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**EFFICIENCY OF PARENTERAL APPLICATION OF PREPARATIONS BASED ON CEPHALOSPORINS FOR MASTITIS TREATMENT IN MILK CATTLE**

**ЭФФЕКТИВНОСТЬ ПАРЕНТЕРАЛЬНОГО ПРИМЕНЕНИЯ ПРЕПАРАТОВ НА ОСНОВЕ ЦЕФАЛОСПОРИНОВ ПРИ ЛЕЧЕНИИ МАСТИТА В МОЛОЧНОМ ЖИВОТНОВОДСТВЕ**

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*Abstract.* Mastitis is the most common disease in dairy farming, in clinical and subclinical form it is diagnosed in more than 50% of cows. Currently, various drugs and methods are used to treat mastitis. The article presents data on the effectiveness of the use of the preparation Ceftonit and preparation on the basis of cefquinome sulfate in the treatment of various forms of clinical mastitis during lactation.

For the treatment of mastitis, the cows used the preparation Ceftonit®, Nita–Farm Russia, containing in its composition 50 mg/ml of Ceftiofur, which is used in comparison with imported drug containing in its composition 25 mg/ml Cefquinome sulfate.

As a result of researches, it is established that the drug Ceftonit in the treatment of clinical mastitis in cows is not found in the milk of BRT by the AIM test and shows 100% therapeutic efficacy with an average recovery time of animals 5.5 days. Disease recurrence in the form of subclinical mastitis in the treatment with the drug Ceftonit was from 25 to 33%, and in the treatment with the drug based on cefkinom sulfate the number of relapses was greater and amounted to 44.44%.

The use of the drug Ceftonit® for the treatment of mastitis does not lead to the culling of milk due to the presence of antibiotics, in contrast to the drug based on Cefquinome sulfate, which has a restriction on milk, as confirmed by our studies.

*Аннотация.* Мастит является самым распространенным заболеванием в молочном животноводстве, в клинической и субклинической форме диагностируется более чем у 50% коров. В настоящее время для лечения мастита используются различные препараты и методы. В статье представлены данные об эффективности применения препарата «Цефтонит» и препарата на основе цефкинома сульфата при лечении различных форм клинического мастита в период лактации.

Для лечения мастита у коров использовали препарат «Цефтонит®», «Нита-Фарм» Россия, содержащий в своем составе 50 мг/мл цефтиофура, который применяли в сравнении с импортным препаратом, содержащим в своем составе 25 мг/мл цефкинома сульфата.

В результате проведенных исследований установлено, что препарат «Цефтонит» при лечении клинических маститов у коров не обнаруживается в молоке BRT тестом фирмы АИМ и проявляет 100% терапевтическую эффективность при средних сроках выздоровления животных 5,5 суток. Рецидив заболевания в форме субклинического мастита при лечении препаратом «Цефтонит®» составил от 25 до 33%, а при лечении препаратом на основе цефкинома сульфата количество рецидивов было больше и составило до 44,44%.

Применение препарата «Цефтонит®» для лечения маститов не приводит к выбраковке молока из-за нахождения в нем антибиотиков, в отличие от препарата на основе цефкинома сульфата, который имеет ограничение по молоку, что подтверждается проведенными нами исследованиями.

*Keywords:* mastitis, cephalosporin drugs, somatic cells, treatment.

*Ключевые слова:* мастит, препараты цефалоспоринового ряда, терапия, соматические клетки, тесты на маститы и антибиотики.

Mastitis is one of the most economically significant diseases of milk cows. According to International Milk Federation data and European Stock-breeders Association reports and also numerous domestic and foreign researchers clinical form of mastitis is diagnosed in some cases in 20–25% of cows, and subclinical in more than 50% of cows in a herd. This form of mastitis can remain during 1–2 lactations without timely and effective treatment [1–3]. In Europe nearly 38% of the total sum of expenses in a milk herd are spent on prevention and treatment of mastitis [4–6]. Loses connected with clinical mastitis are caused by the early cow rejection (genetic potential loss) and cost of medical preparations, veterinary services, salary expenses increase, milk quality decrease and reduction of its volume. Loses caused by mastitis in Europe are \$233 per capita per year [7].

