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Pathogenesis and efficacy of therapeutic and preventive measures for associated bacterial infections of the distal limbs in dairy cows

K. Rodionova*, S. Vyrvykyshka*, M. Morozov*, M. Khimych*, A. Paliy**, I. Korkh***, V. Railean****

**Odesa State Agrarian University, Odesa, Ukraine*
***Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine*
****Livestock Farming Institute of the National Academy of Agrarian Sciences of Ukraine, Kharkiv, Ukraine*
*****"Exvet" Veterinary Clinic, Odesa, Ukraine*

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Odesa State Agrarian University, Panteleimonivska st., 13, Odesa, 65012, Ukraine. Tel.: +38-066-248-68-56. E-mail: katerina.rodionova@ukr.net

Institute of Experimental and Clinical Veterinary Medicine, Skovorody Hryhoriia st., 83, Kharkiv, 61023, Ukraine. Tel.: +38-066-225-34-34. E-mail: paliy.dok@gmail.com

Livestock Farming Institute of NAAS, Tvarynnykiv st., 1A, Kharkiv, 61026, Ukraine. Tel.: +38-095-425-09-58. E-mail: dr.fox2011@ukr.net

"Exvet" Veterinary Clinic, Knyazya Yaroslava Mudroho av., 19A, Odesa, 65000, Ukraine. Tel.: +38-095-064-82-16. E-mail: proffesorul@gmail.com

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The alarming prevalence of distal limb disorders in dairy cows has become one of the most pressing issues in modern dairy farming. A decline in milk production, reduced welfare, shorter reproductive lifespans, and significant economic losses accompany these disorders. Research aimed at determining the effectiveness of therapeutic and preventive measures as the basis for ensuring stable herd productivity is a priority in addressing this problem. Based on the results obtained, specific elements of a comprehensive system for monitoring welfare and implementing preventive and therapeutic measures have been substantiated. The proposed regimen involved three applications of hoof baths with a 0.5% solution of the drug "Septiflex", containing a chelated form of copper, at 14-day intervals. This approach facilitated the clinical recovery of 95% of the animals, restoring their milk production and live weight to the levels recorded before the onset of the disease. Three applications of a 0.5% solution of "Intra Hoof-Fit"- a preparation based on copper and zinc chelates – administered at 12-day intervals also resulted in the recovery of 95% of the animals. At the same time, the average daily milk yield and live weight in these cows remained 7.4% and 3.0% lower, respectively, compared to baseline levels, indicating incomplete recovery of their functional status. The inclusion of the antibiotic "Kocefen 200" in the treatment regimen from the very first day of treatment demonstrated the absence of toxic effects on milk, confirming its safety and suitability for use in veterinary practice without risks to product quality. During the 90 days following treatment completion, no recurrences of associated bacterial infections of the distal limbs were recorded in any experimental group, indicating a sustained therapeutic effect of the administered drugs and the efficacy of the proposed regimens.

Keywords: cows; copper chelates; zinc chelates; distal limbs; microbiota; hoof bath.

Introduction

Dairy farming remains a leading sector of agriculture, providing the population with highly nutritious food products, particularly milk and meat (Faverdin et al., 2022; Michalchenko et al., 2024). The quality and safety of these products depend directly on compliance with technological requirements, sanitary and hygienic conditions in animal husbandry, and the herd's epizootic well-being (Paliy et al., 2020; O'Donoghue et al., 2025). Violations of these conditions lead to the development of diseases of various origins, the consequences of which negatively impact the efficiency of technological processes in livestock production. Among these, orthopedic pathologies of the distal limb segments pose a particular danger and are currently considered one of the global challenges in dairy cattle farming. The industrialization and intensification of production over the past decades have been accompanied by a steady increase in the prevalence of these diseases among cows, as reported in many countries around the world. According to statistical data, the prevalence of this pathology among dairy herds in the United Kingdom is estimated by various researchers to average 35% (Charfeddine & Pérez-Cabal, 2017; Blackie & Maclaurin, 2019), in Norway – 40%, in Malaysia and Canada – up to 47%, Sweden – 72% (Sadiq et al., 2021), the Czech Republic – up to 50% (Novotná et al., 2019), Denmark, the United States, New Zealand, and North America – up to 55% (Adams et al., 2017; Daros et al., 2019; Bach et al., 2021), and in the Netherlands, according to various sources, from 70% to 80% (Charfeddine & Pérez-Cabal, 2017; Sadiq et al., 2021).

In Ukraine, limb disorders in cows, manifested as lameness, affect nearly 50% of the total active herd (Khomyn et al., 2018). High-producing animals are particularly susceptible to musculoskeletal disorders. This is due to significant nutrient loss through milk production and decreased overall body resistance (Ózsvári, 2017). Tissue damage in the cows' distal limbs significantly restricts mobility and leads to a substantial decline in the industry's economic efficiency (Matias et al., 2020; Omontese et al., 2020; Sadiq et al., 2020). In particular, in terms of the magnitude of losses caused, orthopedic pathologies rank third after mastitis and reproductive disorders (Oehm et al., 2019; Silva et al., 2021; Browne et al., 2022c). Direct losses are associated with the treatment of animals, while indirect losses result from reduced profitability due to decreased milk production, reduced reproductive capacity, and the premature culling of affected cows (Nuss et al., 2020; Ninković et al., 2021). Even minor orthopedic disorders in lactating cows lead to a decrease in milk production (Garvey, 2022).

