EFFECTIVENESS OF A NEW MEANS OF ETIOTROPIC THERAPY OF MASTITS IN COWS DURING THE LACTATION PERIOD

Taras Stetsko, Larysa Ostrovska, Yevhen Kostyschyn, Orest Katsaraba, Lidia-Mariia Kostyshyn, Dmytro Morozenko

The aim: is to study the therapeutic effectiveness of the new veterinary medicinal product Revozyn RTU 400 mg/ml (suspension for injections), manufactured by Eurovet Animal Health B.V. (Netherlands), in the treatment of acute (clinical) and hidden (subclinical) mastitis in cows during lactation.

Materials and methods. The research was conducted on dairy cows of the Simmental breed with a milk productivity of 6000-6500 kg of milk per lactation (F “Pchany-Denkovich”, Pchany village, Stryi district, Lviv region). To confirm the diagnosis of “acute mastitis” and to identify cows with hidden (subclinical) mastitis, as well as to establish the effectiveness of the researched drug after treatment, a sample was taken with the California Mastitis Test (CMT), manufactured by Bayer Animal Health GmbH, Germany. For bacteriological research, milk samples were taken from the affected quarters of the mammary gland (1 sample from each cow) in compliance with generally accepted sanitary rules. The sensitivity of bacterial isolates to the drug was determined by diffusion in agar using standard discs with benzylpenicillin.

Results. A clinical examination of 127 Simmental dairy cows of different periods of lactation was carried out. During the clinical-diagnostic examination of animals, 8 cows with clinically pronounced, acute course of mastitis were found. According to the nature of the exudate in 3 cows, mastitis was serous, in 5 cows it was purulent-catarrhal. For serous mastitis, the CM test gave a positive result – thickening, the milk solution looks like a gel. In case of purulent catarrh, a sample with the CMT gave a strongly positive result – the mixture thickened, the gel took on a certain shape and became very viscous. The CMT revealed 12 cows with suspected latent mastitis. Representatives of opportunistic microflora – Streptococcus agalactiae, Staphylococcus aureus, and Escherichia coli bacteria were isolated and identified from mastitis milk. The summarized results of the clinical trial of the drugs Revozyn RTU 400 mg/ml and Procillin® 30 % on cows suffering from the clinical form of mastitis were as follows. On the 10th day of the experiment, a milk sample with the California mastitis test showed that all milk samples from cows of both groups (with the exception of one milk sample from a cow from the control group) gave a negative result (the solution remained liquid, blue or gray and homogeneous, without clots). Taking into account the results of the CMT and the number of somatic cells in the milk after the therapy, the therapeutic effectiveness of the veterinary medicinal product Revozyn RTU 400 mg/ml in the treatment of various forms of mastitis in lactating cows was 100 %, and the one of the comparative drug Procillin® 30 % was 83.3 %.

Conclusions. A clinical study of the veterinary medicinal product Revozyn RTU 400 mg/ml, suspension for injections, manufactured by Eurovet Animal Health B.V. (Netherlands) established its effectiveness in the dosage, recommended by the manufacturer, in the treatment of clinical and subclinical mastitis in lactating cows, caused by microorganisms sensitive to benzylpenicillin. In terms of therapeutic effectiveness, the drug Revozyn RTU 400 mg/ml was not inferior to the comparative drug Procillin® 30 % in its dosage form and active substance when used in the treatment of various forms of mastitis in lactating cows.

Keywords: lactating cows, clinical and subclinical mastitis, milk, sensitivity of microorganisms, benzylpenicillin, therapeutic efficacy


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1. Introduction

Mastitis is considered the most common among all non-infectious diseases of cows, which causes significant economic losses to dairy cattle due to the decrease of milk production, reduction of its quality, premature culling of cows, morbidity of newborn calves, treatment costs, etc. [1]. On average, the total cost of losses due to bovine mastitis is estimated at US$147 per cow per year [2]. The decrease in milk production accounts for 70 % of the total economic losses from mastitis [3].
Numerous scientific works of domestic and foreign researchers over the last decade indicate that the incidence of mastitis in cows in clinical and subclinical forms remains at a high level [1, 2].

