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**SPREAD OF BOVINE NECROBACTERIOSIS IN THE KHMELNYTSKYI REGION****Abstract**

*Bovine necrobacteriosis is a disease that causes significant economic losses in dairy farming. It has been established that the spread of this disease occurs in large farms where highly productive animals are kept.*

*The paper deals with the distribution of fusobacteria in the farms of Khmelnytsky region and the peculiarities of laboratory diagnostics of the pathogen. For bacteriological examination, biological material was collected in vivo - scrapings from lesions on the border of healthy and necrotic tissue. Identification of cultures was performed on the basis of morphological, tinctorial, cultural and biological properties assessed by conventional bacteriological methods, as well as using the Bergie's determinant.*

*Necrobacteriosis in the farms was clinically manifested in most dairy cows, with isolated cases in calves. It was found that the highest percentage of sick cows in the entire dairy herd was observed in the second lactation. Necrobacteriosis of the distal limbs was usually complicated by purulent and putrefactive microflora. *Fusobacterium necrophorum* was always isolated in association with other bacteria: *Clostridium difficile*, *Escherichia coli*, *Staphylococcus aureus* and other opportunistic pathogens. *Fusobacterium necrophorum* was poorly identified on the basis of morphological and biochemical properties, but the biological test on rabbits gave a consistently positive result. In the presence of *F. necrophorum* in the pathological material or in the culture, necrosis developed in the rabbit at the puncture site in 3–4 days.*

**Key words:** *necrobacteriosis, *Fusobacterium necrophorum*, microbiocenosis, bioassay.*

**Introduction.** Bovine necrobacteriosis is recorded annually on Ukrainian livestock farms. Economic losses from the disease are based on reduced milk production of cows (by 14–50%), reduced calf production from cows that have been infected, and the costs of treatment and prevention measures [14]. Under conditions of year-round stall housing of cows, lesions of the distal limbs occur in 79% of animals and are recorded evenly throughout the year. In stall housing with passive motility in winter and camp housing with grazing on pastures in summer, they are found in 13.8% of the available livestock and mainly in summer [15].

It is known that due to purulent-necrotic processes in the area of the toes in cows, the weight gain of fattening animals decreases by 30–50% or more, and sire bulls reduce sperm production and are quickly culled. In cows with necrobacteriosis, depending on the severity of the pathology, milk yields decrease by up to 50%, calf yield – by 15–20% [20]. It also increases herd rotation, disrupts the breeding plan, which does not allow the genetic potential of the breed to be realized and reduces the profitability of the industry [8; 9; 22].

The disease among animals is most often recorded as an associated infection that manifests itself against the background of reduced body resistance, with immunodeficiency of the B-cell immune system, due to the negative impact of exogenous and endogenous stress factors on the animal body [7; 10; 17].

A retrospective analysis showed that over the past 15 years, the spread of necrobacillosis in Ukraine has been closely linked to the import of breeding cattle from Germany, Hungary, the Netherlands and other European countries where the disease has been recorded for more than 100 years [21].

In order to increase the milk yield of cows, many livestock farms in the country are switching their dairy herds to silage and silage-concentrate feeding types, which are able to provide the maximum amount of milk. However, in most cases, these types of feeding have a negative impact on animal homeostasis and become one of the causative factors of necrobacteriosis. The use of a significant proportion of acidic feed, even benign ones, in the volume of diets for dairy cattle changes the direction of metabolic processes in the body of dairy cattle due to disruption of carbohydrate metabolism and the ratio between lactic acid and VFAs [13].

It is known that the quality of silage is directly related to the pH value. The reaction of the silage medium with a pH value of 4.4 indicates its preservation through fermentation processes with the formation of lactic acid, which ensures its highest quality. Silage pH values above 4.4 indicate proteolytic fermentation of silage with the formation of butyric, propionic and other acids, amines and ammonia, which change the direction of metabolism [18]. Such changes provoke the gradual development of metabolic acidosis and functional disorders, including water-salt metabolism. At the same time, due to increased water retention in the interstitial environment, geodynamics is disturbed, and tissues become hydrophilic. In addition, the progression of metabolic acidosis causes the mobilization of Ca, Na and P ions from bones, which negatively affects the general condition of bone tissue and hoof horn tissue. At this time, the stomach reflexively reduces the secretion of hydrochloric acid, which leads to ulcers. In the fore stomachs of cattle, favorable conditions are created for the development of opportunistic microflora, which is constantly present in them [23].