In spite of widespread introduction of mastitis prevention technologies at present mastitis is one of the most widespread diseases in milk cattle in the world [8]. Different pathogenic organisms can cause the disease: bacteria, mycoplasma, yeasts, and algae, in all there are more than 137 species of microorganisms that can cause the disease [9], but only 20 of them are well studied. Mastitis can be classified into two types: infectious pathogenesis mastitis and environmental pathogenesis mastitis [10].

Infectious pathogenesis pathogens exist inside infected parts of the udder. They cause subclinical infection or subclinical mastitis, which, as a rule, reveals itself in quantitative increase of somatic cells (leucocytes [mainly neutrophils] and epithelial cells) in milk from the infected quarter.

Pathogens are transferred from cow to cow, or from one udder part to another and also during milking through milkmaid hand towel and milking apparatuses. The most widespread pathogens of

this type are *Staphylococcus aureus*, *Streptococcus agalactia*, *Streptococcus disgalactia*, *Corynebacterium bovis* [11].

Environmental pathogens — bacteria exist in the environment, they are especially numerous in cow's litter. Udder invasion occurs during intervals between milking, cows are also subjected to these bacteria at the beginning of their “dry” period, when teat channels are open. The most widespread environmental microorganisms are *Escherichia coli* and *Streptococcus uberis*.

The majority of mastitis of bacterial origin in 80% of cases is caused by the following five bacterial species: *Escherichia coli*, *Streptococcus uberis*, *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae* [12].

Economic consequences of mastitis in cattle are the reason of development of different therapeutic strategies of milk gland infections treatment: preparations belonging to different therapeutic classes (antimicrobial, antiphlogistic and other preparations [13–16], vitamins, vaccines [17–19], cytokines [20–21] and even homoeopathy, and different methods of medical preparation introduction (systemic, intramammary or local (putting medicine on teat or udder skin)) [22] are being used. However, in spite of considerable progress in mastitis therapy some animals still suffer from this disease.

In Russia preparations on the basis of penicillin, streptomycin, neomycin, erythromycin and some other antibiotics, which have insufficient efficiency, are used more often for systemic treatment of mastitis. Recently preparations on the basis of highly effective antibiotics of new generations (for example, cephalosporin) start to appear in the market. Cephalosporins are one of the most important classes of semisynthetic antimicrobial preparations used for mastitis treatment in milk cows.

One of the representatives of the third generation of cephalosporins is Ceftiofur, specially synthesized for application in cattle. Ceftiofur is highly active against gram–negative bacteria, and at the same time it is highly active against gram–positive bacteria; its increased hydrolytic stability to  $\beta$ –lactamases, which are active against early generation cephalosporins and penicillins should also be mentioned. Oxymine group in side chains increased  $\beta$ –lactam ring stability to  $\beta$ –lactamases [23–24].

Ceftiofur like sodium salt was introduced into veterinary practice in 1988 by Upjohn Company in the form of Naxcel® sterile powder preparation, which was used for making intramuscular injection solutions, for treatment of cattle respiratory diseases; ceftiofur hydrochloride in the form of oil suspension, known as Excenel® RTU, started to be used at the same time. Intracisternal injection suspensions SPECTRAMAST DC, used for prevention and treatment of mastitis recently came into use. Preparations on the basis of Ceftiofur are distinguished by the possibility to use them by lactating animals without milk use restriction.

Systemic application of antibiotic preparations for treatment of lactating cows with clinical mastitis is usually the only way of quick problem solution, treatment course consists of 3–5 injections, remember about milk use restriction during 2 days with penicillin being used, and 21 and more days with the use of tetracyclin.

