

DOI: <https://doi.org/10.36719/2663-4619/127/251-257>

**Vusala Sardarly** 

Azerbaijan State Agrarian University  
Ganja, Azerbaijan  
PhD of Biology  
vusalasardar@hotmail.com

**Ramil Mammadov** 

Azerbaijan State Agrarian University  
Ganja, Azerbaijan  
PhD of Agrarian Sciences  
m.ramil201979@gmail.com

**Ayten Aghayeva** 

Azerbaijan State Agrarian University  
Ganja, Azerbaijan  
aytan.agayeva@adau.edu.az

**Turkan Babayeva** 

Azerbaijan State Agrarian University  
Ganja, Azerbaijan  
babayevaturk88@gmail.com

**Rahile Farmanli** 

Azerbaijan State Agrarian University  
Ganja, Azerbaijan  
rahila.fermanli@adau.edu.az

## Experimental Evaluation of an Alternative Treatment Method for Necrobacteriosis in Lambs

### Abstract

Necrobacteriosis remains an important infectious disease in livestock, leading to significant economic losses and severe systemic complications, particularly in young animals. The search for effective therapeutic approaches aimed at improving local antimicrobial activity and host immune response remains relevant.

The aim of this study was to evaluate the therapeutic efficacy of combined administration of naftalan oil, oxytetracycline, and the immunomodulatory preparation mixoferon in animals affected by necrobacteriosis.

The therapeutic effect of topical application of a naftalan oil–oxytetracycline mixture on lesions of the oral mucosa was investigated under experimental conditions. Clinical recovery dynamics and general condition of animals receiving combined therapy were assessed and compared with conventional treatment approaches.

Application of the naftalan oil–oxytetracycline combination was associated with improvement of local tissue condition and reduction of inflammatory manifestations, which contributed to accelerated clinical recovery. Additional administration of mixoferon was accompanied by improvement of nonspecific resistance indicators and a reduced tendency toward systemic spread of infection.

The obtained results indicate the potential effectiveness of complex therapy combining antimicrobial and immunomodulatory components in the treatment of necrobacteriosis. However, further studies using randomized controlled experimental designs, standardized outcome measures, and molecular diagnostic confirmation are required to validate these findings.

**Keywords:** *necrobacteriosis, Fusobacterium necrophorum, naftalan oil, oxytetracycline, immunomodulation, veterinary therapy*

**Vüsalə Sərdarlı** 

Azərbaycan Dövlət Aqrar Universiteti  
Gəncə, Azərbaycan  
biologiya üzrə fəlsəfə doktoru  
vusalasardar@hotmail.com

**Ramil Məmmədov** 


Azərbaycan Dövlət Aqrar Universiteti  
Gəncə, Azərbaycan  
aqrar elmlər üzrə fəlsəfə doktoru  
m.ramil201979@gmail.com

**Aytən Ağayeva** 

Azərbaycan Dövlət Aqrar Universiteti  
Gəncə, Azərbaycan  
aytan.agayeva@adau.edu.az

**Türkan Babayeva** 

Azərbaycan Dövlət Aqrar Universiteti  
Gəncə, Azərbaycan  
babayevaturk88@gmail.com

**Rahilə Fərmanlı** 

Azərbaycan Dövlət Aqrar Universiteti  
Gəncə, Azərbaycan  
rahila.fermanli@adau.edu.az

## Quzularda nekrobakteriozun alternativ müalicə üsulunun eksperimental qiymətləndirilməsi

### Xülasə

Nekrobakterioz heyvandarlıqda mühüm yoluxucu xəstəliklərdən biri olaraq qalır və xüsusilə cavan heyvanlarda ciddi sinir pozğunluqlarına və əhəmiyyətli iqtisadi itkilərə səbəb olur. Yerli antimikrob təsirin gücləndirilməsinə və orqanizmin immun cavabının yaxşılaşdırılmasına yönəlmiş effektiv kompleks müalicə yanaşmalarının axtarışı aktual olaraq qalmaqdadır.

Tədqiqatın məqsədi nekrobakteriozla xəstələnmiş heyvanlarda naftalan nefti, oksitetrasiklin və immunomodulyator preparat olan miksoferonun birgə tətbiqinin terapevtik effektivliyini qiymətləndirmək olmuşdur.