According to some estimates, the decrease in productivity in cows with pathology of the distal limb can reach 20%. This is due to several factors. First, it is associated with a direct decrease in milk yield and a deterioration in milk quality (fat, protein, and lactose content) due to pain, stress, and reduced feed intake. These animals spend most of the day lying down, which increases the risk of udder dysfunction, particularly the development of mastitis (Afonso et al., 2020; Hisira et al., 2020). Second, production losses result from significant culling of milk during antibiotic treatment of animals (Wrzecinska et al., 2021b; Browne et al., 2022c). Limb diseases negatively impact the reproductive function of cows; in lame animals, stress causes elevated cortisol levels, which suppress the secretion of reproductive hormones. This leads to a longer period from the onset of estrus to ovulation, as well as an increase in the interval between calving and the next estrus and conception. It has been established that lameness reduces conception rates and the time to conception by

9 days, toe dermatitis by 20 days, and sole ulcers by 40 days (Omontese et al., 2020; Ratanapob et al., 2020; Wrzecińska et al., 2021a). Therefore, without proper maintenance of the hooves in an optimal morphofunctional state, timely detection and treatment of lesions, it is impossible to increase productivity and ensure high retention of the milking herd.

Diseases of the distal limbs are typically classified according to their etiology. Infectious lesions that cause lameness include digital dermatitis, interdigital dermatitis, heel horn erosion, and sole rot. Non-infectious lesions include laminitis, sole ulceration, toe ulceration, sole hemorrhage, and white line disease (Fabbri et al., 2020; Langova et al., 2020). Inflammatory processes in the limbs are caused by opportunistic microflora (streptococci, staphylococci, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* sp., enterococci, etc.), whose pathogenic properties are exacerbated by stress and a decrease in the body's natural resistance under the influence of various negative factors (Bay et al., 2023; Wong et al., 2024). The etiology of lameness is multifactorial and complex, with the main risk factors for limb diseases being housing and care conditions, feeding practices, and the animal's physiological state (Moreira et al., 2019). Given the multifactorial nature of the etiological factors that may contribute to the onset and development of diseases of the distal limbs in cattle, particularly the hooves, it is necessary to conduct a comprehensive assessment of the condition of livestock housing, including the flooring, and their compliance with sanitary and hygiene standards (Shkromada et al., 2019; French et al., 2023). The decisive risk factors for orthopedic injuries and lameness in cows are precisely the conditions of housing and care. It has been proven that violations of housing parameters, such as shortened stalls, hard flooring, excessive indoor humidity, irregular cleaning, lack of sufficient exercise, the presence of traumatic factors, and delayed health screenings, contribute to the emergence of widespread orthopedic diseases (Matzhold et al., 2021; Browne et al., 2022b). It has also been established that cows kept without access to pastures are more likely to suffer from lesions of the distal limb (Novotná et al., 2019; Crossley et al., 2021). Animals with hoof pathologies have shorter strides and longer stride durations compared to healthy cows (Aliiev et al., 2024).

Bedding has a significant impact on the strength and structure of the hoof horn. Prolonged exposure of productive animals to manure leads to softening of the hoof horn and an increased susceptibility to hoof damage, sole erosion, and bacterial infections, which spread rapidly in a moist environment. Contamination of bedding with mycotoxins and mold fungi further exacerbates their harmful effects on the animals' bodies (Abdugheni et al., 2023; Orobchenko et al., 2023). It is well established that a balanced diet is a key factor in the healthy growth of the hoof horn. Its strength and structure are influenced by the composition and balance of the diet, particularly the content of amino acids (cysteine and methionine), minerals (zinc, copper, selenium, manganese, cobalt), and vitamins (biotin, vitamins E and D) (Singh et al., 2019; Winter et al., 2022). Additionally, some researchers have concluded that disturbances in rumen microflora and its metabolites, caused by diets with excessively high levels of carbohydrates and proteins, an unbalanced ratio of these nutrients in concentrates, lead to changes in metabolic processes, thereby reducing the natural resistance of highly productive cows and contributing to the development of diseases of the distal limbs (Zhang et al., 2018; Guo et al., 2021). In particular, acidosis, which develops as a result of excessive production of lactic acid, histamine, and endotoxin release, also negatively affects hoof health and leads to hemorrhages in the sole, laminitis, and various forms of sole ulcers (Langova et al., 2020). In addition, the presence of toxic substances in the diet, particularly feed containing high levels of toxin-producing microfungi and mycotoxin residues, weakens the body's resistance and is an additional factor in the development of limb diseases (Franchino et al., 2024; Zhu et al., 2025).

Calving and the start of lactation can contribute to stress, immune dysfunction, and negative energy balance. During these periods, the cows' bodies are subjected to significant stress, leading to a loss of physiological tone and increased susceptibility to pathological processes. Hoof damage and the development of lameness in high-producing cows after calving are often associated with hormonal changes, particularly the action of hormones that cause relaxation of the pelvic ligaments during preparation for calving. In the early lactation period, animals exhibit a reduction in the thickness of the digital pad distal to the navicular bone and in adipose tissue in the caudal part of the sole, which creates conditions for the development of hoof pathologies (Nuss et al., 2020; Bach et al., 2021; Pirkkalainen et al., 2022). However, regardless of the primary etiological factor causing limb damage, numerous studies confirm the key role of bacterial microflora in the development of these diseases. Even cases of aseptic injuries often serve as a trigger for the development of severe pathologies, complicated by purulent-inflammatory and purulent-necrotic processes with superimposed fungal and bacterial infections (Kofler, 2017; Fabbri et al., 2020; Browne et al., 2022a).

This study aims to comprehensively assess the course of the pathological process and determine the effectiveness of therapeutic and preventive measures for associated bacterial infections of the distal limbs in cows.

Materials and methods

During the experimental studies presented in this paper, all manipulations with the cows involved in the research were carried out taking into account the basic principles of bioethics, according to Article 26 of the Law of Ukraine "On Protection of Animals from Cruelty", the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the "General Ethical Principles for Animal Experiments" adopted by the First National Congress on Bioethics (2012). This study was reviewed and approved by the Bioethics Committee of Odesa State Agrarian University. The sample collection procedure, feeding conditions, laboratory tests, and animal handling comply with ethical standards (Protocol No. 1, April 10, 2024).

