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Research Article

Diagnosis and Therapy of Subclinical Mastitis in Lactating Dairy Cows

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Abstract | Subclinical mastitis is one of the most common diseases in dairy cattle and is diagnosed in more than 25-45% of cows. Despite of extensive economic losses, no effective treatment is yet available. The present study was sought to compare the therapeutic efficacy of ceftiofur and tsefkinoma sulfate based drugs in the treatment of subclinical mastitis during lactation periods. At the first stage, three commentary tests were applied to not only identify the clinical but also to sub-clinical cases of mastitis, including Ketotest, Masttest, and California mastitis test. These tests were simultaneously applied on two analogous groups: Group 1 (n=389) was treated with drug based on ceftiofur whereas Group 2 (n=392) was treated with drug based on tsefkinoma sulfate. Sick animals in each group were divided into two similar subgroups according to the frequency of the use of drugs. Blood sampling and mammary secretion were collected before the application of the drugs and after treatment at 24, 48 and 72 hours. Haematological and biochemical studies were carried out to calculate the number of somatic cells and the presence of antibiotics in mammary secretions. Results revealed that the drug based on ceftiofur was not found in the milk and its therapeutic efficiency is proved at 100% in the treatment of subclinical mastitis in cows. Furthermore, treatment with ceftiofur and tsefkinoma sulfate based drugs causes a decrease in somatic cell counts, lactoperoxidase; and an increase in lactoferrin activity. Taken together, results demonstrated the effective application of tested drugs for the treatment of sub-clinical mastitis and may alone or in combination appear to be reliable control strategies for this deadly illness.

Keywords | Subclinical mastitis, Blood and milk biochemical indicators, Ceftiofur, tsefkinoma sulfate, Cattle

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INTRODUCTION

One of the major challenges of developed dairy farming worldwide is to increase the productivity of cows and to improve nutritional and sanitary qualities of milk (Abdrakhmanov, 2002; Avdeenko, 2009; Gamayunov and Novikov, 2002; Kartashova, 1980; Parikov et al., 2005). However, several diseases challenge this aim and significantly reduce not only the quantity but also the quality of the milk. Mastitis, inflammation of the teats, is one of such diseases, which are considered major constraints in the supply of food especially to the developing nations. According to the International Dairy Federation and the Communications of the European Association of Animal Production the clinical mastitis is diagnosed in 20-25% whereas subclinical mastitis is reported in 35-50% of dairy cattle (Aknazarov et al., 2009; Aliev, 2007; Avdeenko, 2009). Moreover, the subclinical mastitis may persist for one to two lactations in ignored cases (Vachevsky et al., 2005).

Financial losses associated with the diseases of the udder and teats in lactating animals may be due to treatment of animals, veterinary services, increase in labour costs and the milk quality deterioration. In Europe, these costs are estimated to be 233$ per head per year (Bradley and Green, 2000; Shakhov et al., 2001). Diseases of the udder and teats in postpartum currently occupy the first place in morbidity structure of these animals (Akhmetov, 2012; Bradley and Green, 2000; Erskine et al., 2002; Slobodjanik, 1999).

Table 1: Doses and administration plan of ceftiofur and tsefkinoma sulfate for treatment of subclinical mastitis in cows

Groups of animals	Drug	Drug dose	Frequency	Parameters control
1st experimental group	Ceftiofur	1.0 ml/ 50 Kg., 1 time per day (n=20)	Twice	Morphological and biochemical parameters of blood, the number of somatic cells, CFU, camphene, the antibiotic residues
			Thrice	
2nd experimental group	Tsefkinoma sulfate	2.0 ml/ 50 Kg., 1 time per day (n=20)	Twice	
			Thrice	

In Europe, more than 38% of the total direct costs in the dairy herd is necessary for treatment and prevention of subclinical mastitis (Erskine et al., 1993; Kartashova, 1995; Klimov et al., 2009). These heavy economic losses are further aggravated by the lack of reporting of subclinical infections as these cases are only often registered in countries with most intensive exploitation of animals, developed dairy cattle, and high level of mechanization and automation of production (Kartashova and Ivanov, 1993).

To investigate the causes of subclinical mastitis, efforts have been made for a long time without any satisfactory success. Despite it is recognized by a wider community of researchers that microbial factors play crucial roles in the development of mastitis, subclinical mastitis has not yet been declared as an infectious disease in nosological profile (Avdeenko, 2009; Klimov and Slobodjanik, 2012). It is suggested that disease is mainly contributed by environmental factors (associated with feeding and milking) (Bagmanov, 2011; Bradley, 2002), or the illness is developed through polyetiologyque infection.

