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Study of the vaccination effects against *Staphylococcus aureus*, causing mastitis and endometritis in cows

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ABSTRACT

The high contagiousness of staphylococcal infections and emergence of antimicrobial resistant strains call for search and development of new highly effective drugs and vaccines against infectious animal diseases. Twenty adult pregnant black pied cows were used to form a test and a control groups (10 animals per group). The vaccine was administered twice subcutaneously in the middle third of the neck of the test animals: the first dose in a volume of 3 mL 55–70 days before calving, the second dose in the same volume 25–30 days before the expected calving. Control animals were injected subcutaneously with the same volume of sterile saline at the same dates. To evaluate the antigenicity of the vaccine against *Staphylococcus aureus*, blood was collected from animals of both groups: in the test group 14–16 days after booster vaccination, in the control group 14–16 days after second injection of the sterile saline. For bacteriological testing, milk samples from both groups were collected during the first month of lactation after calving. According to the results of serological testing, the antibody titer against *Staphylococcus aureus* in the test group ranged from 4.01 to 4.61 Ig, its mean value was (4.34 ± 0.06) Ig. In the test group, the mean antibody titers against *Staphylococcus aureus* were 5.8 times lower and were equal to (0.75 ± 0.09) Ig with fluctuations from 0.3 to 1.2 Ig. The bacteriological tests of milk in the control group revealed *Staphylococcus aureus* in 5 out of 10 samples, which is 50%. In the test group, the pathogen was detected in 20% of cases, which is 2.5 times lower than in the control group.

Keywords: mastitis, endometritis, cows, vaccination, *Staphylococcus aureus*, antibodies, potency, milk

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Изучение воздействия вакцинации в отношении *Staphylococcus aureus*, вызывающего маститы и эндометриты у коров

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РЕЗЮМЕ

Высокая contagiousность стафилококковых инфекций и появление антибиотикостойчивых штаммов микроорганизмов диктует необходимость поиска и разработки новых высокоэффективных средств лечения и профилактики инфекционных болезней животных. Для опытной и контрольной групп отобраны взрослые стельные коровы черно-пестрой породы по 10 гол. в каждой. Животным опытной группы вакцину вводили подкожно в область средней трети шеи двукратно: первую дозу в объеме 3 см³ – 55–70 дней до отела, вторую – за 25–30 дней до предполагаемого отела в том же объеме. Животным контрольной группы подкожно вводили стерильный физиологический раствор в сопоставимом объеме и с тем же интервалом. Для изучения антигенной активности вакцины в отношении *Staphylococcus aureus* у животных обеих групп отбирали кровь: в опытной группе – через 14–16 сут после двукратной иммунизации, в контрольной группе – через 14–16 сут после двукратного введения животным стерильного физиологического раствора. Для проведения бактериологического исследования пробы молока в обеих группах отбирали в первый месяц лактации коров после отела. Как показали результаты серологических исследований, в опытной группе коров титр антител к *Staphylococcus aureus* колебался от 4,01 до 4,61 Ig, его среднее значение составило

(4,34 ± 0,06) lg. В контрольной группе животных средние значения титра антител к *Staphylococcus aureus* были в 5,8 раза ниже и составили (0,75 ± 0,09) lg с колебаниями от 0,3 до 1,2 lg. В результате бактериологических исследований молока в контрольной группе *Staphylococcus aureus* выделили в 5 из 10 образцов, что составило 50%. В опытной группе возбудитель был обнаружен в 20% случаев, что в 2,5 раза ниже по сравнению с контрольной группой.

Ключевые слова: мастит, эндометрит, коровы, вакцинация, *Staphylococcus aureus*, антитела, иммуногенность, молоко

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INTRODUCTION

Staphylococcal diseases are the most frequent pathologies of animals and require highly qualified, long-term and expensive therapy [1, 2]. Staphylococci can affect any tissue or organ and cause more than 100 different diseases: mastitis, endometritis, dermatitis, pneumonia, arthritis, purulent and wound infections, food poisoning, sepsis, etc. Enterotoxins, secreted by staphylococci in large quantities, have a complex effect on the animal immune system, resulting in its limited resistance. All staphylococcal enterotoxins are proteins with a relatively small molecular weight: from 26,900 to 29,600 Da. The classic staphylococcal enterotoxins comprise five main types: A, B, C, D and E (SEA-SEE), which are believed to be responsible for 95% of all staphylococcal poisoning cases. The prevention of food-borne diseases relies on the effectiveness of early diagnosis, that is, on the detection of enterotoxigenic staphylococci in milk, dairy and other food products [3, 4].