Eksperimental şəraitdə ağız boşluğunun selikli qişasında yaranmış zədələrə naftalan nefti–oksitetrasiklin qarışığının yerli tətbiqinin müalicəvi təsiri öyrənilmişdir. Kompleks terapiya alan heyvanlarda klinik sağalma dinamikası və ümumi vəziyyət qiymətləndirilmiş və ənənəvi müalicə üsulları ilə müqayisə edilmişdir.

Naftalan nefti–oksitetrasiklin kombinasiyasının tətbiqi yerli toxumaların vəziyyətinin yaxşılaşması və iltihabi əlamətlərin azalması ilə müşayiət olunmuş, bu da klinik sağalmanın sürətlənməsinə səbəb olmuşdur. Miksoferonun əlavə tətbiqi qeyri-spesifik rezistentlik göstəricilərinin yüksəlməsi və infeksiyanın sistem şəkildə yayılma meylinin azalması ilə müşahidə edilmişdir.

Əldə olunan nəticələr göstərir ki, antimikrob və immunomodulyator komponentləri birləşdirən kompleks terapiya nekrobakteriozun müalicəsində perspektivli hesab oluna bilər. Lakin, bu nəticələrin təsdiqlənməsi üçün randomizə olunmuş nəzarətli eksperimental dizayn, standartlaşdırılmış qiymətləndirmə meyarları və molekulyar diaqnostik təsdiqlə aparılan əlavə tədqiqatlara ehtiyac vardır.

**Açar sözlər:** nekrobakterioz, *Fusobacterium necrophorum*, naftalan nefti, oksitetrasiklin, immunomodulyasiya, baytarlıq terapiyası

## Introduction

Infectious diseases of livestock remain one of the major constraints affecting sustainable animal production worldwide despite substantial progress achieved in veterinary medicine. Diseases such as brucellosis, tuberculosis, leptospirosis, listeriosis and necrobacteriosis continue to cause considerable economic losses associated with decreased productivity, treatment costs and increased mortality of young animals (Shevchenko, 2018, Terekhov, 2025).

Necrobacteriosis is considered one of the most widespread bacterial infections affecting small ruminants and represents a significant veterinary and economic concern in sheep breeding systems. (Sato, 2021, Rizgar, 2024). The disease is characterized by purulent-necrotic lesions of soft tissues and mucous membranes, impaired growth performance and, in severe cases, systemic complications leading to organ damage and mortality. The etiological agent, *Fusobacterium necrophorum*, is widely distributed in the environment and may constitute part of the normal gastrointestinal microbiota of animals. However, under predisposing conditions such as tissue injury, stress, poor hygienic management and decreased immune resistance, the microorganism acquires pronounced pathogenic potential (Nagaraja, 2005, Manuel 2003, Aliyev, 2025).

Despite advances in understanding the epidemiology and pathogenesis of necrobacteriosis, effective therapeutic management remains challenging. Conventional antimicrobial therapy does not always provide satisfactory clinical outcomes due to tissue necrosis, impaired local circulation and reduced penetration of antibacterial agents into affected areas. Therefore, the development of complex therapeutic approaches aimed at improving local drug delivery while simultaneously enhancing host immune responsiveness represents an important direction of contemporary veterinary research (Bennett, 2001, Carvallo, 2020).

Naftalan oil possesses biologically active properties potentially capable of improving local microcirculation and inflammatory response modulation, which may enhance antimicrobial treatment efficacy. In addition, immunomodulatory agents may contribute to improved resistance of the host organism and prevention of systemic dissemination of infection, particularly in young animals characterized by immature immune responses. However, scientific evidence supporting the combined therapeutic use of these agents in necrobacteriosis remains limited (Juan, 2019, Kolychev, 2025, Kislenko, 2012).

**The aim of the present study** was to evaluate the clinical effectiveness of combined therapy including naftalan oil, oxytetracycline and the immunomodulatory preparation mixoferon in the treatment of oral mucosal necrobacteriosis in young farm animals under field conditions.

To achieve this aim, the following objectives were established: to characterize clinical manifestations of necrobacteriosis in lambs; to assess the therapeutic effect of topical application of naftalan oil in combination with oxytetracycline; to compare the effectiveness of the proposed treatment protocol with conventional therapeutic approaches; to evaluate the potential immunomodulatory contribution of mixoferon in preventing disease complications; to develop practical recommendations for veterinary application based on obtained results.

### Materials and Methods

The study was conducted at a private sheep farm located in the Kara-Arkh settlement of the Samukh district (Azerbaijan) and at the regional veterinary diagnostic laboratory.