Recently, the market of pharmaceutical veterinary industry acquired popular products based on cephalosporins (Erskine et al., 2002). Ceftiofur represents the third generation of cephalosporins, which is specifically synthesized for use in veterinary medicine (Wenz et al., 2005). Tsefkinoma sulfate is a part of cephalosporin of the fourth generation, possesses a wide range of antibacterial action against majority of gram-positive and gram-negative bacteria (Avdeenko et al., 2016).

The primary purpose of the study was to compare the therapeutic efficacy of ceftiofur and tsefkinoma sulfate for the treatment of subclinical mastitis during lactation.

MATERIALS AND METHODS

Animal Experiment

The work was performed in 2011-2013 at the Department of Therapy, Obstetrics and Pharmacology, Saratov State Agrarian University, JSC Nita-Farm, and at farms of various patterns of ownership of Saratov region (Uchkhoz RGAU-ICCA them Timiryazev "Mummovskoe" Atkarsky District, JSC "Agri" Volga "Marxian district, K (F) X FE "AV Akimov "Bazarno-Karabulaksky District). A total of 781 milking cows of Simmental breed from the 7th to the 27th day after delivery, were used in the study. Studies were carried out under the guidelines of the European Convention for protection of animals.

During external examination of cows, attention was paid to hair condition, the shape of udder, and symmetry of quarters, skin colour, teat's size, and the sphincter of the teat canal. Using palpation the local temperature was defined, and the mammary gland consistency and presence of pain reaction was observed. Holding alternatively palpation of the right and left half mammary tissue by probing from the base to the top of teat, after that we determine the state of udder's lymph nodes, their size, consistency and pain reaction. Decanting to test the sphincter tonus of the teat canal and its permeability ends the clinical study. The quantity, homogeneity, colour, viscosity and appearance of milk secretion from each mammary quarter were examined and the presence of clots, flakes, blood and watery secretions were observed.

For the diagnosis of subclinical mastitis following test were performed: Ketotest (Intervet, the Netherlands), Masttest (Agrofarm, Russia), CMT - California mastitis test (Pfizer, USA). In addition, the somatic cell count was performed in the counting chamber with a grid and using Somatos mini.

According to the results of diagnostic test, two experimental groups were constituted. Group 1 consists of 389 cows and were treated with ceftiofur (ZAO "Nita-Farm" series - 004 211 212) whereas Group 2 cows (n=392) were treated with tsefkinoma sulfate. Sick animals in each group were divided into two subgroups according to the frequency of the drugs used. Blood and mammary secretion sampling were performed before the application of the preparations, and after treatment at 24, 48, and 72 hours on the 5th day. Drugs were used through subcutaneous injection (SC) route in a therapeutic dose according to the instructions (Table 1).

Laboratory Analyses

For haematological studies, an automatic haematology analyser (Abacus Junior Pse 90 Vet, Germany) and biochemical blood analyser (Chem Well combi Models 2902 and 2910, USA) were used. The number of somatic cells and the presence of antibiotics were determined in mammary secretions and BRT-test (AIM, Germany) was used for the determination of antibiotics.

Table 2: The clinical effects of ceftiofur and tsefkinoma sulfate on subclinical mastitis in cows

Groups of animals	Drug	Frequency	Clinical effect		Term of recovery (days)
			n	%	
1st experimental group (n=20)	(Ceftiofur) (n=10)	Twice	8	80.0	2.64±0.03
	(Ceftiofur) (n=10)	Thrice	10	100.0	3.23±0.02
Total			**18**	**90.0**	**2.93±0.02**
2nd experimental group (n=20)	Tsefkinoma sulfate (n=10)	Twice	9	90 .0	2.41±0.03
	Tsefkinoma sulfate (n=10)	Thrice	10	100.0	3.24±0.02
Total			**19**	**95.0**	**2.32±0.03**

Table 3: Analysis of different antibacterial parameters of udder secretion during the treatment of subclinical mastitis by ceftiofur

