветеринария. – 2002. – № 3.– С. 15 – 16.

## **ВЛИЯНИЕ МЕТАБОЛИТОВ ВАКЦИННОГО ШТАММА E. COLI IBM-1 НА ЛАБОРАТОРНЫХ ЖИВОТНЫХ**

*А.А. ЗАВИРЮХА, Т.Б. ВАСИЛЬЕВА*

Представлены результаты изучения влияния метаболитов вакцинного штамма E. coli IBM-1 на белых мышей. Установлено, что метаболиты E. coli IBM-1 безвредны для здоровья лабораторных животных, и в составе вакцины Метакол потенцируют развитие ГСТ, что свидетельствует о наличии у вакцины специфических иммуностимулирующих свойств.

*Ключевые слова: E. coli IBM-1, метаболит, лабораторные животные, безвредность, гиперчувствительность замедленного типа.*

## **ВПЛИВ МЕТАБОЛІТІВ ВАКЦИННОГО ШТАМУ E. COLI IBM-1 НА ЛАБОРАТОРНИХ ТВАРИН**

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Представлені результати вивчення впливу метаболітів вакцинного штаму E. coli IBM-1 на білих мишах. Встановлено, що метаболіти E. coli IBM-1 нешкідливі для здоров'я лабораторних тварин і в складі вакцини Метакол потенціюють розвиток ГСТ, що свідчить про наявність у вакцини специфічних імуностимулюючих властивостей.

*Ключові слова: E. coli IBM-1, метаболіт, лабораторні тварини, нешкідливість, гіперчутливість сповільненого типу.*