The study was conducted at dairy farms in Odesa and Cherkasy regions, the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", the Multidisciplinary Laboratory of Veterinary Medicine, and the Department of Infectious Pathology, Biosafety, and Veterinary and Sanitary Inspection named after Prof. V. Y. Atamas at Odesa State Agrarian University, as part of the initiative research project "Ensuring Animal Welfare, Food Safety, and Quality in the Context of European Integration" (State Registration No. 0123U103523).

In the first stage of the study, a surgical (clinical-orthopedic) examination of herds of Ukrainian Red and Holstein cows was conducted on the farms to identify limb abnormalities by assessing their posture and static and dynamic functions (Khulek, 2015). Locomotion (degree of lameness) was assessed by observing their behavior at rest (standing) and during movement, focusing primarily on the position of the backline and gait characteristics, using a 5-point scale, where 1 point corresponded to the absence of abnormalities, while 5 corresponded to severe lameness of the highest degree (Klymas & Syrlyk, 2024). Animals that showed signs of lameness received surgical treatment on their hooves, followed by the collection of biological samples for laboratory analysis. For this purpose, the animals were secured in a restraint device and anesthetized using a circular novocaine block. Hoof treatment was performed using a disc knife and a trimming kit (manufactured by Kerb, Buchbach, Germany, 2024). A circular novocaine block was performed using a 0.5% novocaine solution, injected above the affected area, in the upper third of the metacarpus (metacarpal bone), to infiltrate all neurovascular bundles of the tissues. The extensor and flexor tendons of the digits served as landmarks for injection. The injection was performed laterally and medially from the tendons' edge to the bone, saturating all tissues with 100 mL of novocaine solution (Mysak et al., 2021).

In addition, studies were conducted to isolate and identify microorganisms involved in the development of purulent-necrotic inflammation of the distal limbs in cows, in accordance with current regulatory documents. The study material was examined for the presence of pathogens, including *Escherichia coli*, streptococci, enterococci, clostridia, pseudomonads, staphylococci, and other opportunistic and fungal microflora capable of causing disease. To isolate microorganisms from biological material and veterinary control objects in the barn, as well as to evaluate their cultural properties, both simple and selective culture media, discs, and strips for the differential diagnosis of microorganisms were used (manufactured by Farmaktiv LLC, Kyiv, Ukraine, 2024; and HiMedia Laboratories Private Limited, Thane, India, 2024). The species affiliation of the isolated microbial cultures was determined using tests described in the international reference book "Bergey's Manual of Systematic Bacteriology" (2007).

In the second phase of the study, tests were conducted to determine the sensitivity of microorganisms to antimicrobial agents using pure bacterial cultures. For this purpose, sets of antimicrobial agents (manufactured by Aspect LLC, Kyiv, Ukraine, 2024, and HiMedia Laboratories Private Limited, Thane, India, 2024) were used, and Mueller-Hinton agar was used as the culture medium. The susceptibility of microorganisms was determined to drugs from various groups: the penicillin group (amoxicillin, benzylpenicillin, ampicillin, amoxiclav); cephalosporins (cefazolin, cephalexin, cefotaxime, ceftazidime); macrolides (erythromycin, azithromycin, tylosin); lincosamides (lincomycin, clindamycin); fluoroquinolones (ofloxacin, enrofloxacin, ciprofloxacin, gatifloxacin, norfloxacin, levofloxacin); aminoglycosides (gentamicin, kanamycin, neomycin, amikacin); tetracyclines (tetracycline, doxycycline, oxytetracycline), amphenicols (chloramphenicol/levomycetin), polymyxins (colistin), and others (fosfomycin, furazolidone, co-trimoxazole). The results of the antibiotic susceptibility testing were classified into three categories: susceptible, moderately resistant, and resistant culture, in accordance with Order No. 167 "Determination of Microorganism Sensitivity to Antibacterial Agents" and the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

In the third stage of the study, two hoof baths were set up, each measuring 2.5 m in length, 1 m in width, and 20 cm in depth, with sloped entrances and exits located 2 m apart. The first bath was used for cleaning and rinsing off dirt, filled with a 0.1% solution of DK HOOF PRO Cleaner (manufacturer: "DK VET" Uman, Ukraine, 2024): 100 mL of the product was dissolved in 100 liters of water directly in the bath. In just 15 to 20 seconds, the solution effectively penetrated the dirt, removed it from the surface, and neutralized uric acid. An experimental disinfectant solution was added to the second bath. After 150 animals had passed through the baths, they were cleaned and refilled with fresh solutions. The treatment was performed three times.

A total of 46 lactating cows aged 30 to 36 months with lesions in the distal limbs were included in the study: the experimental group (n = 20) – cows treated with a 0.5% solution of the drug "Septiflex" (manufacturer: ABM-Trade LLC, Lutsk, Ukraine, 2024) based on copper chelate. Baths with the experimental solution were administered once every two weeks for one month. Experimental group (n = 20) – cows treated with a 5.0% solution of the drug "Intra Hoof-Fit" (manufacturer: Intracare B.V., Veghel, Netherlands, 2024) based on zinc and copper chelates. Baths with the experimental solution were administered for two consecutive days, every 12 days for two months. The control group consisted of cows with lesions of the distal limb segments that did not receive treatment (n = 6).

At the same time, animals in the experimental groups received intramuscular injections of the antibacterial drug "Kocefen 200" (manufactured by Vetsintez LLC, Kharkiv, Ukraine, 2024) from the third-generation cephalosporin group (injection suspension, 1 ml of which contains the active ingredients: ceftiofur hydrochloride – 50 mg; ketoprofen – 150 mg) at a rate of 1 mL per 50 kg of body weight. Before hoof treatment, livestock facilities (floors, stall walls, and cleaning equipment) were cleaned and disinfected using a wet method with a disinfectant solution containing: didecyl dimethylammonium chloride – 0.05%, glutaraldehyde – 0.25%, propan-2-ol – 0.15%, fetalcohol polyglycol ether – 0.05%, nitrilotrimethylene tris – 0.05%, water – 99.45%. Disinfection was performed for one hour according to current methodological recommendations for testing disinfectants in agriculture (Wales et al., 2021).