Parenteral use of preparations, based on Ceftiofur, for clinical mastitis treatment is mentioned in the literature [25–26].

*The purpose of this research* is to define therapeutic effect of systemic use of Ceftonit® preparation, based on Ceftiofur with different forms of clinical mastitis (serofibrinous and purulent catarrhal) during lactation period in comparison with the preparation based on Cefquinome sulfate and find out possible milk use restrictions due to the presence of antibiotics.

#### *Materials and methods*

The experiment was done in “Volga” agrofirma closed joint–stock company in Marx district and in “Mummovskoye” training farm of Moscow Agricultural Academy named after K. A. Timiryazev in Atkarsk district of Saratov region. 114 lactating cows of Simmental and black and many–colored breeds with serofibrinous and purulent catarrhal mastitis were involved in the

research. Diagnostics of serofibrinous and purulent catarrhal mastitis was done according to clinical symptoms.

*Results and discussion*

Sick animals with serofibrinous mastitis had no appetite, chewing process, belching and rumination. Tachycardia, hammering sound, rapid weak pulse was observed. General body temperature was higher than 41°C. Back extremities lameness was observed from the infected udder quarter or part side. Lymphatic nodes above the udder were enlarged and painful. Infected quarter or the whole udder is sharply enlarged, its skin is inflamed and edematous, and parenchyma is dense, painful, and hot. With profound palpation of infected quarters crepitation is observed at the bottom of the teat. The teat of the infected quarter or all quarters (when the whole udder is infected) is edematous and painful. Its mucous membrane is edematous. Some drops of sticky viscid liquid with admixture of fibrin flakes, of yellow color, often with reddish shading can hardly be milked from the infected quarters.

With purulent catarrhal mastitis animals had no appetite, chewing process, belching and rumination. Tachycardia, hammering sound, rapid weak pulse was observed. Body temperature was higher than 41°C. Back extremities lameness was observed from the infected udder quarter or part side. Lymphatic nodes above the udder were enlarged and painful. Infected quarter or the whole udder are sharply enlarged, painful, local temperature is high. Udder skin is strained and hyperemic. The teat is strained, enlarged and painful. Whitish and cream-colored thick creamlike purulent catarrhal odor-free exudation can be milked from the infected quarter. Clinical picture of udder disease in lactating cows was observed during the whole experiment till milk gland function recovery. For mastitis treatment in cows we used Ceftonit® preparation, Nita-Farm, Russia, containing 50 mg/ml of Ceftiofur, which was applied in comparison with foreign preparation containing 25 mg/ml of Cefquinome sulfate.

Preparations were introduced parenterally in accordance with the scheme given in Table 1.

Table 1.

PREPARATION INTRODUCTION SCHEME

<i>Groups</i>	<i>Active substance</i>	<i>Preparation dose</i>	<i>Repetition factor of application</i>	<i>Number of animals in the group</i>
1 experimental (serofibrinous)	Ceftiofur hydrochloride (Ceftonit)	1,0 ml/ 50 kg animal weight, 1 time per day	twofold	10
			threefold	10
			fourfold	4
			fivefold	4
2 experimental (purulent catarrhal)	Ceftiofur hydrochloride (Ceftonit)	1,0 ml/ 50 kg animal weight, 1 time per day	twofold	10
			threefold	10
			fourfold	4
			fivefold	5
3 experimental (serofibrinous)	Cefquinome sulfate	2,0 ml/ 50 kg animal weight, 1 time per day	twofold	10
			threefold	10
			fourfold	4
			fivefold	4
4 experimental (purulent catarrhal)	Cefquinome sulfate	2,0 ml/ 50 kg animal weight, 1 time per day	twofold	10
			threefold	10
			fourfold	4
			fivefold	5

Therapeutic efficiency results were evaluated in accordance with the clinical symptoms and test results: “Mastotest” produced by “Agrofarm” closed joint-stock company (Russia, Voronezh) and sedimentation samples. Animal were considered healthy if tests results were negative. We also took milk and milk gland secretion samples before using preparations, in 24, 48, and 72 hours and on the fifth day after starting the treatment.