The majority of scientists consider the main cause of mastitis in cows to be pathogenic microorganisms [4, 5], among which the main role in the development of the inflammatory process in the mammary gland is assigned to bacterial opportunistic microflora [6, 7]. Mastitis in cows can be caused by both gram-positive (Staphylococcus aureus, Streptococcus spp. (for example, Strep. uberis)) and gram-negative (E. coli, Klebsiella spp., Enterobacter spp., Pseudomonas spp.) bacteria [8]. In the vast majority of cases, mastitis in cows is caused by streptococci and staphylococci. Thus, Streptococcus agalactiae (serogroup B according to the Landsfield classification) is a specific causative agent of mastitis in cows, causing subclinical mastitis with a high level of somatic cells in milk and low milk productivity [9]. Staphylococcus aureus is also one of the most common etiological factors of acute and chronic mastitis in dairy herds [10]. Mastitis, caused by E. Coli, is less common in cows [11].

Today, antibiotic therapy remains the basis of treatment of bacterial mastitis in cows. For the treatment of mastitis in lactating cows, both systemic antibacterial therapy in the form of solutions and suspensions for intramuscular or subcutaneous administration and drugs for intramammary infusion are used [12, 13]. Currently, Ukraine has a number of preparations for intracisternal use, which have a wide spectrum of antibacterial action. They are administered through the milk duct using catheters, special tubes or syringe machines [14, 15]. However, in connection with the progressive development of antibiotic resistance among bacterial strains, causative agents of mastitis [16], especially staphylococci and escherichia [17, 18], the effectiveness of intracisternal drugs in the treatment of mastitis in cows has significantly decreased.

There are a number of injectable antibacterial drugs for the systemic treatment of mastitis in cows. Parenteral administration of antibiotics provides a quick effect when the causative agent enters the animal’s bloodstream, especially in the initial stage of the inflammatory process. First of all, this applies to those antibiotics that are inactivated in the digestive tract, such as, for example, benzylpenicillin, which is destroyed by the hydrochloric acid of gastric juice.

Many specialists of veterinary medicine believe that the treatment of mastitis should be complex, when along with the local intramammary administration of antimicrobial drugs, it is necessary to use injectable antimicrobials for the systemic treatment of the animal [12, 19]. To choose an effective etiotropic agent for the treatment of bacterial mastitis in cows, it is important to establish what is the causative agent (or causative agents) of the disease and determine the degree of its sensitivity to antibiotics [19, 20].

Currently, in Ukraine there is no registered, commercially available veterinary medicinal product for parenteral administration for the treatment of mastitis in cows, the active substance of which is penetratame hydroiodide. Penethamate in an aqueous medium is hydrolyzed to benzylpenicillin and diethylaminoethanol. An antimicrobial activity of benzylpenicillin is unique. The antimicrobial spectrum of penetratame corresponds to the antimicrobial spectrum of benzylpenicillin, which is effective against non-beta-lactamase-producing Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis and Staphylococcus aureus. Useful properties of penetratame after intramuscular administration are its rapid absorption from the injection site, rapid therapeutic effect and short elimination period. An effective therapeutic effect is provided by the ability of penetratame to cross the blood-milk barrier and concentrate in the udder.

The company Eurovet Animal Health B.V. developed a veterinary medicine Revozyn RTU 400 mg/ml in the form of suspension for injections for the treatment of mastitis in lactating cows, the active substance of which is penetratame hydroiodide.

The aims of the research is to study the therapeutic effectiveness of the new veterinary medicinal product Revozyn RTU 400 mg/ml (suspension for injections), manufactured by Eurovet Animal Health B.V. (Netherlands), in the treatment of acute (clinical) and hidden (subclinical) mastitis in cows during lactation.

2. Materials and methods

The research was conducted on dairy cows of the Simmental breed with a milk productivity of 6000–6500 kg of milk per lactation (F “Pchany-Denkovich”, Pchany village, Striy district, Lviv region).

The diagnosis of "bovine mastitis" was made on the basis of the anamnesis, clinical signs of the disease, the results of a sample with the California mastitis test, determination of the number of somatic cells in milk, as well as the results of a bacteriological examination of the milk of animals suffering from mastitis.

Clinical and diagnostic research of animals was carried out in accordance with methodical recommendations for diagnosis and research of the general state of the animal organism [21]. During the entire clinical experiment, the animals were under observation, which included the registration of changes in the general condition and clinical signs.

To confirm the diagnosis of "acute mastitis" and to identify cows with hidden (subclinical) mastitis, as well as to establish the effectiveness of the researched drug after treatment, a sample was taken with the California Mastitis Test (CMT), manufactured by Bayer Animal Health GmbH, Germany. For this purpose, the first drops of milk were expressed, milk samples from each quarter of the udder were introduced into separate wells of the milk control plate (2–3 ml). 2–3 ml of CMT was added to each well. The mixture was blended in the wells with circular movements, observing the reaction of the mixture's viscosity change. After 10 seconds, the result was read. The result was evaluated as follows:

- negative result: the solution remains liquid, blue or gray and homogeneous, there are no clots (no mastitis);
- weakly positive result: slight thickening of the solution, and the reaction may disappear after about 10 seconds (suspicion of mastitis, hidden mastitis);
- positive result: thickening, the solution may resemble a gel (mastitis);
The number of somatic cells in milk was determined using the Ekomilk device. For bacteriological research, milk samples were taken from the affected quarters of the mammary gland (1 sample from each cow) in compliance with generally accepted sanitary rules [22].