The live weight of the cows was determined by weighing them once every two weeks at the same time on 4BDU600X-1212-P-B electronic scales (manufacturer: "Vagi AKSIS Ukraine", Lviv, Ukraine, 2025), with a measurement accuracy of ± 0.5 kg.

Average daily milk yields were determined in accordance with the "Instruction on maintaining pedigree records in dairy and dairy-beef cattle breeding" (with amendments made in accordance with Order No. 50 (z0380-17) of the Ministry of Agrarian Policy and Food dated February 10, 2025). https://zakon.rada.gov.ua/laws/show/z0096-04#Text), using the control milking method and the "Uniform Agri" dairy farm accounting and management software (manufacturer: "UNIFORM-Agri B.V.", Assen, Netherlands, 2024).

For the biochemical analysis of cow blood samples, we used the BK-240 automatic veterinary biochemical analyzer (manufactured by Sinothinker, Shenzhen Sinothinker Technology Co., Ltd., Shenzhen, China, 2024) with a built-in operating system and a touchscreen. In-laboratory quality control of the tests was performed in accordance with the analyzer manufacturer's recommendations. Blood samples were collected at 5:00 a.m., two hours before feeding. The venipuncture site in cows was the tail vein. For sample collection, sterile disposable 22G needles with a length of 1.5″ (manufacturer F.L. Medical s.r.l. Unipersonale, Torrelia, Italy, 2024) and sterile vacuum blood collection tubes without preservatives were used (manufacturer: Cangzhou Yongkang Medical Devices Co., Ltd., Cangzhou, China, 2024).

In the final stage of the study, the overall toxicity of the milk obtained from the experimental groups of cows was assessed. For this purpose, a test culture of the infusoria *Colpoda steinii* was prepared: 2.0 cm³ of nutrient medium was added to a vial containing the dry culture, and the mixture was incubated in a thermostat for 24 hours at a temperature of 26–28 °C. For analysis, a 10 cm³ milk sample was collected, extracted with 10.0 cm³ of acetone, shaken for 20 minutes, and filtered. Then, 60 cm³ of Lozin-Lozinsky's solution was added to 0.5 cm³ of the filtrate. Next, 2.0 cm³ of the resulting acetone extract solution was added to a vial containing a prepared test culture of the ciliate *Colpoda steinii* and placed in a thermostat at a temperature of 26–28 °C. Three minutes after the start of incubation, the working mixture was examined under a microscope at × 80–120 magnification to assess the condition of the test culture (growth inhibition, pathological forms), its behavior (motility, abnormal movements), and the percentage of live and dead ciliates was counted. If 100% of the ciliates died, the experiment was terminated. If some ciliates remained alive, thermostating of the working mixture was continued for up to 1 hour, followed by a repeat microscopic examination. The final assessment of the working mixture's toxicity level was performed according to the scale (Table 1).

Table 1
Criteria for assessing the toxicity of a working mixture

The condition and behavior of a test culture of the infusoria *Colpoda steinii*	Level of toxicity			
	non-toxic	slightly toxic	toxic	highly toxic
Live (active/mobile) infusoria, %	90–100	80–85	5–10	0
Abnormal movements, %	0–2	3–5	3–5	0
Growth inhibition, %	0–2	3–5	3–5	0
Abnormal forms, %	0–2	3–5	3–5	0
Dead infusoria, %	0–5	5–10	70–90	100

The obtained results were processed by methods of variation statistics using the software package for analysis of variance (ANOVA) StatPlus 5 (6.7.0.3) (AnalystSoft Inc., USA). The results are presented as mean ± SD. To compare the mean values between groups, the Tukey test was applied, with statistical significance defined as $P < 0.05$ for all data.

Results

To determine the nature of local lesions in the distal extremities, a clinical examination was conducted on 700 cows in the milking herd through visual inspection and palpation with test forceps. During mechanical hoof cleaning, dirt, stones, and dried manure were removed from the hooves with a hoof knife. Attention was paid to the condition of the lateral and plantar parts of the hooves and hoof horn (e.g., cracks, wounds, pigmented areas, hemorrhages, and tenderness), as well as to the presence or absence of soft tissue ulcers (e.g., coronets, soles, and interdigital clefts), pododermatitis, laminitis, body ulcers, Rusterholz ulcers, Mortellaro disease, purulent wounds, and bruises.

An assessment of locomotion (degree of lameness) revealed noticeable lameness in most of the cows. Specifically, 7% received a score of 1, 18% received a score of 2, and 75% received a score of 3. The results of the clinical and orthopedic examinations showed that 350 of the 700 milking cows (50%) had hoof lesions. The largest share of the identified lesions was pododermatitis (serous, ulcerative, or purulent), accounting for 20.9–38.0% of the lesions. Hoof deformities accounted for 6.4–9.1% of the lesions, sole wounds accounted for 2.7–4.4%, and interdigital phlegmon accounted for 0.5–1.2% (Fig. 1–7).