The number of somatic cells and presence of antibiotics were defined. BRT tests of AIM firm, Germany were used to define antibiotics.

Under state control with the use of latent mastitis test was being done during 21 days after animal’s recovery. The effect of parenteral use of preparations containing Cefquinome sulfate and Ceftiofur hydrochloride (Ceftonit) is presented in Tables 2 and 3.

Table 2.

**THERAPEUTIC EFFICIENCY OF PREPARATIONS CONTAINING CEFQUINOME SULFATE AND CEFTIOFUR HYDROCHLORIDE (CEFTONIT) WITH SEROFIBRINOUS AND PURULENT CATARRHAL MASTITIS**

Animal groups	Active substance	Repetition factor of application	Clinical effect		Absence of effect		Disease relapse	
			n	%	n	%	n	%
1 experimental (serofibrinous mastitis)	Ceftiofur hydrochloride (Ceftonit)	twofold (n = 10)	5	50.0	5	50.0	2	20.0
		threefold (n = 10)	7	70.0	3	30.0	1	10.0
2 experimental (purulent catarrhal mastitis)	Ceftiofur hydrochloride (Ceftonit)	twofold (n = 10)	3	30.0	7	70.0	4	40.0
		threefold (n = 10)	6	60.0	4	40.0	1	10.0
3 experimental (serofibrinous mastitis)	Cefquinome sulfate	twofold (n = 10)	5	50.0	5	50.0	2	20.0
		threefold (n = 10)	7	70.0	3	30.0	1	10.0
4 experimental (purulent catarrhal mastitis)	Cefquinome sulfate	twofold (n = 10)	3	30.0	7	70.0	3	30.0
		threefold (n = 10)	6	60.0	4	40.0	2	20.0

The given data state that twofold and threefold application of Ceftonit® preparation based on Ceftiofur hydrochloride at a dose of 1.0 ml/50 kg animal weight 1 time for 24 hours and preparation based on Cefquinome sulfate at a dose of 2.0/50 kg animal weight 1 time during 24 hours with serofibrinous and purulent catarrhal mastitis in cows do not have sufficient effect (lower than 70%).

So, twofold application of Ceftonit® preparation with serofibrinous mastitis has therapeutic effect of 50%. And 20% of animals had a disease relapse. Threefold application gives therapeutic effect of 70%, and 10% of recovered animals had a relapse. Twofold application of Ceftonit® preparation with purulent catarrhal mastitis has therapeutic effect of 30%. And 40% of animals had a disease relapse. Threefold application of the preparation increases its efficiency twice, and only 10% of animals had a disease relapse.

Twofold application of the preparation based on Cefquinome sulfate with serofibrinous mastitis demonstrates 50% efficiency, 20% of animals had a disease relapse. Threefold application of the preparation increases its clinical effect up to 70%, and 20% of animals had a relapse. Treatment of purulent catarrhal mastitis with the preparation based on Cefquinome sulfate with twofold application gives a positive clinical effect only in 30% of cases, 30% of animals had a relapse, and threefold application the preparation increases its efficiency up to 60%, with 20% of relapse cases.

Thus, with serofibrinous and purulent catarrhal mastitis in cows twofold and threefold application of Ceftonit® preparation at a dose of 1.0 ml/50 kg animal weight 1 time for 24 hours and preparation based on Cefquinome sulfate at a dose of 2.0 ml/kg animal weight 1 time for 24 hours do not provide sufficient efficiency and a high percentage of relapse.

The best results during experimental research of preparation efficiency were received with increased repetition factor of preparation application (4 and 5 times).

Table 3.