During routine clinical examination (dispensary survey), 24 lambs aged 2 months showing clinical signs consistent with necrobacteriosis were identified and included in the study. Affected animals demonstrated excessive salivation characterized by foamy viscous discharge with a putrid odor, reduced feed intake, depression, and predominantly recumbent behavior.

Clinical examination of the oral cavity revealed deep purulent-necrotic ulcerative lesions localized on the lips, gums, cheeks, and tongue, frequently covered with grayish diphtheritic plaques. In several cases, lesions extended to the mucous membranes of the larynx and nasal cavity. Gingival involvement was occasionally associated with signs of periodontitis.

Animals were selected based on clinical manifestation of oral necrobacteriosis and comparable age characteristics.

Biological samples were collected from ulcerative-necrotic lesions after mechanical removal of necrotic tissue at the border between affected and healthy areas under aseptic conditions.

Smear imprints were prepared from pathological material and stained using Gram staining for preliminary microscopic evaluation. Samples were subsequently cultured under anaerobic conditions on Kitt–Tarozzi medium for isolation of suspected *Fusobacterium necrophorum*.

For biological confirmation of pathogen virulence, laboratory animals (white mice and rabbits) were experimentally inoculated. All procedures involving laboratory animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123, 1986).

Molecular confirmation of the pathogen was carried out using polymerase chain reaction (PCR) performed on a “Tertsik” thermal cyler (Russia).

Genomic DNA extraction from biological samples included proteinase K digestion (10 mg/mL), phenol–chloroform extraction (1:1), and ethanol precipitation.

A deposited reference strain of *Fusobacterium necrophorum* (№ 8TS630501) was used as a positive control. PCR products were separated by electrophoresis in 0.8% agarose gel at 35–40 mA for 30–40 minutes using pUC18 DNA digested with AluI restriction enzyme as a molecular size marker.

Amplified DNA fragments were documented digitally, and selected amplicons were sequenced according to standard molecular biology protocols.

Indirect fluorescent antibody testing (IFAT) was used for serological confirmation of necrobacteriosis.

Hyperimmune serum was obtained from clinically healthy rabbits immunized with formalin-inactivated epizootic strains of *F. necrophorum*. Bacterial suspensions were prepared in isotonic sodium chloride solution and administered in increasing doses (0.5–1.2 mL) at 4-day intervals over a 1.5-month immunization period.

Smears prepared from clinical material were ethanol-fixed and incubated with hyperimmune serum followed by fluorescein-labeled anti-rabbit globulin conjugate. Preparations were examined using a fluorescence microscope at  $\times 400$  and  $\times 1000$  magnification.

Positive controls consisted of known *F. necrophorum* cultures, whereas negative controls included preparations treated with normal rabbit serum or without primary antiserum.

To evaluate nonspecific resistance in lambs, blood serum samples were analyzed for: bactericidal activity; lysozyme activity.

Serum bactericidal activity was determined using flow cytometry-based assessment of bacterial viability after exposure to serum samples. Lysozyme activity was evaluated using immunochemical analytical methods based on antigen–antibody interaction.

Therapeutic evaluation included combined administration of:

naftalan oil (topical application), oxytetracycline (antibacterial therapy), mixoferon (immunomodulatory preparation).

Following mechanical cleaning of necrotic lesions, a mixture of naftalan oil and oxytetracycline was applied locally to affected oral mucosa.

Oxytetracycline was additionally administered according to recommended veterinary therapeutic dosages. Mixoferon was used as adjunct immunomodulatory therapy aimed at supporting host immune response and reducing the risk of systemic infection development.

Naftalan oil was used as a topical biologically active agent possessing anti-inflammatory and regenerative properties facilitating local tissue recovery.

Oxytetracycline, a broad-spectrum tetracycline antibiotic, was applied as the primary antimicrobial component of therapy.

Mixoferon, an interferon-based immunomodulatory preparation, was administered to stimulate cellular immune mechanisms and enhance nonspecific resistance of the organism.

All procedures involving animals complied with international standards for animal welfare and were performed under veterinary supervision with measures taken to minimize animal stress and suffering.

### Results

Microscopic analysis of smears revealed Gram-negative bacteria morphologically consistent with *Fusobacterium necrophorum*, the causative agent of necrobacillosis. Bacteria were thin, filamentous, and varied in length, with a granular structure and pale staining.