Therefore, the introduction of silage or silage-concentrate feeding in dairy cattle breeding is an endogenous causal factor for the emergence and rapid spread of necrobacteriosis with a significant impact on the dairy herd in farms [24]. According to foreign scientists, the use of concentrate feeding also ensures high milk yields, but increases the cost per unit of production. Therefore, this practice is not widely used in livestock farms [11]. The emergence and spread of the disease is facilitated by improper animal housing and feeding conditions, short stalls, untimely manure removal, improper use of preventive foot baths, untimely hoof cleaning, and inadequate housing and feeding conditions for heifers and high-yield cows imported from the Netherlands and Germany. Significant economic losses are suffered by those farms that import heifers [1; 2; 16].

**Objective.** In view of this, the aim of our research was to investigate the spread of fusobacteria in farms of Khmelnytskyi region and to determine the peculiarities of laboratory diagnostics of the pathogen.

**Presentation of the main research material. Materials and methods.** Clinical studies were conducted on the farms of Dunayevetskyi and Bilohirskyi districts of Khmelnytskyi region. Necrobacteriosis of cows was determined taking into account the epizootic situation and on the basis of clinical signs. In case of purulent necrotic lesions of the skin and adjacent connective and muscle tissues, mainly on the lower parts of the limbs, pathological material was collected for bacteriological examination. Scrapings from lesions at the border of healthy and necrotic tissue were sent to the Khmelnytskyi Regional Laboratory of Veterinary Medicine for in vivo examination ( $n = 20$ ). In two cases, parts of parenchymal organs with necrotic foci were sent.

Isolation and identification of *Fusobacterium necrophorum* was performed in accordance with the Working Instruction «Scheme of bacteriological examination for necrobacteriosis» PI BIQ 5.4–139 of 12.07.2013. Stages of bacteriological testing: sample preparation; inoculation on nutrient media; smear microscopy; biological tests; isolation of the pathogen culture; identification of the pathogen; recording of results. Fixed smears were made from necrotized tissues, and impression smears were made from pieces of parenchymal organs, which were stained with Gram, Romanowski-Gimzy and Loeffler's blue. Cultures from pathological material were made in Kitt-Tarozzi medium, which was previously regenerated at 100°C for 20–30 min. and cooled to 45–50°C; MPB (meat-peptone broth); MPA (meat-peptone agar) and on serum-glucose agar. Agar plates were placed in anaerobic conditions at 37°C for 5 days [5].

At the same time, 0.5–1 ml of a suspension of pathological material diluted at a concentration of 1:10 was injected subcutaneously into the middle third of the outer surface of the ear. Also, if necessary, the rabbit was infected with 0.5–1 ml of daily broth culture of the pathogen subcutaneously in the middle third of the outer surface of the ear. In the presence of *F. necrophorum* in the pathological material or in the culture, necrosis developed in the rabbit at the puncture site in 3–4 days. Smears were made from the necrotic area, stained with Gram's and Romanowski-Gimzy stains. If granular stained filaments characteristic of the pathogen were found in the smears, the biological sample was considered positive.

To determine the associations of microorganisms in the necrotic focus of the limbs, pathological material was inoculated on conventional and selective nutrient media, namely, meat-peptone broth, meat-peptone agar, Kitt-Tarozzi broth, salt-blood agar for streptococci, KODA medium, Endo agar, sucrose broth and 6.5% salt agar. Cultures were identified on the basis of morphological, tinctorial, cultural and biological properties assessed by conventional bacteriological methods, as well as using the Bergey's identifier [4].

**Research results.** Animals in the farm of the Dunayevetskyi district were kept in typical cowsheds, tethered during the stall period, there was practically no exercise in winter, and from spring to autumn the animals were kept in a fenced summer camp. The bedding in the barns was made of straw, and manure was removed by scraper conveyors.

Animals in the farms of Bilohirskyi district were kept without ties. The floors in the cowsheds are partly tiled and partly wooden (where the cows are kept). The farm uses modern fattening, housing and milking technologies.

The diet of cows throughout the year in all farms changed slightly and was calculated by groups depending on the milk production of the animal. Attention was paid to the feeding of dry cows, because the viability of future young animals and the milk production of females depend on its quality.

Necrobacteriosis in the farms was clinically manifested in most dairy cows, with isolated cases in calves. Therefore, we did not take into account the entire livestock population in our studies.

The data of the study of the spread of necrobacteriosis in cows in accordance with lactation are shown in fig. 1.

It was found that the highest percentage of sick cows in the entire dairy herd was observed in the second lactation. During this period, the animals' immune system becomes more stressed, which can be undermined by unfavorable factors, such as feeding, housing and animal care conditions. The decrease in sick cows in the fifth and sixth lactation is explained by the fact that the number of 8–9-year-old animals in the farm does not exceed 4% of the total number of dairy cows and, as a rule, only mastitis-resistant cows were kept until that age.