Fig. 1. Pododermatitis

Fig. 2. Ulcerative pododermatitis

Fig. 3. Sole ulcer

Fig. 4. Purulent pododermatitis

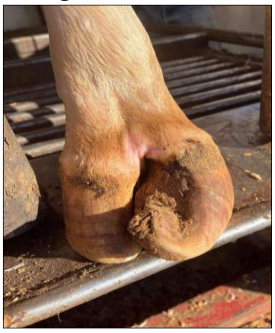

Fig. 5. Hoof deformation

Fig. 6. Asymmetrical hoof

Fig. 7. Wound on the sole + interdigital phlegmon

In the next stage, microorganisms involved in the onset and development of purulent-necrotic inflammation of the distal limbs in cows were isolated and identified. Based on the results of bacteriological analysis of biological material from the affected limbs, the following cultures were isolated: *Escherichia coli* (100%), *Staphylococcus epidermidis* (100%), *Staphylococcus saprophyticus* (100%), and saprophytic microorganisms of the genus *Bacillus* spp. (87.5%), *Enterococcus faecium* (62.5%), *Clostridium perfringens* (50.0%), *Citrobacter freundii* (25.0%), *Streptococcus pyogenes* (25.0%), *Klebsiella pneumoniae* (12.5%), and *Staphylococcus aureus* (12.5%). A similar composition of microflora was detected in swab samples from the floor, walls, bedding, and cleaning equipment (Table 2). No microscopic keratomycete fungi were identified in biological material from affected limbs and livestock objects. Thus, the sources of infection and the sites of pathogen spread in the environment are not only sick animals but also livestock facilities.

The results obtained indicate the prevalence of multidrug-resistant microorganisms among the pathogens causing purulent-inflammatory lesions of the limbs in cows and confirm the need for preliminary antibiotic susceptibility testing before administering antimicrobial therapy. The informed selection of antibacterial drugs based on antibiotic susceptibility testing has enhanced treatment effectiveness, minimized the risk of antibiotic resistance, and optimized the use of antimicrobial agents in veterinary practice (Table 3).

The isolated cultures of *Escherichia coli*, *Enterococcus* spp. / *Streptococcus* spp., *Staphylococcus* spp., and *Clostridium* spp. exhibited variable susceptibility to the studied antimicrobial agents. Third-generation cephalosporins, such as cefotaxime and ceftriaxone, as well as fluoroquinolones, including enrofloxacin, ciprofloxacin, ofloxacin, and marbofloxacin, exhibited the highest antimicrobial activity against all tested cultures, and most isolates remained susceptible to these agents. *Escherichia coli*, *Enterococcus* spp. / *Streptococcus* spp., and *Staphylococcus* spp. exhibited high susceptibility to neomycin and florfenicol. Conversely, *Enterococcus* spp. / *Streptococcus* spp. and *Staphylococcus* spp. cultures exhibited resistance to most penicillin-derived agents, macrolides, and tetracyclines. *Clostridium* spp. were resistant to cefazolin, macrolides, aminoglycosides, co-trimoxazole, and furazolidone. *E. coli* isolates showed intermediate susceptibility to tetracycline, doxycycline, oxytetracycline, and levofloxacin.

Table 2
Results of microbiological screening of environmental swabs collected from livestock facilities and pieces of processing equipment (n = 50)

Microorganisms	Positive results out of the total number of swab samples	
	number	%
Citrobacter freundii	7	14
Escherichia coli	50	100
Klebsiella pneumoniae	not detected	not detected
Proteus spp.	not detected	not detected
Salmonella spp.	not detected	not detected
Bacillus spp.	12	24
Staphylococcus epidermidis	21	42
Staphylococcus saprophyticus	17	34
Staphylococcus aureus	10	5
Enterococcus faecium	42	84
Streptococcus pyogenes	11	22
Clostridium perfringens	27	54

Microbiological studies confirm the need to further refine the treatment and prevention protocol for pododermatitis in cows, in conjunction with mandatory disinfection of livestock facilities and equipment. To reduce microbial contamination, all surfaces in the livestock facility were washed and subjected to forced disinfection using a proprietary disinfectant based on quaternary ammonium compounds and glutaraldehyde with a 60-minute exposure time. The use of this agent enabled complete (100%) disinfection of veterinary control objects in the cowshed made of various materials: concrete, rubber, wood, metal, plastic, and glass.

Table 3
Parameters for determining the susceptibility to anti-bacterial drugs of pathogens isolated from pathological material obtained from cows with limb diseases

Class of antibiotics	Antimicrobial drug	*Escherichia coli*	*Enterococcus* spp. / *Streptococcus* spp.	*Staphylococcus* spp.	*Clostridium* spp.
Penicillins	Amoxicillin	R	R	R	R
	Ampicillin	R	R	R	R
	Benzylpenicillin	R	R	R	I
	Amoxiclav	R	R	R	I
Cephalosporins	Cefazolin	R	R	R	R
	Cefotaxime	S	S	S	S
	Ceftazidime	S	R	S	R
	Ceftriaxone	S	S	S	S
	Cefuroxime	S	R	S	S
	Sulbactam	S	S	S	R
Tetracyclines	Tetracycline	I	R	R	R
	Doxycycline	I	R	R	R
	Oxytetracycline	I	R	R	R
Macrolides	Erythromycin	R	R	R	R
	Azithromycin	R	R	R	R
	Tylosin	R	R	R	R
Fluoroquinolones	Levofloxacin	I	R	R	S
	Enrofloxacin	S	S	S	S
	Ciprofloxacin	S	S	S	S
	Ofloxacin	S	S	S	S
	Norfloxacin	S	S	S	R
	Marbofloxacin	S	S	S	S
Aminoglycosides	Gentamicin	I	S	S	R
	Kanamycin	S	I	I	R
	Neomycin	S	S	S	R
	Amikacin	R	S	S	R
Amphenicols	Chloramphenicol	I	R	R	R
Lincosamides	Lincomycin	R	R	S	R
	Clindamycin	R	R	S	R
Others	Fosfomycin	S	S	R	R
	Furazolidone	R	R	R	R
	Co-trimoxazole (sulfamethoxazole/	R	R	R	R
	trimethoprim)	S	S	S	R

Note: S – susceptible (growth inhibited by the corresponding antimicrobial agent); I – intermediate susceptibility; R – resistant (not susceptible to the antimicrobial agent at standard therapeutic concentrations).