Parameter	Before treatment (n = 20)	After treatment (n = 20)		
		1st day	3rd day	5th day
Somatic cells (thousand/ml)	4003.7 ± 534.7	1513.4 ± 157.6	954.7 ± 85.6	954.7 ± 85.6
IgG (mg/ml)	3.55 ± 0.13	2.36 ± 0.17	2.00 ± 0.24	1.90 ± 0.12
IgM (mg/ml)	0.22 ± 0.02	0.32 ± 0.03	0.36 ± 0.04	0.20 ± 0.03
M3	0.39 ± 0.04	0.57 ± 0.05	0.67 ± 0.04	0.65 ± 0.05
Lactoperoxidase (U/ml)	992.7 ± 47.5	802.4 ± 72.3	635.0 ± 64.5	532.4 ± 49.1
Lactoferrin (mg/ml)	359.5 ± 64.8	274.4 ± 22.2	110.2 ± 29.5	101.5 ± 14.5

Statistical Analysis

Statistical analyses were performed using the standard Microsoft Excel 2000 and SPSS 10.0.5 for Windows and results were presented as mean± SEM. The significance level was considered when $P < 0.05$.

RESULTS AND DISCUSSION

The therapeutic efficiency of the ceftiofur was appeared to be 90.0%, with a recovery average time of the udder function 2.93 ± 0.02 days. At the same time, the use of the drug based on tsefkinoma sulfate showed clinical benefit in 95.0% of cows, with an average recovery time of the udder function of 2.32 ± 0.03 days, from the beginning of treatment (Table 2). These data suggest high therapeutic efficiency of the drugs administered to cows with subclinical mastitis (90.0-100.0%) with a sufficiently good recovery timing of 2.64 ± 0.03-3.23 ± 0.02 days, and no disease recurrence (Table 2).

The absence of antibiotics in milk facilitates the use of these drugs in dairy herd. Drugs based on ceftiofur can be used without restriction in lactating animals, which is confirmed by special studies conducted in Russia (Klimov and Slobodjanik, 2012). However, there was apprehension due to changes of the tissues caused by mastitis, ceftiofur can be found in the produced milk in quantities that exceed the acceptable permitted limit. Therefore, investigation on the presence of antimicrobials in milk is imperative.

Results of antibacterial substances found in mammary secretions have been summarized in Table 3. Significant changes in the content of immunoglobulin class G and M, and an increase in antibody titre, a decrease of phagocytic index was observed which indicates the beginning of antibody production after 5 days (Parikov et al., 2009).

After the treatment of subclinical mastitis with the drug based on ceftiofur, on the 5th day from the beginning of treatment in comparison to the before treatment, there was a significant decrease in the udder somatic cells secretion by 14.78 times. The concentration of lactoferrin (LF) was decreased by 3.54 times, lactoperoxidase (LPO) by 1.86 times (Marek et al., 2011), and the activity muramidase (M3) has increased by 1.67 times (Riaz et al., 2012).

High blood levels of white blood cells due to the increase in neutrophil forms; stab by 63.6% and 25.4% segmented ones was recorded. At the same time, the number of lymphocytes was lowered by 6.4% and monocytes by 46.7%. The development of neutropenia was redistributed from the bloodstream into the mammary gland at a significant number (Parikov et al., 2009).

The number of erythrocytes reduced by 1.23 times. In 29.4% of animals, erythrocyte sedimentation rate (ESR) was within normal limits (moderate in 25.1%, and the average in 30.2%). Thrombocytes contents in the blood were reduced by 26.6-39.6% and hemoglobin by 13.9% ($p < 0.05$) (Table 4).

Table 4: Blood biochemical and hematological parameters analysed during the treatment of subclinical mastitis by ceftiofur and tsefkinoma sulfate

Parameters	Ceftiofur		Tsefkinoma sulfate	
	Before treatment	After treatment	Before treatment	After treatment
Total protein (g / l)	85.59 ± 2.35	85.6 ± 3.47	82.4 ± 4.41	83.48 ± 3.92
Erythrocytes (10^{12} / l)	5.57 ± 0.18	6.4 ± 0.3	5.37 ± 0.09	5.5 ± 0.4
Thrombocytes (10^{9} /l)	627.5 ± 12.7	544.75 ± 37.9	638.5 ± 17.5	564.65 ± 72.5
Hemoglobin (g / l)	95.0 ± 3.4	120.3 ± 2.6	97.0 ± 4.5	126.4 ± 9.9
ESR (mm / hr)	3.27 ± 0.35	2.64 ± 0.32	3.07 ± 0.31	2.76 ± 0.21
C3	17.6 ± 3.1	18.4 ± 2.0	1.6 ± 2.0	18.6 ± 1.7
C4	24.0 ± 3.6	33.3 ± 1.3	28.4 ± 2.0	42.5 ± 2.6
Phagocytic index (%)	1.02 ± 0.07	7.0 ± 0.1	1.00 ± 0.04	5.6 ± 0.4