Isolation and identification of microorganisms, the causative agents of mastitis in cows [23], was carried out according to generally accepted microbiological methods [24, 25] by sowing the selected milk secretion on general nutrient media (meat-peptone agar – MPa, meat-peptone broth – MPB), blood agar, Endo medium in order to study the species and cultural properties of pathogens mastitis, and smear microscopy was also performed to study the morphological properties of the selected cultures.

The sensitivity of bacterial isolates to the drug was determined by diffusion in agar using standard discs with benzylpenicillin [26]. To determine the antimicrobial sensitivity by the disk diffusion method, the Mueller-Hinton nutrient medium and standard disks with benzylpenicillin (10 μg), manufactured by HiMedia Laboratories Pvt Ltd (India), were used. The results of the sensitivity test to benzylpenicillin were interpreted as follows: the diameter of growth retardation around the disk with the antibiotic ≥28 mm – the strain of the microorganism is sensitive; 20–27 mm – moderately sensitive; ≤19 mm – resistant.

The conducted research met the requirements of the "General Ethical Principles of Animal Experiments" (Kyiv, 2001), are consistent with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Scientific Experiments or for Other Scientific Purposes (Strasbourg, 1986), correspond to Law of Ukraine No. 3447 -IV dated February 21, 2006 "On the protection of animals from cruelty" and Directive 2010/63/EU "On the protection of animals used for scientific purposes". The research materials were reviewed and approved at a meeting of the bioethics committee, protocol No. 12 dated 05/29/2023.

### 3. Research results

A clinical examination of 127 Simmental dairy cows of different periods of lactation was carried out. During the clinical-diagnostic examination of animals, 8 cows with clinically pronounced, acute course of mastitis were found. According to the nature of the exudate in 3 cows, mastitis was serous, in 5 cows it was purulent-cataarrhal. Serous mastitis was characterized by hypere mia, significant sweating of serous exudate into the subcutaneous connective tissue between the lobes of the mammary gland. Depression of the cow’s general condition, decreased appetite, slight increase in body temperature was observed. The affected quarter of the udder was enlarged in volume, dense, "stony", the skin of the affected quarter of the udder was swollen, tense, reddened. The local temperature was slightly increased, a slight soreness was felt. Milk secretion was reduced. Initially, visually, the milk from these animals did not differ from the milk of healthy cows. In the future, the viscosity of milk decreased, it became bluish or cyanotic in color and liquid in consistency, with casein clots that could clog the milk ducts. A sample with CMT gave a positive result – thickening, the milk solution looks like a gel.

With purulent-cataarrhal mastitis, a depressed state of the animal, a slight increase in temperature, a decrease in appetite and a significant decrease in milk production were observed. The milk was initially watery, later yellowish or gray-dirty in color with admixtures of flakes and purulent blood clots. The cow was worried when palpating the udder – it was swollen and painful, dense nodules and compaction were felt. Visually affected udder quarters were enlarged. Obstruction of the ducts of the affected quarters of the mammary gland was observed. A sample with CMT gave a very positive result – the mixture thickened, the gel took on a certain shape and became very viscous.

The CM test revealed 12 cows with suspicion of hidden mastitis. The test gave a weakly positive result - a slight thickening of the solution (the reaction could disappear after about 10 seconds). The number of somatic cells in the milk of these animals confirmed the presence of subclinical mastitis. A generalized assessment of the results of the milk research is given in Table 1.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of animals</th>
<th>Result samples from CMT</th>
<th>Number of somatic cells, thousands/cm³*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute serous mastitis</td>
<td>3</td>
<td>positive</td>
<td>4069±153</td>
</tr>
<tr>
<td>Acute purulent-cataarrhal mastitis</td>
<td>5</td>
<td>very positive</td>
<td>5980±238</td>
</tr>
<tr>
<td>Subclinical mastitis</td>
<td>12</td>
<td>weakly positive</td>
<td>1780±87</td>
</tr>
<tr>
<td>Healthy cows</td>
<td>107</td>
<td>negative</td>
<td>336±38</td>
</tr>
</tbody>
</table>

Note: * – standards of DSTU 3662:2018 Cow’s raw milk. Technical conditions: extra – up to 400 thousand/cm³; higher grade – up to 400 thousand/cm³; the first grade – up to 500 thousand/cm³

The results of studies of the number of somatic cells in milk showed that in the secretion of sick animals, compared to clinically healthy ones, the total content of somatic cells was significantly higher and amounted to: 4069±153 thousand/cm³ – in acute serous mastitis, 5980±238 thousand/cm³ – in acute cataarrhal mastitis, 1780±87 thousand/cm³ – in case of subclinical mastitis.