Studies evaluating the effectiveness of therapeutic and preventive measures for associated bacterial infections of the limbs using hoof baths revealed significant differences between groups. Analysis of serum biochemical parameters in cows before treatment with the drugs (Table 4) indicates that the total protein content in samples from animals in both experimental groups was within the physiological norm and amounted to 82.70 ± 3.11 and 86.32 ± 3.50 g/L, respectively. In the control group, this indicator was slightly higher, at 89.41 ± 4.50 g/L. Glucose concentration in animals of all groups was below the physiological norm, which may indicate impaired energy metabolism and the development of a pathological process. Similar changes were observed in the levels of the liver enzymes AST and ALT and cholesterol, with no statistically significant intergroup differences for any of the parameters studied.

During the 10 days between the orthopedic examination and the start of treatment, the average daily milk yield in sick animals treated with "Septiflex" decreased by 25.50 ± 2.72%, while in cows treated with "Intra Hoof-Fit" it decreased by 24.80 ± 2.83%. The live weight of cows in these groups also decreased by 16% and 22%, respectively. When selecting therapeutic drugs and their formulations, their comprehensive effect on the microflora (bactericidal, fungicidal) and positive impact on the cows' bodies were taken into account.

To assess the antibiotic sensitivity of the microflora on the first day of treatment, all experimental animals underwent surgical hoof treatment followed by intra-aortic administration of a 0.5% novocaine solution with a third-generation cephalosporin antibiotic. On the 2nd and 3rd days of treatment (at 24-hour intervals), cows in the experimental groups received repeated intra-aortic administration of a 0.5% novocaine solution with an antibiotic. Thus, the total course of etiotropic and pathogenetic therapy lasted three days. In cases of deep tissue damage to the sole following mechanical treatment, an exacerbation of the inflammatory process was observed in cows starting on the second day of the experiment. Those treated with "Septiflex" were treated for three days, while those treated with "Intra Hoof-Fit" were treated for five days. Manifestations of the disease included increased swelling of the coronary band and interdigital space, increased pain and temperature, pulsation of the digital arteries, and varying degrees of pronounced lameness. At rest, these animals turned their limbs to the side and leaned on their hoof walls. In animals treated with hoof baths containing "Septiflex" starting on the fourth day and "Intra Hoof-Fit" starting on the fifth day, signs of inflammation gradually subsided. Lameness, pain, and body temperature decreased, and swelling in the interdigital arch and coronary band areas subsided. By the fifth day of treatment, animals in both groups began to rely more confidently on their affected limb while moving. Pronounced signs of healing, such as granulation tissue, were observed in the affected area. The cleansing processes of the wound were indicated by the presence of debris. Fibrin flakes were observed in the deep areas of the ulcer. At the junction of the hoof horn and the wound surface, the granulation tissue had turned pink. The pain response to palpation of the sole of the hoof became less pronounced in these experimental animals.

Table 4
Biochemical parameters of cow serum before treatment of associated bacterial limb infections using hoof baths (mean ± SD)

Indicator	Drug for treating the limbs		
	Septiflex (n = 20)	Intra hoof-fit (n = 20)	Control group (n = 6)
Total protein, g/L	82.70 ± 3.11	86.32 ± 3.50	89.41 ± 4.50
Calcium, mmol/L	2.23 ± 0.11	2.22 ± 0.61	2.19 ± 0.12
Inorganic phosphorus, mmol/L	1.82 ± 0.05	1.70 ± 0.12	1.91 ± 0.38
Magnesium, mmol/L	1.15 ± 0.12	1.10 ± 0.11	0.90 ± 0.14
Glucose, mmol/L	0.25 ± 0.11	0.29 ± 0.12	0.33 ± 0.19
Total bilirubin, mmol/L	2.57 ± 0.37	2.64± 0.21	2.83 ± 0.71
ALT, U/L	29.27 ± 0.37	28.34 ± 4.10	27.40 ± 1.37
AST, U/L	104.83 ± 4.37	139.58 ± 23.74	162.47 ± 34.71
Alpha-amylase, U/L	73.87 ± 2.94	62.02 ± 7.49	67.52 ± 7.04
Cholesterol, mmol/L	5.99 ± 0.32	5.32 ± 0.70	5.63 ± 0.52
Urea, mmol/L	5.73 ± 0.21	5.57 ± 0.28	5.37 ± 0.45
Alkaline phosphatase, U/L	64.27 ± 5.23	61.78 ± 6.10	56.80 ± 9.52

Note: none of the selected values differ from one another within a row.

In the control group of cows, an exacerbation of the inflammatory process in the hoof area was observed during the first 24 hours of the experiment and in the absence of treatment, along with grade 5 lameness, a 32–37% decrease in average daily milk yield, an increase in body temperature to 40 °C, and leukocytosis. The animals spent most of the day lying down, refused feed, and experienced a decreased rumen motility rate of one movement per minute. In accordance with the principles of humane veterinary medicine, these animals were removed from the experiment and sent for treatment.

On the sixteenth day of treatment for cows treated with "Septiflex" and on the twenty-first day for cows treated with "Intra Hoof-Fit", signs of lameness, increased pain, body temperature, and swelling in the interdigital space and coronary band of the affected limb were nearly absent. The animals confidently supported themselves on the affected limb during movement, and only a slight pain response was observed upon deep palpation in some animals. The sole wound was filled with pale pink granulation tissue, and signs of keratinization and new hoof horn formation were observed at the hoof horn-wound surface junction. The area of the wound surface had significantly decreased. Twenty-one days after treatment began, the efficacy of the therapeutic and preventive measures for bacterial limb infections was 80% for animals treated with "Septiflex" and 70% for cows treated with "Intra Hoof-Fit". The wound surface area decreased significantly in sick animals of the experimental groups, and a small amount of light pink or white mature granulation tissue was observed in the center of the wound. The peripheral part of the wound surface was covered with young hoof horn.