The main causative agent of staphylococcosis in cattle is *Staphylococcus aureus*, which is isolated in 69.5% of staphylococcosis cases [5]. In biological samples collected from cows demonstrating signs of mastitis and endometritis, *S. aureus* bacteria are most frequent [6, 7, 8]. According to researchers, *S. aureus* was isolated from 8.8% of milk samples from healthy cows; in 59.3 to 62.8% of cases *S. aureus* was detected in milk samples from cows with subclinical mastitis; it was also found in udder secretions of mastitic cows and pooled milk in 28.8 and 18% of samples, respectively [6, 9]. The bacteriological testing of vaginal swabs showed that one of the most frequent species responsible for postpartum endometritis was *S. aureus* (15.3% of the total tested cultures) [10].

The recovery rate from diseases caused by *S. aureus* is lower compared to other bacteria, which is explained by antimicrobial resistance and biofilm formation ability [11, 12].

In recent years, *S. aureus* isolated from animals have become increasingly resistant to antimicrobials, including through the production of the enzyme β -lactamase, capable of cleaving penicillins and cephalosporins [13, 14, 15, 16, 17]. Staphylococcal mastitis caused by resistant strains of *S. aureus* is reported in almost 90% of large farms and commercial holdings where antibiotics are used [3, 18, 19, 20]. Penicillin-resistant *S. aureus* are most common strains, which are identified as the first wave of resistance, and the second wave of resistance are methi-

cillin-resistant *S. aureus*. Artemyeva O. A. et al. found that the highest resistance of *S. aureus* isolates *in vitro* was observed to erythromycin (82.5%) and fusidin (75.7%). Only seven isolated strains (6.8%) showed susceptibility to all tested antimicrobials, whereas 96 isolates were resistant to at least one of them [21]. According to I. S. Abdina et al., *S. aureus* antimicrobial resistance varied significantly, with the highest resistance reported to ampicillin (up to 57%), benzylpenicillin (up to 45%), doxycycline (up to 38%), oxacillin (up to 48%), streptomycin (up to 55%) and tetracycline (up to 45%) [22]. Other researchers established that of the 64 isolated *S. aureus* strains, 60 (93.7%) showed resistance to one or more antimicrobials. Multidrug resistance was observed in the tested *S. aureus* strains [11].

Despite the large increase in the incidence of staphylococcal infections in animals, effective drugs against them have not yet been developed. The spread of multidrug resistant *S. aureus* strains only complicates the disease control [23]. The successful treatment of animals with antibiotics brings risks of their consumption by humans. The high contagiousness of staphylococcal infections and the emergence of antimicrobial-resistant strains are the most serious problems and emerging threats for cattle industry, leading to decreased milk yields, impaired hygiene and quality of milk, increased costs for veterinary medicines and services, early culling of cows and their restricted performance. In this regard, it is necessary to search for alternative options based on staphylococci suppression, limiting the use of antimicrobials and minimizing their negative effects on the organism. One of such options is specific prevention, i.e. vaccines that provide reliable protection of animals from infectious diseases, contribute to the reduction in the use of antibiotics and prevent antimicrobial resistance of microorganisms [24, 25]. Scientists have proven the effectiveness of the vaccines containing *S. aureus* antigen against mastitis and endometritis of cows. The studies revealed that immunization of animals against mastitis has a positive effect. After 6 months after first vaccination in the breeding facility and farm, the number of mastitis cases decreased by 16.6 and 7.3%, respectively, and somatic cell counts in the milk of high yielding cows decreased by 26.5 and 10.7%. The immunization remained effective even after 12 months post vaccination [26]. "Combovac-Endomast" vaccine (Vetbiochim, Russia) decreased the number of clinical mastitis cases by 7.8 times, of subclinical mastitis by 5.4 times, and

endometritis by 3.7 times [27]. The experiments with the “Mastivac” vaccine in Laboratorios Ovejero S. A. Spain established that clinical mastitis cases decreased by 32% in the experimental group compared to the control one [9].