Representatives of opportunistic microflora – *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Escherichia coli* bacteria were isolated and identified from mastitis milk. Most *Streptococcus agalactiae* cultures (17 isolates) were isolated from mastitis milk, fol-
lowed by 12 strains of *Staphylococcus aureus* and 3 strains of *Escherichia coli* (Table 2). These bacteria, which are representatives of opportunistic microflora [4, 5], are the most common etiological factors of mastitis in cows during lactation [10, 11]. In 12 cows mastitis was caused by an association of microorganisms (60 %), in 8 cows (40 %) monoinfection was registered. The results of the test for sensitivity to benzylpenicillin of Streptococcus agalactiae and Staphylococcus aureus bacterial isolates are shown in Fig. 1 and 2. Of the 17 strains of Streptococcus agalactiae, 12 were sensitive (70.6 %), 4 were moderately sensitive (23.5 %), and only one was resistant (5.9 %) to benzylpenicillin.

### Table 2

<table>
<thead>
<tr>
<th>Form mastitis</th>
<th>Number of samples</th>
<th>Selected microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Str. agalactiae</em></td>
</tr>
<tr>
<td>Clinical mastitis</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Subclinical mastitis</td>
<td>12</td>
<td>3</td>
</tr>
</tbody>
</table>

*Staphylococcus aureus* cultures showed a fairly high degree of sensitivity to benzylpenicillin. Thus, 9 isolates (75 %) were sensitive to penicillin G, 3 isolates (25 %) of *Staphylococcus aureus* were moderately sensitive. No resistant strain was detected.

Such a high level of sensitivity of gram-positive bacteria *Str. agalactiae* and *S. aureus* to benzylpenicillin is explained by the mechanism of its antimicrobial action, which consists in disrupting the synthesis of peptidoglycan, a mucopeptide of the cell membrane, which leads to the inhibition of the synthesis of the microorganism's cell wall, inhibiting the growth and reproduction of bacteria [27]. Since the peptidoglycan layer is much thicker in gram-positive bacteria (20–80 nm) than in gram-negative bacteria (7–8 nm), and it makes up to 90 % of the dry mass of gram-positive bacteria (only
10% – gram-negative), penicillin G effectively acts specifically on cocci [28].

All Escherichia coli isolates were resistant to benzylpenicillin, since most gram-negative bacteria usually have primary (natural) resistance to natural penicillins [29].

To establish the effectiveness of the veterinary medicinal product Revozyn RTU 400 mg/ml (suspension for injections), manufactured by Eurovet Animal Health B.V. (Netherlands), in the treatment of both clinical and subclinical mastitis in lactating cows, its clinical trial was carried out under the production conditions of a dairy farm.

All cows suffering from mastitis (20 heads) were divided into 2 groups (control and experimental) of 10 cows each. In each group there were 4 cows with clinically expressed inflammation of the mammary gland and 6 cows with a hidden form of mastitis.

The animals of the research group were intramuscularly injected with the drug Revozyn RTU 400 mg/ml (suspension for injections) in the neck area in the dose: cows with clinical mastitis – 15,000 IU (15 mg of penicillamine hydroiodide) per kg of body weight, which corresponds to 3.75 ml of the drug per 100 kg of body weight for 3 days; cows with a subclinical form of mastitis – 10,000 IU (10 mg of penicillamine hydroiodide) per kg of body weight, which corresponds to 2.5 ml of the drug per 100 kg of body weight for 3 days. Before use, the bottle with the drug was thoroughly shaken to obtain a homogeneous suspension. The drug was administered alternately, in the left and right side.

Animals of the control group were injected with the drug Procillin® 30% (suspension for injections), manufactured by Lohmann Pharma Herstellung GmbH (Germany). The active substance of the drug is benzylpenicillin in the form of novocaine salt. Procillin® 30% was administered intramuscularly in the dose: cows with clinical mastitis – 20,000 IU of procaine benzylpenicillin per 1 kg of body weight, which corresponds to 1.0 ml of the drug per 15 kg of body weight once a day for 3 days; cows with a subclinical form of mastitis – 10,000 IU of procaine benzylpenicillin per 1 kg of body weight, which corresponds to 0.5 ml of the drug per 15 kg of body weight once a day for 3 days. Before use, the vial with the drug was thoroughly shaken.