The total protein content in the blood serum of animals treated with "Septiflex" during this period was 79.02 ± 1.11 g/L ($P < 0.05$). In cows treated with "Intra Hoof-Fit", it was 78.10 ± 0.75 g/L ($P < 0.05$), which corresponds to the physiological norm. However, serum glucose levels increased to 1.78 ± 0.05 and 1.76 ± 0.03 mmol/L, respectively, in cows from these groups.

Over the course of three weeks from the start of treatment, the average daily milk yield increased by 1.2 kg in sick cows treated with "Septiflex" and by 0.9 kg in cows treated with "Intra Hoof-Fit". Live weight increased by 9% and 14%, respectively.

After the third course of hoof baths, the effectiveness of the therapeutic and preventive measures was 95% for both experimental groups: the affected animals were healthy, and there were no signs of disease in the distal limb segments. The sole wounds had completely healed. There was complete regeneration of the tissues of the affected hoof soles. Normalization of most serum biochemical parameters was also observed in these animals compared to the results obtained before therapy began (Table 5).

Table 5
Biochemical parameters of cows' blood serum after the third course of treatment for associated bacterial infections of the limbs using hoof baths (mean ± SD)

Indicator	Drug for treating the limbs	
	Septiflex (n = 20)	Intra hoof-fit (n = 20)
Total protein, g/L	78.62 ± 1.47[a]	78.74 ± 1.60[a]
Calcium, mmol/L	2.39 ± 0.27[a]	2.38 ± 0.12[a]
Inorganic phosphorus, mmol/L	1.82 ± 0.04[a]	1.73 ± 0.22[a]
Magnesium, mmol/L	1.08 ± 0.12[a]	1.04 ± 0.11[a]
Glucose, mmol/L	1.78 ± 0.05[b]	1.76 ± 0.03[b]
Total bilirubin, mmol/L	0.46 ± 0.16[a]	0.47± 0.19[a]
ALT, U/L	39.24 ± 2.88[a]	38.64 ± 3.10[a]
AST, U/L	51.54 ± 11.15[b]	50.47 ± 14.41[b]
Alpha-amylase, U/L	47.87 ± 3.64[b]	56.52 ± 9.07[a]
Cholesterol, mmol/L	3.21 ± 0.32[b]	3.45 ± 0.52[b]
Urea, mmol/L	5.63 ± 0.51[a]	5.53 ± 0.45[a]
Alkaline phosphatase, U/L	73.36 ± 12.10[a]	72.87 ± 11.30[a]

Note: different letters denote values that differ significantly from one another between the start and end of treatment; Reference values according to Kaneko et al. (2008).

Specifically, glucose concentration increased by 1.53 mmol/L (7.1-fold, $P < 0.001$) in cows treated with "Septiflex" and by 1.47 mmol/L (6.1-fold, $P < 0.001$) in animals treated with "Intra Hoof-Fit", compared to baseline values. The results obtained fell within the normal physiological reference ranges, indicating stabilization of energy metabolism, reduction of intoxication signs, and decreased necrotic processes. Concurrently, AST activity decreased by 53.3 U/L, or 2.3-fold ($P < 0.001$), in cows treated with "Septiflex" and by 89.1 U/L, or 2.8-fold ($P < 0.01$), in those treated with "Intra Hoof-Fit". Cholesterol concentration decreased by 2.8 mmol/L (1.9-fold, $P < 0.001$) and by 1.9 mmol/L (1.5-fold, $P < 0.05$), respectively, reaching the upper limit of the physiological norm. Total protein levels normalized in both groups: in cows treated with "Septiflex", from 82.70 ± 3.11 g/L to 78.62 ± 1.47 g/L; and in animals treated with "Intra Hoof-Fit", from 86.32 ± 3.50 g/L to 78.74 ± 1.60 g/L. After treatment, calcium, phosphorus, magnesium, total bilirubin, ALT, alpha-amylase, urea, and alkaline phosphatase activity levels in the blood serum of both groups of animals were within the physiological range.

The average daily milk yield increased by 1.4 kg in sick animals treated with "Septiflex" and by 1.1 kg in cows treated with "Intra Hoof-Fit", compared to the initial treatment levels. However, when comparing the average daily milk yield and live weight of the experimental cows before and after recovery from the disease, it was found that, in cows treated with "Septiflex", these parameters fully returned to their initial levels. Meanwhile, average daily milk yields in animals treated with "Intra Hoof-Fit" were 3.8 kg lower than before the disease (23.1 ± 1.1 kg), and live weight was 17.13 kg lower, or 3.0% of the pre-illness live weight (571 ± 18.5 kg).

The results of an assessment of the overall toxicity of milk obtained from animals during treatment with third-generation cephalosporin antibiotics (based on ceftiofur hydrochloride) revealed that 99–100% of *Colpoda steinii* ciliate protozoa remained alive and actively moving. No pathological forms or dead ciliate protozoa were detected. The results of the study indicate that milk obtained after treatment with third-generation cephalosporin antibiotics is safe for human consumption and does not exhibit toxic properties.

Discussion

Years of research by scientists indicate that high-yielding cows are the most susceptible group of animals to musculoskeletal disorders in dairy farming. This is due to significant nutrient loss through milk production, which weakens overall resistance and reduces the animals' adaptive capacity (Alvergnas et al., 2019; Necula et al., 2024; Hassani et al., 2025).

In dairy complexes, the prevalence of sole ulcers and acute and chronic laminitis increases in high-producing cows, and the most common toe region pathologies among them are hoof horn deformities (54.2%), chronic laminitis (23.9%), interdigital dermatitis (13.3%), papillomatous digital dermatitis (11.0%), sole ulcers (7.8%), and acute laminitis (1.5%) (Khomyn et al., 2017). According to Mysak et al. (2021), 85.5% of significant surgical diseases are primarily located in the distal part of the limbs. Ulcers (of the coronet band, sole, interdigital cleft, and Mortellaro's disease) are the most common purulent-necrotic diseases in the distal part of the limbs (71.8%), followed by pododermatitis and laminitis (11.2%), phlegmon (5.9%), Rusterholz's ulcer (3.6%), and purulent wounds and contusions (2.7%). Other surgical diseases occur in fewer than 2.0 % of cases.