The aim was to study the effect of immunization against *S. aureus*, which causes mastitis and endometritis in cows.

MATERIALS AND METHODS

The study has been performed starting from 2021 in a family-operated farm in the Republic of Mordovia. Twenty adult pregnant black pied cows were used to form a test and control groups (10 animals per group). The animals were selected based on conformity principle, that is, all animals in both groups had standard parameters of the body weight, age, health status and management conditions. The vaccine was administered twice subcutaneously to the middle third of the neck of test animals. The first dose in a volume of 3 mL was administered 55–70 days before calving, the second dose of the same volume 25–30 days before the expected calving. In one immunizing dose the vaccine contains the following strains: *Escherichia coli* UR-10, *Streptococcus agalactiae* UR-7, *Streptococcus dysgalactiae* UR-16, *Streptococcus uberis* OB-5, *Streptococcus pyogenes* OB-4, *Staphylococcus aureus* OB-14, *Klebsiella pneumoniae* K-2 (at least 3.5×10^9 CFU of each), inactivated with formalin (0.3% solution) and adsorbed on carbomer gel (10% by volume). The vaccine is intended to prevent mastitis and endometritis in cows. Control animals were injected subcutaneously with the same volume of sterile saline at the same dates.

Before the start of the experiment, milk and blood samples were collected from 20 cows with clinical mastitis, not included in any of the groups, for microbiological testing for *S. aureus* and serological testing for antibodies against *S. aureus*.

Blood was collected from animals of both groups to evaluate the antigenicity of the vaccine against *S. aureus*

and milk samples for bacteriological testing. Blood sampling in the test group was performed 14–16 days after booster immunization, in the control group 14–16 days after second administration of sterile saline (placebo).

Animals were handled in compliance with the ethical standards adopted by the European Convention ETS No. 123.

The vaccine antigenicity was evaluated by the increase in the antibody titer against *S. aureus* measured by agglutination test. For the purposes of testing serum was diluted with sterile saline from 1:2 to 1:4096 and 0.5 mL of *S. aureus* OB-14 antigen with concentration of $\sim 5 \times 10^8$ CFU/mL was added to 0.5 mL of each serum dilution. The serum-antigen mixture was thoroughly mixed, placed in a thermostat and kept for 16–18 hours at $(37 \pm 1)^\circ\text{C}$, and then for another 3–4 hours at room temperature. After that, they were examined for agglutination occurrence. The results were interpreted with the agglutination viewer and a four-point visual scale was used: ++++ is 100% of cells agglutinated, complete liquid clearing; +++ means 75% of cells agglutinated, slight turbidity of the liquid; ++ means 50% of cells agglutinated, medium turbidity of the liquid; + is 25% of cells agglutinated, heavy turbidity of the liquid; – means no agglutination, homogeneous bacterial suspension.

Milk was pooled from all four udder lobes, but milk samples from different animals were tested individually, that is, they were not pooled with each other. The bacteriological testing of milk was performed during the first month of lactation after calving in accordance with the “Guidelines for the bacteriological testing of milk and udder secretion of cows”¹. *S. aureus* was identified in accordance with GOST 30347-2016 “Milk and milk products. Methods for determination of *Staphylococcus aureus*”², and by mass spectrometry (MALDI-ToF)³.

The results were statistically processed using generally accepted methods with Microsoft Office Excel 2010, Stat Plus 2009 software.

RESULTS AND DISCUSSION

According to the results of serological testing, the antibody titer against *S. aureus* in the test group ranged from 4.01 to 4.61 lg, its mean value was (4.34 ± 0.06) lg. In the test group, the mean antibody titers against *S. aureus* were 5.8 times lower and were equal to (0.75 ± 0.09) lg with fluctuations from 0.3 to 1.2 lg (Table).