THERAPEUTIC EFFICIENCY OF PREPARATIONS CONTAINING CEFQUINOME SULFATE AND CEFTIOFUR HYDROCHLORIDE WITH REPETITION FACTOR OF PREPARATION APPLICATION OF 4 AND 5 TIMES WITH SEROFIBRINOUS AND PURULENT CATARRHAL MASTITIS

Animal groups	Active substance	Repetition factor of application	Therapeutic effect		Disease relapse		Recovery period, days
			n	%	n	%	
1 experimental (serofibrinous mastitis)	Ceftiofur hydrochloride (Ceftonit) (n = 8)	fourfold (n = 4)	4	50.0	1	25	4.3±0,01
		fivefold (n = 4)	4	100.0	1	25	5.1±0,03
total			8	100.0	2	25	4.7±0,02
2 experimental (purulent catarrhal mastitis)	Ceftiofur hydrochloride (Ceftonit) (n = 9)	fourfold (n = 4)	4	44.44	2	50.0	4.4±0,03
		fivefold (n = 5)	5	100	1	20.0	5.5±0,03
total			9	100	3	33.33	4.95±0,03
3 experimental (serofibrinous mastitis)	Cefquinome sulfate (n = 8)	fourfold (n = 4)	4	50.0	1	25.0	4.2±0,04
		fivefold (n = 4)	4	100.0	0	0	5.1±0,02
total			8	100.0	1	12.5	4.65±0,03
4 experimental (purulent catarrhal mastitis)	Cefquinome sulfate (n = 9)	fourfold (n = 4)	4	44.44	2	50.0	4.5±0,03
		fivefold (n = 5)	5	100	2	40.0	5.5±0,02
total			9	100	4	44.44	4.95±0,02

Received data prove high therapeutic efficiency of fivefold application of Ceftonit® preparation at the dose of 1.0 ml/kg animal weight 1 time during 24 hours with serofibrinous mastitis, effect was 100% with the average recovery period of 4.7±0.02 days. Relapse in the form of sub clinical mastitis, revealed during 14–21 days, was 25%. Fivefold application of Ceftonit® preparation with purulent catarrhal mastitis at the dose of 1,0 ml / kg animal weight 1 time during 24 hours, provided 100% effect with the average recovery period of 4.95±0.03 days. Relapse in the form of sub clinical mastitis was 33.33%. Fivefold application of preparation based on Cefquinome sulfate at the dose of 1.0 ml / 50 kg animal weight 1 time during 24 hours with serofibrinous mastitis provided 100% effect with the average recovery period of 4.65±0,03 days, with purulent catarrhal mastitis (100%) with relatively good period of sick animal's recovery (4.95±0,02) days. Relapse was 44.44%.

Consequently, for receiving therapeutic effect of serofibrinous and purulent catarrhal mastitis fivefold application of Ceftonit® preparation at the dose of 1.0 ml/50 kg animal weight with the interval of 24 hours is necessary. Received data prove sufficient therapeutic efficiency of preparations used with clinical mastitis in cows; 100.0% efficiency with relatively short recovery

periods ( $5.5 \pm 0.03$  —  $5.5 \pm 0.02$  days) and admissible percentage of relapse in the form of subclinical mastitis (max — 33.33%).

Considering rather hard and hardly susceptible to treatment form of mastitis (purulent catarrhal) received data allows to recommend these preparations for therapy of this kind of mastitis in cows, as well as a compound part of complex therapy. Presence of antibiotics in milk is one of the factors that define the possibility of preparation use in a milk herd without milk use restrictions. Preparations based on Ceftiofur belong to a small group of preparations which can be used by lactating cows without restrictions. It is so due to the fact that with a recommended dose (1 ml / 50 kg) per day during five days therapeutic preparation concentration in organs, tissues and biological liquids do not exceed standards imposed by Sanitary epidemiological rules and regulations 2.3.2.2871-11 for milk.