The diagnosis of bacteriosis was made on the basis of epizootic data, clinical presentation and bacteriological examination. Clinically, the disease was detected during daily examinations and was manifested by lesions of the distal limbs, which began with redness and swelling of the coronet, soft tissue and arch of the interlocks gap (fig. 2). Initially, serous exudate was released, which gradually turned into purulent exudate.

Animals began to limp. *Necrobacteriosis* of the distal limbs was usually complicated by purulent and putrefactive microflora. In this case, the disease became chronic, and deeply located limb tissues were involved in the process, undergoing necrosis.

The diagnosis of necrobacteriosis was confirmed bacteriologically. We found that out of 20 samples of pathological material, *Fusobacterium necrophorum* was isolated from 17 samples (fig. 3).

*Fusobacterium necrophorum* has always been isolated in association with other bacteria: *Clostridium difficile*, *Escherichia coli*, *Staphylococcus aureus* and other opportunistic pathogens.

It should be noted that the pathogen did not always grow on the nutrient media, but the biological test on rabbits was always positive (fig. 4). The pure culture of *Fusobacterium necrophorum* was obtained using the biological sample.

*Fusobacterium necrophorum* is a strict anaerobe, a Gram-negative, non-motile, non-spore-forming polymorphic bacillus. On Kitt-Tarozzi medium, *F. necrophorum* formed intense turbidity first in the lower layers of the medium,

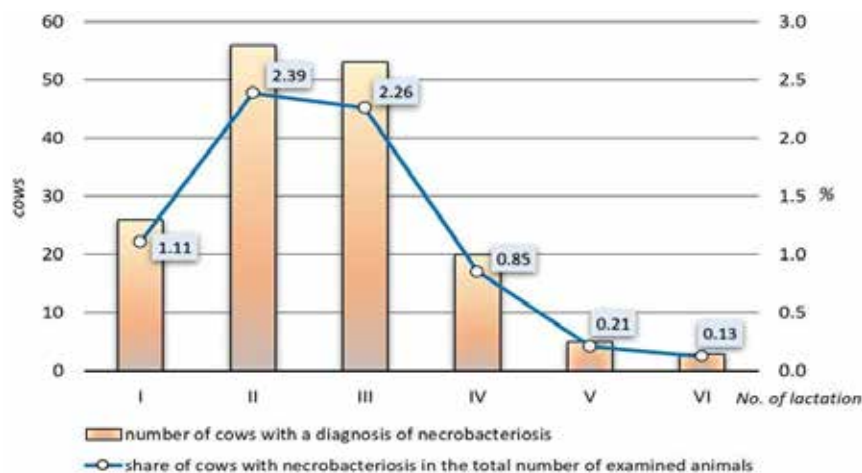


Fig. 1. Incidence of necrobacteriosis in cows in Khmel'nitsky region



Fig. 2. Clinical picture of necrobacteriosis

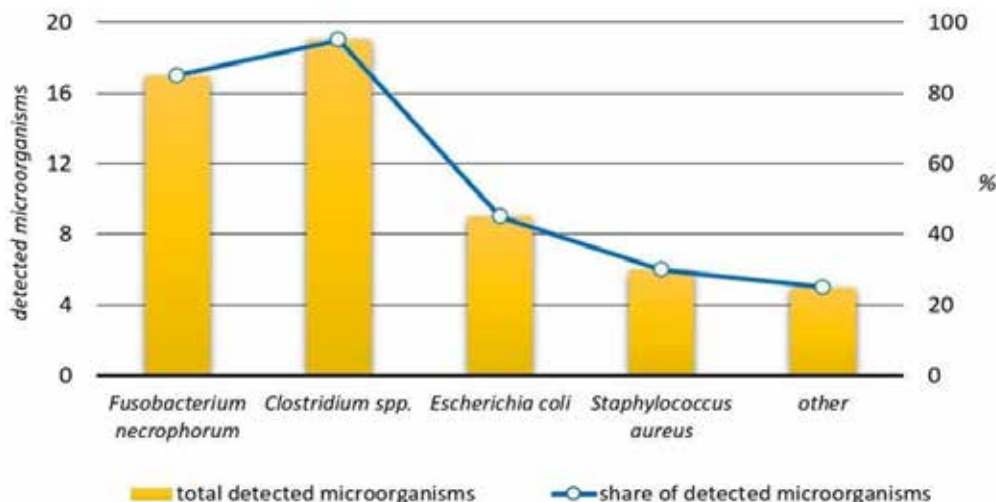


Fig. 3. Microbial associations in cow necrobiosis

and later in the upper layers; gas formation was weak. Broth clarification occurred on day 5–8, with a fragile precipitate falling to the bottom of the tube. Under microscopy, granularly stained long filaments were found in the culture.