Pathological material was inoculated onto Kitt–Tarozzi anaerobic nutrient medium. Within 18–24 hours of incubation, intense turbidity developed initially in the lower layers, gradually spreading upwards, accompanied by slight gas formation. Pure cultures of *F. necrophorum* were successfully isolated from the lesions.

In vivo pathogenicity tests were conducted on white mice. Subcutaneous injection of 0.3 mL of the isolated culture into the tail root resulted in animal death within 14 days. Post-mortem examination revealed muscle tissue necrosis and purulent foci in the liver, lungs, and heart.

In rabbits, subcutaneous administration of 1 mL culture into the outer ear surface induced necrotic lesions within 24 hours, followed by death and extensive necrotic changes in the head, liver, and heart.

Molecular diagnostics were performed using PCR targeting the leukotoxin gene of *F. necrophorum* with the diagnostic PCR system produced by “NIVI of Siberia and the Far East,” following the manufacturer’s instructions. PCR was conducted in two stages using external and nested primers. Amplicon analysis showed the characteristic pattern, confirming the presence of *F. necrophorum* DNA in the tested samples.

The IFA reaction was considered positive upon detection of bright green fluorescence of bacterial cells against a dark background, consistent with *F. necrophorum* morphology. Absence of specific fluorescence or weak diffuse background was interpreted as negative.

Ten strains of *F. necrophorum* were identified from diseased lambs.

### Nonspecific Serum Reactivity

Bactericidal activity in the serum of diseased lambs was significantly lower than in the control group ( $26.1 \pm 0.63\%$  vs.  $52.9 \pm 0.49\%$ ,  $p < 0.05$ ). Lysozyme activity was also reduced,  $26.8 \pm 1.26\%$  vs.  $48.0 \pm 0.51\%$  in controls ( $p < 0.001$ ).

Twenty diseased lambs were divided into two groups (n = 10 each):

**Group 1 (experimental)** – topical application of a mixture of naftalan oil and oxytetracycline to affected mucous membranes with subsequent intramuscular injection of Mixoferon (0.1 mL/kg once daily).

**Group 2 (control)** – identical topical treatment without Mixoferon.

In Group 1, improvement was observed by Day 3, with reduced salivation and normalization of body temperature. By Day 5, appetite and general condition were restored.

In Group 2, recovery was slower and less pronounced.

### Bactericidal Activity of Serum (%)

Day	Group 1	Group 2
3	$51.0 \pm 0.83$	$45.8 \pm 0.20$
5	$52.4 \pm 0.50$	$47.3 \pm 0.35$
7	$52.5 \pm 0.52$	$48.2 \pm 0.22$

( $p < 0.05$  between groups and relative to baseline)

### Lysozyme Activity of Serum (%)

Day	Group 1	Group 2
3	44.6 ± 0.28	40.9 ± 0.82
5	46.8 ± 0.28	41.0 ± 0.44
7	47.9 ± 0.64	41.3 ± 0.46

( $p < 0.05$  between groups and relative to baseline)

### Conclusion

The obtained results confirm that necrobacillosis in lambs is accompanied by pronounced local purulent-necrotic lesions of the mucous membranes as well as a significant decrease in indicators of nonspecific resistance of the organism. The detected reduction in serum bactericidal and lysozyme activities indicates the development of secondary immunodeficiency, which is consistent with current concepts of the pathogenesis of infections caused by *Fusobacterium necrophorum*.

It is well known that the effectiveness of antibacterial therapy in necrobacillosis is often limited by the presence of necrotic tissues, impaired microcirculation, and insufficient penetration of antibiotics into inflammatory lesions. Consequently, the use of systemic antimicrobial agents alone frequently results in prolonged disease progression and an increased risk of infection generalization.

The results of the present study demonstrated that topical application of a mixture of naftalan oil and oxytetracycline provides a more pronounced therapeutic effect compared with conventional treatment approaches. This effect is likely associated with the ability of naftalan oil to improve local blood circulation, stimulate physiological hyperemia, and enhance penetration of antibacterial agents into affected tissues. In addition, the anti-inflammatory and reparative properties of naftalan oil promote faster cleansing of ulcerative-necrotic lesions and activation of tissue regeneration processes.

Particular importance should be attributed to the use of the immunomodulatory preparation mixoferon. The obtained data indicate a significant increase in serum bactericidal and lysozyme activities in animals of the experimental group, suggesting restoration of innate immune mechanisms. Enhancement of nonspecific resistance likely contributed to limitation of infection spread and accelerated clinical recovery of the animals.