The main etiological factor in inflammatory processes of the limbs is opportunistic microflora (Dashko, 2021), whose pathogenic properties are significantly enhanced under conditions of stress and a decrease in the animals' natural resistance due to a combination of adverse factors (Kučević et al., 2022; Melnikov et al., 2022). The main causes of diseases of the distal parts of the limbs include poor sanitary conditions in the premises (Holzhauer et al., 2024; Weber et al., 2025), the use of low-quality or injury-prone flooring (Führer et al., 2019; Espiritu et al., 2025), stalls that are too short (Patoliya et al., 2024), lack of active movement during the stabling period (Browne et al., 2022c), and untimely orthopedic examinations (Moreira et al., 2019; Yaremchuk et al., 2019; Vyrvykyshka et al., 2026).

During the microbiological analysis of samples collected from necrotic lesions on the lower limbs of cows, the most frequently isolated bacteria were *Escherichia coli* (80.3%), *Staphylococcus aureus* (76.3%), *Proteus vulgaris* (62.9%), and *Streptococcus faecalis* (59.9%). Less frequently isolated organisms include *Pseudomonas aeruginosa* (44.4 %), *Streptococcus pyogenes* (43.4%), *Staphylococcus epidermidis* (37.3%), *Staphylococcus saprophiticus* (34.9%), *Streptococcus agalactiae* (26.5%), *Fusobacterium necrophorum* (30.1%), *Proteus mirabilis* (17.3%), and *Klebsiella pneumoniae* (12.5%). In a few cases, spore-forming bacteria such as *Clostridium perfringens* (8.4%), *C. oedematiens* (4.8%), and *C. septicum* (4.7%) were found (Ulko, 2013).

For the treatment of various hoof diseases, including necrobacteriosis, soft tissue hoof rot, and verrucous pododermatitis, therapeutic soaks are recommended: copper sulfate (10% copper sulfate solution) or mixed (5.0% formalin solution and 10.0% copper sulfate solution), especially in cases of widespread infection without the application of bandages (open treatment method) (Mysak et al., 2021). The use of such measures helps accelerate the demarcation process, stimulates the formation of granulation tissue, and activates wound epithelialization. In particular, the regular use of hoof baths over four months, combined with a two-month course of the "Farmpak" feed supplement administered twice daily for one month followed by a two-month break, significantly reduced the incidence of hoof diseases in cows (Ulko, 2013).

A summary of data from various studies indicates that the primary pathogens associated with infectious lesions of the distal limb include enterococci, *Escherichia*, staphylococci, streptococci, *Proteus*, fusobacteria, and *Pseudomonas aeruginosa*. In less common cases, clostridia may also be responsible for the disease, with isolated instances involving *Klebsiella*. Our findings align with existing literature, although some differences may arise due to specific husbandry conditions and the state of the microbial environment on different farms.

In particular, during studies conducted on biomaterial collected from affected distal limb segments, a wide range of microorganisms was isolated, forming a specific microbiocenosis in the area of the pathological process. The following were detected in the samples: *E. coli* (100%), *S. epidermidis* (100%), *S. saprophyticus* (100%), saprophytic microorganisms of the genus *Bacillus* (87.5%), *Enterococcus faecium* (62.5%), *Enterococcus faecalis* (62.5%), *Clostridium* (50.0%), *Candida* (37.5%), *Citrobacter freundii* (25.0%), *Streptococcus pyogenes* (25.0%), *K. pneumonia* (12.5%), and *S. aureus* (12.5%). Furthermore, a comparative analysis of the obtained results with data from other researchers demonstrates that the microbial profile in cases of distal limb lesions can vary significantly depending on the conditions of the host farm (microclimate characteristics, housing conditions, sanitary status of the premises, the immune status of the animals, etc.) and may change over time against the backdrop of a decline in the body's resistance.

Studies have demonstrated the high therapeutic efficacy of a comprehensive treatment and prevention regimen that includes hoof baths with organic chelates (0.5% solutions of "Septiflex" or "Intra Hoof-Fit"), a circular Novocain block, and third-generation cephalosporin antibiotic therapy. This method led to complete recovery in up to 95% of cows with orthopedic diseases of bacterial origin affecting their distal limbs. No cases of disease recurrence were recorded during the 90-day study period.

Thus, the results confirm the feasibility of implementing a comprehensive system to monitor the health and welfare of high-yielding dairy herds that takes into account modern, innovative methods for preventing and treating distal limb diseases.

Conclusion

The main etiological agents causing bacterial infections of the distal limbs in dairy cows have been identified as staphylococci (*Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*), *Clostridium* (*Clostridium perfringens*), *Escherichia coli*, *Streptococcus* (*Streptococcus pyogenes*), and saprophytic bacteria of the genus *Bacillus*.

A system has been created to monitor the welfare of dairy cows and to implement therapeutic and preventive measures for bacterial infections related to their limbs. This system involves hoof baths with a 0.5% solution of the drug "Septiflex", which is based on copper chelate, at 14-day intervals. This regimen ensured the recovery of 95% of affected animals, fully restoring their milk production and body weight to baseline levels.

Using a 0.5% solution of the drug "Intra Hoof-Fit", which is based on copper and zinc chelates, at 12-day intervals led to the recovery of 95% of the affected animals. However, their average daily milk yield and body weight were 7.4% and 3.0% lower, respectively, compared to the baseline data before the illness.

Adding the third-generation cephalosporin antibiotic "Kocefen 200" (active ingredient: ceftiofur hydrochloride) to the treatment regimen showed no toxic effects on milk, indicating that it is safe for human consumption.

The authors declare they have no conflict of interest.

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