**Discussion.** According to a number of authors, up to 18 species of bacteria are isolated and identified from the necrotic focus, which confirms the polyetiological of necrobacteriosis. However, *Fusobacterium necrophorum* plays the main role in the pathogenesis of the disease. These studies have shown that the disease does not develop without the presence of *F. necrophorum* [19].

Most studies also indicate that the etiology of the disease includes associates in addition to the main pathogen *F. necrophorum*: *Staphylococcus aureus*, *Clostridium perfringens*, *Diplococcus spp.*, *Klebsiella pneumoniae*, *Citrobacter spp.*, *Str. zooepidermicus*, *Micrococcus spp.*, *Streptococcus faecalis*, *Salmonella typhimurium* [12].

To date, scientists have identified a synergy between *Fusobacterium necrophorum* and *Actinobacillus pyogenes* that increases the virulence of pathogens through the production of leukotoxin by fusobacteria, which promotes the spread of *Actinobacillus pyogenes* in body tissues, and actionability, in turn, produce a growth factor for *Fusobacterium necrophorum*, enhancing the activity of its enzymes and toxins [3]. It has been established that associations of microorganisms *Cl. pyogenes*, *S. aureus*, *S. pyogenes*, *Fusififormis nodosus* with their enzyme systems enhance the effect of *F. necrophorum* through the activation of its pathogenicity factors [6].

Taking into account the causative factors of purulent necrotic lesions of necrobacteriosis in cattle, the prevention of the disease is based on the following principles: prevention of intestinal dysbiosis; control of mineral nutrition; control of hoof condition; technological disinfection; organization of pasture or walking maintenance. Preventing the development of intestinal dysbiosis can be achieved by increasing the proportion of good quality roughage in the diet, as fibre is an essential element of nutrition for ruminants. Protein is supplied to the body in cows mainly due to the biomass of microorganisms involved in the breakdown of fibre. Observations show that cows experiencing a fibre deficiency make up for it on their own by eating straw used for bedding.

Concentrate feeding should be optimized and rational. The energy content of the diet can be increased by using carbohydrate feeds, such as molasses, dry beet pulp, molasses, and milled cereals. There are reports of the high efficiency of including propylene glycol or preparations based on it in the diets of highly productive cows. Feed conversion can also be increased by feeding ergotropics from the group of intestinal stabilizers, which include probiotics. In this regard, the most technologically advanced is the introduction of organic acids (lactic, succinic, propionic) into feed mixtures together with molasses [15; 21].

**Conclusions.** According to the results of the research, it was found that *Fusobacterium necrophorum* was most often manifested in dairy cows of the second lactation by purulent-necrotic lesions of the limbs. The pathogen was always isolated in association with opportunistic pathogens. *Fusobacterium necrophorum* was poorly identified on the basis of morphological and biochemical properties, but the biological test on rabbits gave a consistently positive result.



Fig. 4. Biological sample for necrobacteriosis

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## ПОШИРЕННЯ НЕКРОБАКТЕРІОЗУ ВЕЛИКОЇ РОГАТОЇ ХУДОБИ В ХМЕЛЬНИЦЬКОМУ РЕГІОНІ

### Анотація

Некробактеріоз великої рогатої худоби – захворювання, яке зумовлює істотні економічні збитки в молочному скотарстві. Встановлено, що поширення даного захворювання відбувається саме у великих господарствах, де утримуються високопродуктивні тварини.

У роботі розглянуто розповсюдження фузобактерій у господарствах Хмельницької області та особливості лабораторної діагностики збудника. Для бактеріологічного дослідження відбирали при житті біологічний матеріал – зіскрібки з місць ураження на межі здорової та некротизованої тканин. Ідентифікацію культур проводили на підставі морфологічних, тінкторіальних, культуральних і біологічних властивостей, оцінених за загальноприйнятими бактеріологічними методиками, а також користуючись визначником Берджі.

Некробактеріоз у господарствах клінічно проявлявся переважно у дійних корів, у телят спостерігалися поодинокі випадки. Встановлено, що найбільший відсоток хворих корів від усього дійного стада спостерігався на другій лактації. Некробактеріоз дистальних відділів кінцівок, як правило, ускладнювався гнійно-гнильною мікрофлорою. *Fusobacterium necrophorum* виділявся завжди в асоціаціях з іншими бактеріями – клостридіями, ешерихіями, золотистим стафілококом та іншими умовно патогенними мікроорганізмами. *Fusobacterium necrophorum* погано ідентифікувався на основі морфолого-біохімічних властивостей, проте біологічна проба на крілях давала постійно позитивний результат. За наявності в патологічному матеріалі або в досліджуваній культурі *F. necrophorum* у кріля на місці зараження через 3–4 дні розвивався некроз.

**Ключові слова:** некробактеріоз, *Fusobacterium necrophorum*, мікробіоценоз, біопроба.

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