The more rapid disappearance of clinical symptoms observed in lambs receiving combined therapy confirms the effectiveness of an integrated therapeutic approach combining local antimicrobial treatment with immune correction. These findings are consistent with contemporary concepts of veterinary infectious disease therapy, according to which maximum treatment efficacy is achieved through simultaneous action on both the pathogen and the host immune system.

It should be noted that the study was conducted under field production conditions, which increases the practical relevance of the obtained results. However, the limited number of animals and the absence of randomized experimental design indicate the need for further studies involving larger sample sizes and standardized experimental models.

### References

1. Aliyev, A. S., Danko, Y. Y., Eshchenko, I. D., (2025). *Epizootiology with Fundamentals of Microbiology: Textbook*— Saint Petersburg: Lan, 432.
2. Bennett, P. M., & McEwen, S. A. (2001). Necrobacillosis in domestic animals. *Veterinary Record*, 149(22), 683–686.
3. Carvallo, F. R., Uzal, F. A., Flores, K., Diab, S. S., Giannitti, F., Crossley, B., Wünschmann, A. (2020). *Journal of Veterinary Diagnostic Investigation*, 32(2), 339–343. doi:10.1177/1040638720906409
4. Dmitriev, A. F. (2014). *Sheep Diseases*: Stavropol: — 168 ISBN 978-5-9596-1010-4.

5. Juan, M. R., Ignacio, G. B., Rafael, Z. L., Pilar, M. R., Alba, G. R. (2019). *Manuales clínicos de Veterinaria*, Elsevier 152. ISBN: 9788491135951
6. Kolychev, N. M. (2025). *Veterinary Microbiology and Mycology*. Lan.
7. Kislenko, V. N. (2012). *Veterinary Microbiology and Immunology*. GEOTAR-Media.
8. Kumar, A., Anderson, D., Amachawadi, R. G., Nagaraja, T. G., Narayanan, S. K. (2013). Alimentary necrobacillosis in alpacas. *Journal of Veterinary Diagnostic Investigation*, 25(4), 502–507. doi:10.1177/1040638713491407
9. Chirino-Trejo, M., Woodbury, M. R., & Huang, F. (2003). Antibiotic sensitivity and biochemical characterization of *Fusobacterium* spp. and *Arcanobacterium pyogenes* isolated from farmed white-tailed deer (*Odocoileus virginianus*) with necrobacillosis. *Journal of Zoo and Wildlife Medicine*, 34(3), 262–268. <https://doi.org/10.1638/02-019>
10. Nagaraja, T. G., Narayanan, S. K., Stewart, G. C., Chengappa, M. M. (2005). *Fusobacterium necrophorum* infections in animals. *Vet Res Commun* 29 (1), 55–70.
11. Petryankin, F. P., Petrova, O. Yu. (2014). *Diseases of Young Farm Animals: Study Guide*. Saint Petersburg: Lan Publishing House, 352.
12. Sulaiman, R. R., Marif, H. F., Ali, B. A., Ali, K. N., & Baba Shekh, M. O. (2024). Genetic variability and antibacterial sensitivity of *Fusobacterium necrophorum* in sheep. *Iraqi Journal of Veterinary Medicine*, 48(1), 93–100. <https://doi.org/10.30539/xxn4db22>
13. Sato, K., Matsubara, T., Imai, S., Hatada, K., Mitsuma, W., Kawasaki, S., Hama, I., Kamura, T. (2021). *Fusobacterium necrophorum* Endocarditis with Liver Abscesses: A Case Report and Review of the Literature. *Intern Med* 60 (15):2445-2449 doi: 10.2169 /internalmedicine. 6348-20. PMID: PMC8381182
14. Shevchenko, A. A., et al (2018). *Diagnosis of Infectious Diseases of Farm Animals: Bacterial Diseases: Monograph*. Kuban State Agrarian University, 701.
15. Skorodumov, D. I., Rodionova, V. B., Kostenko, T. S. (2023). *Practical Course in Veterinary Microbiology and Immunology*: Lan, 336. ISBN 978-5-507-47839-2.
16. Terekhov, V. I. (2025). *Infectious Diseases of Animals: Clostridial and Other Anaerobic Infections*: Lan, 220.

Received: 11.11.2025

Accepted: 14.03.2026