As the data obtained show, the immunization facilitated the increase in antibodies against *S. aureus* in cows of the test group, which confirms the high potency of the vaccine. In addition, the titer of antibodies to *S. aureus* in the blood of animals during preliminary serological testing was almost identical to the titer in the control group and was equal to (0.70 ± 0.05) lg.

Loskutova I. V. et al. also found that clinically healthy cows immunized with the Mastivac vaccine (Laboratorios Ovejero S. A., Spain) containing *S. aureus* induced

Table
***S. aureus* antibody titer in blood of cows from test and control groups**

Microorganism	Antibody titer	
	test group (10 animals), lg	control group (10 animals), lg
<i>S. aureus</i>	4.31	0.6
	4.01	0.3
	4.61	1.2
	4.01	0.3
	4.61	0.9
	4.01	0.6
	4.31	1.2
	4.31	0.3
	4.61	0.9
	4.61	1.2
<i>M ± m</i>	4.34 ± 0.06	0.75 ± 0.09

¹ Guidelines for the bacteriological testing of milk and udder secretion of cows: approved by Chief Veterinary Department under the USSR Ministry of Agriculture No. 115-69 on 30.12.1983. <https://base.garant.ru/72125912>

² GOST 30347-2016 Milk and milk products. Methods for determination of *Staphylococcus aureus*. <https://docs.cntd.ru/document/1200142424>

³ Guidelines for microorganism identification using MALDI Biotyper mass spectrometer when testing food raw materials and food products (approved by the Rosselkhoz nadzor Scientific Technical Committees on 03.04.2014).

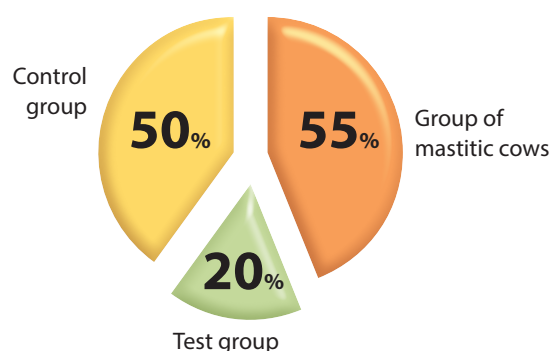


Fig. *S. aureus* isolation rate in the tested milk samples

antibodies to *S. aureus* enterotoxins which is evident of the vaccine ability to induce an immune response in animals against the bacterium [12]. Hadimli H. H. et al. evaluated the effectiveness of the staphylococcal vaccine on humoral immunity against *S. aureus* in vaccinated animals [28].

At the next stage, a bacteriological testing of milk for *S. aureus* was performed.

During a preliminary testing of milk from 20 cows with clinical mastitis, *S. aureus* was found in 11 samples, which amounted to 55% of the total number of samples tested. In the control group, *S. aureus* was isolated in half of the milk samples tested (the bacterium was detected in 5 out of 10 samples (50%). In the milk of cows from the test group, *S. aureus* was found in 20% of samples (2 samples), which is 2.7 and 2.5 times lower compared to the group of cows before the experiment and the control group, respectively.

The figure shows that *S. aureus* isolation rate from non-vaccinated animals (the control group and the group of cows with clinical mastitis) is almost the same; in immunized cows, it was significantly lower.

The effectiveness of *S. aureus* vaccination against mastitis was also established by other researchers, who isolated the bacterium from 73.3% of milk samples before vaccination, and 6 months after the first immunization the isolation rate decreased to 26.6%, that is, by 2.7 times [29].

CONCLUSION

It was found that double vaccination facilitated the increase in antibodies levels against *S. aureus*; the mean titers in the test group were 5.8 times higher than in the control group. In milk samples collected from vaccinated animals, the *S. aureus* isolation rate decreased by 2.7 and 2.5 times compared with groups of non-immunized animals. The results obtained show that the vaccination induces immune response in animals against *S. aureus*.

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