The effectiveness of the treatment of cows with the clinical form of mastitis was evaluated, taking into account the following indicators: normalization of the local temperature, improvement of the general condition and appetite, absence of foci of mammary gland complications, flakes and clots in milk, development of complications and transition to another form, percentage of recovery, average recovery time, indicators of recovery of milk productivity, therapeutic effectiveness.

Already after the second administration of both drugs, a decrease in soreness and a decrease in the local temperature of the affected quarters of the udder were registered in most cows. In general, normalization of the local udder temperature in cows occurred on average 3-4 day after the start of treatment. Improvement of the general condition and recovery of appetite, normalization of the local udder temperature, and reduction of the number of flakes and clots in milk after milking were recorded. Complete resorption of the foci of compaction, normalization of milk secretion, absence of clots and flakes in milk was recorded on 5–6 day. Recovery of milk productivity of cows of both groups was recorded on the 9–11th day after the start of treatment. In one cow from the control group, after treatment with the drug Procillin®, 30% of the disease went into a latent form, which was confirmed by the results of a sample with CMT (weakly positive) and an increased number of somatic cells in the milk of cows after the therapy (1230 thousand/cm³).

The summarized results of the clinical trial of the drugs Revozyn RTU 400 mg/ml and Procillin® 30% on cows suffering from the clinical form of mastitis are shown in the Table 3.

Table 3

<table>
<thead>
<tr>
<th>Indicators of the drug</th>
<th>Revozyn RTU 400 mg/ml</th>
<th>Procillin® 30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalization of the local temperature, days</td>
<td>3.3±0.43</td>
<td>3.5±0.50</td>
</tr>
<tr>
<td>Improvement of the general condition and resoration of appetite, days</td>
<td>3.5±0.50</td>
<td>3.5±0.50</td>
</tr>
<tr>
<td>Absence of foci of udder seals, days</td>
<td>5.7±0.43</td>
<td>5.3±0.43</td>
</tr>
<tr>
<td>Absence of flakes and clots in milk, days</td>
<td>5.3±0.43</td>
<td>5.5±0.50</td>
</tr>
<tr>
<td>Recovery period, days</td>
<td>5.7±0.43</td>
<td>5.5±0.50</td>
</tr>
<tr>
<td>Recovery of milk productivity, days</td>
<td>10.2±0.57</td>
<td>10.3±0.43</td>
</tr>
<tr>
<td>Number of recovered animals, total</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Therapeutic efficiency, %</td>
<td>100</td>
<td>83.3</td>
</tr>
</tbody>
</table>

On the 10th day of the experiment (7 days after the last administration of drugs), milk samples were taken from all cows, included in the study, a repeated sample was made with the California mastitis test, and the number of somatic cells in the milk was determined (Table 4).
A milk sample with the California Mastitis Test showed that all milk samples from cows in both groups (with the exception of one milk sample from a control group cow) gave a negative result (the solution remained liquid, blue or grey and homogeneous, without clots).

Taking into account the results of the CMT and the number of somatic cells in the milk after the therapy, the therapeutic effectiveness of the veterinary medicinal product Revozin RTU 400 mg/ml in the treatment of various forms of mastitis in lactating cows was 100 %, and the one of the comparative drug Procillin® 30 % was 83.3 %.

**Research limitations.** The authors did not have the opportunity to evaluate laboratory parameters of blood during the examination of cows with mastitis.

**Prospects for further research.** A promising direction of research is the wide clinical use of the veterinary medicinal product Revozin RTU 400 mg/ml for cows in Ukrainian farms and the study of its effect on milk quality.

### 4. Conclusions

A clinical study of the veterinary medicinal product Revozin RTU 400 mg/ml, suspension for injections, manufactured by Eurovet Animal Health B.V. (Netherlands), established its effectiveness in the dosage, recommended by the manufacturer, in the treatment of clinical and subclinical mastitis in lactating cows, caused by microorganisms sensitive to benzylpenicillin. In terms of therapeutic effectiveness, the drug Revozin RTU 400 mg/ml was not inferior to the comparative drug Procillin® 30 % in its dosage form and active substance when used in the treatment of various forms of mastitis in lactating cows.

**Conflict of interests**
The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results, presented in this article.

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**Data availability**
Data will be made available on reasonable request.

**References**


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