Table 5: Therapeutic efficiency of ceftiofur and tsefkinoma sulfate for treatment of subclinical mastitis in cows

Method of therapy	Subjected to treatment		Recovered (%)		Left with violation functions (%)	
	Cows	Udder quarters	Cows	Udder quarters	Cows	Udder quarters
Ceftiofur	389	467	91.00	91.37	9.00	8.63
Tsefkinoma sulfate	392	542	91.84	91.25	8.16	8.75

Table 6: Results of milk BRT- test before and after treatment of subclinical mastitis in cows

Antibiotic	Indicators	Before treatment	After 24 hours	After 48 hours	After 72 hours	After 144 hours
Ceftiofur	BRT- test	-	-	-	-	-
	Presence of mastitis	+	+	+ /-	+/-	-
Tsefkinoma sulfate	BRT-test	-	+	+	+	+
	Presence of mastitis	+	+	+ /-	+ /-	-

Table 4 also shows the total protein level in blood at the beginning of lactation was increased to 10.2-18.1% reflecting the metabolic processes intensity after calving. In cows with subclinical mastitis, a decreased content of γ-globulins was observed (38.1%), and increased proportion of α- and β-globulins was noticed. Decrease in blood γ-globulin occurred due to active transport into the mammary gland. This reduction of immunoglobulin G by 26.3% was noticed due to the decrease of antigen binding activity of the formed immunoglobulin. This is indicated by the low concentration (11.9 ± 1.13 EU) of the circulating immune complexes (CIC, C3) and their small sizes (C4: C3 = 2.10-2.16) (Matei et al., 2010). Strengthening the antigen binding activity of the humoral factors of subclinical mastitis in cows in early lactation due to the presence in the blood of a heterogeneous by specific population of autoantibodies generated during involution process of genital organs after calving (Parikov et al., 2009). Overall, both therapeutic agents were found more than 90% effective for the treatment of subclinical mastitis in cows (Table 5).

The results showed that after single and subsequent administration of the drug based on ceftiofur in the milk of cows with subclinical mastitis, antibiotics were not detected in any of the samples (Table 6). After administration of the drug on the basis of tsefkinoma sulfate, antibiotic was discovered in all milk samples, which corresponded to the limitations specified in the instructions (Avdeenko, 2009).

During haematological studies, it was revealed that a subclinical mastitis was observed with leukocytosis in 29.5 % of cows from 15 to 20 thousands / mm3, while 51.5 % of the animals - from 20.6 to 43.9 thousands / mm3.

Table 7: Economic efficiency of the treatment of subclinical mastitis in cows with ceftiofur and tsefkinoma sulfate

Indicators	Method of therapy	
	Tsefkinoma sulfate	Ceftiofur
Cows treated (No.)	392	389
Recovered (No.)	360	354
Duration of treatment (days)	2.5	2.7
Treatment costs of one animal (USD)	3.19	2.28
Economic efficiency of 1 USD cost (USD)	0.11	0.17

The calculation of economic losses from lower milk production was carried out according to the instructions of the Veterinary Department of Agriculture of the Russian Federation (2010) and results are shown in Table 7.

Cost-effectiveness of the treatment of subclinical mastitis with the drug based on ceftiofur estimates 0.17 USD, and the preparation based on tsefkinoma sulfate costs 0.11 USD.

CONCLUSIONS

The analysis of the data showed that after treatment with ceftiofur and tsefkinoma sulfate, a decrease in somatic cell number, lactoperoxidase, lactoferrin occurred. When applying the drug thrice with ceftiofur (dose - 1.0 ml/50 kg live body weight, 1 time in 24 h) and tsefkinoma sulfate (dose - 2.0 ml/kg of 50 live body weight, 1 time in 24 h) therapeutic efficiency reached 100.0% with an average recovery duration of 2.64 ± 0. 03-3.23 ± 0.02 days, with total absence of disease recurrence. The drug based on ceftiofur unlike tsefkinoma sulfate was not found by the BRT-test in milk and thus it can be recommended for use in dairy herd without any restrictions.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS' CONTRIBUTION

All the authors contributed equally.

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