It is confirmed by registrational research of preparations based on Ceftiofur for parenteral use made in Russia and abroad. But with all that, there was an apprehension that due to changes caused by mastitis, Ceftiofur would be excreted with milk in quantities exceeding admissible standard. That is why we made a research investigating the presence of antibacterial preparations in milk. Different stage data were analyzed: before introduction of preparations, during the treatment and after animal's recovery. Analysis was done with the use of BRT test of AIM firm, Germany.

Results are given in Tables 4 and 5.

Table 4.

MILK GLAND SECRETION INDICATORS IN COWS BEFORE AND AFTER CEFTONIT® PREPARATION INTRODUCTION

Indicators	before introduction		in 24 hours	in 48 hours	in 72 hours	in 144 hours
with serofibrinous mastitis						
antibiotics test	—		—	—	—	—
latent mastitis test	++		++	+	+	—
quantity of somatic cells, thousands	500–100		170–500	170–500	170–500	0–170
presence of mastitis	+		+	+/-	+/-	—
with purulent catarrhal mastitis						
antibiotics test	—		—	—	—	—
latent mastitis test	+++		+++	+++	+	—
quantity of somatic cells, thousands	1000–5000		1000–5000	1000–5000	170–500	0–170
presence of mastitis	+		+	+	+/-	—

After a single and subsequent introduction of Ceftonit® preparation the presence of antibiotics was not detected in any milk samples of cows with different mastitis forms. After the introduction of preparation based on Cefquinome sulfate antibiotics were detected in all milk samples, that comply with the restrictions registered in instruction for use. On the basis of the received data it can surely be said, that application of Ceftonit® preparation for mastitis treatment do not lead to milk restrictions caused by the presence of antibiotics.

Table 5.

MILK GLAND SECRETION INDICATORS IN COWS BEFORE AND AFTER CEFQUINOME SULFATE PREPARATION INTRODUCTION

<i>Indicators</i>	<i>before introduction</i>	<i>in 24 hours</i>	<i>in 48 hours</i>	<i>in 72 hours</i>	<i>in 144 hours</i>
with serofibrinous mastitis					
antibiotics test	—	++	+	+	+/-
latent mastitis test	++	+	+	+	—
quantity of somatic cells, thousands	500–100	170–500	170–500	170–500	0–170
presence of mastitis	+	+	+/-	+/-	—
with purulent catarrhal mastitis					
antibiotics test	---	+--+	+	+	+
latent mastitis test	+++	+++	++	++	—
quantity of somatic cells, thousands	> 5000	1000–5000	170–500	170–500	0–170
presence of mastitis	+	+	+/-	+/-	—

*Conclusions*

–Received data prove sufficient therapeutic efficiency of preparations used with clinical mastitis in cows; 100.0% efficiency with relatively short recovery periods ( $5.5 \pm 0,03$  days) and admissible percentage of relapse in the form of subclinical mastitis (max — 44.44%).

–Both examined preparations demonstrated practically identical, high therapeutic efficiency in treatment of studied mastitis forms in cows with average recovery period of 5 days.

–Disease relapse in the form of subclinical mastitis with Ceftonit® preparation treatment was 25–33%, and with preparation based on Cefquinome sulfate the number of relapses was higher (up to 44.44%). Ceftonit® preparation has convenient dosage — 1ml / 50 kg, and it does not cause milk restrictions in contrast to the preparation based on Cefquinome sulfate with its dose of 1ml / 25kg and milk restrictions confirmed by the experiments.

On the basis of above mentioned experiments it is possible to make the following practical suggestions:

–Ceftonit® preparation can be recommended for treatment of serofibrinous and purulent catarrhal mastitis in cows by fivefold application at a dose of 1.0 ml / 50 kg 1 time during 24 hours with the obligatory recovery control by means of latent mastitis test.

–Ceftonit® preparation cannot be detected in milk by BRT test of AIM firm, Germany, in contrast to the preparation based on Cefquinome sulfate, that makes it possible to recommend Ceftonit® preparation in a milk herd.

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