



Pathomorphological changes in the organs of the peripheral immune system in mycobacteriosis of cattle

Volodymyr Busol*

Doctor of Veterinary Sciences, Professor
National scientific center "Institute of Experimental and Clinical Veterinary Medicine"
61023, 83 Pushkinska Str., Kharkiv, Ukraine
<https://orcid.org/0000-0002-9618-0493>

Petro Boiko

Doctor of Veterinary Sciences, Associate Professor
Lesya Ukrainka Volyn National University
43025, 13 Voli Ave., Lutsk, Ukraine
<https://orcid.org/0000-0003-1418-1159>

Michał Bednarski

Doctor of Veterinary Medicine, PhD
Wrocław University of Environmental and Life Sciences
50-375, 25 C.K. Norwida Str., Wrocław, Poland
<https://orcid.org/0000-0003-0292-7929>

Viktor Shevchuk

PhD in Veterinary Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-5937-3333>

Valery Mazur

PhD in Veterinary Sciences
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-1311-9882>

Suggested Citation:

Busol, V., Boiko, P., Bednarski, M., Shevchuk, V., & Mazur, V. (2023). Pathomorphological changes in the organs of the peripheral immune system in mycobacteriosis of cattle. *Ukrainian Journal of Veterinary Sciences*, 14(2), 9-27. doi: 10.31548/veterinary2.2023.09.

*Corresponding author



Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (<https://creativecommons.org/licenses/by/4.0/>)

Abstract. Conducting routine allergy tests of cattle with a simultaneous sample in TB-prone farms is necessary to confirm the stage of the epizootic process. The purpose of the research – to explore the pathomorphological changes in cows upon detection of para allergic reactions during diagnostic testing for tuberculosis. The diagnosis of tuberculosis in animals was performed comprehensively using all methods for tuberculosis control provided for in the guidelines – epidemiological, clinical, allergic, pathological, histomorphological, bacteriological and biological. It allowed establishing that the reactions to tuberculin in cows were of a paraallergic nature, caused by nontuberculous mycobacteria or so-called atypical mycobacteria. It was determined that cows that reacted positively to tuberculin and an atypical mycobacterial allergen and were sent for slaughter for diagnostic purposes did not demonstrate pathological changes characteristic of tuberculosis. However, pathological changes (granulomatous foci, atrophy of lymphoid and epithelioid tissue, hyperplasia of varying intensity and nature, dotted haemorrhages in the cortical zone) were observed in many lymph nodes, including mediastinal, mesenteric, pharyngeal and mandibular lymph nodes, indicating an active infectious process in the body of animals caused by nontuberculous mycobacteria. Therewith, several changes were observed in the lymph nodes during histomorphological examination: development of primary nodules of lymphocytes and reticular tissue in reactive centres, degradation and necrosis of lymphocytes, the establishment of cellular detritus in the centre of the nodules and capsules of epithelioid elements around the nodules, etc., which can be used to confirm the patterns of pathology development and, indirectly, the mechanism of sensitisation of the cow's body, and therefore the nature of paraallergic reactions in animals. The obtained results are important for the timely detection and identification at the histomorphological level of a complex of changes in the organs and tissues of cows due to the penetration of nontuberculous mycobacteria, which is the key to the successful diagnosis of primary foci of mycobacteriosis and their effective prevention

Keywords: tuberculosis; tuberculin; antigen of atypical mycobacteria; infectious process; atypical mycobacteria; allergic reactions; diagnostics

Introduction

Mycobacteriosis is an infectious disease of animals and humans caused by pathogens belonging to a large group of nontuberculous mycobacteria (NTMB) or so-called atypical mycobacteria (AMB).

Currently, it has been identified more than 180 species of mycobacteria, of which 30-60 are etiological agents of mycobacteriosis (Adjemian *et al.*, 2017, 2018; Armstrong *et al.*, 2023).

The relevance of mycobacteriosis was raised after an increase in mortality of AIDS patients with generalised NTMB infection was noted (Ose *et al.*, 2021; Thomas *et al.*, 2023). The

increase in the incidence of mycobacteriosis is associated with immunosuppression resulting from glucocorticoid and cytostatic therapy, treatment with tumour necrosis factor α blockers (Taira *et al.*, 2018), a decrease in the spread of tuberculosis (Madzinga *et al.*, 2022), and improved methods of isolation and identification of NTMB (Haworth *et al.*, 2017; Kok *et al.*, 2022).

Recently, scientists in many countries around the world have been focusing on the growing problem of NTMB infection in mammals. Thus, since 2000, North America, Europe, New Zealand, and Australia have recorded a

steady increase in the prevalence of NTMB (Prevots & Marras, 2015). The authors state that the prevalence of NTMB in the setting of pulmonary disease continues to increase, and the number of such cases is lower in Europe than in North America and Australia. Global surveys of tertiary care facilities in East Asia (Japan, South Korea, Taiwan) indicate a high and growing prevalence of NTBIs. In several African countries, NTMBs were detected in 4.2-15% of cases of suspected tuberculosis and in 18-20% of individuals with “chronic” suspected multidrug-resistant tuberculosis.

The first documented cases of NTMB pulmonary infections in the English medical literature from Ecuador were reported by Echeverria *et al.* (2023). In Denmark (Dahl *et al.*, 2023), the incidence rate fluctuated from 1.3 (2013) to 2.5 (2021) per 100 thousand people. Scientists conclude that there is a highly positive linear correlation between the incidence of NTMB over the years ($r=0.75$, $p=0.010$), which indicates an upward trend. Vonasek *et al.* (2023) reported a stable annual incidence of NTMB infection in Wisconsin (USA) in 2011-2018. The authors report more than 90% of cases of respiratory tract NTMB infection, the vast majority of which were caused by *M. avium complex*. Among the fast-growing mycobacteria, skin and soft tissue pathogens predominated and were considered secondary respiratory pathogens.

Within the genus *Mycobacterium*, of the more than 170 different species of mycobacteria, the largest number (more than 160 species) belonged to nontuberculous mycobacteria. They occurred in the environment, were highly resistant and adaptable to growth and development. They frequently infected humans and animals (Cvetnić *et al.*, 2018).

Nontuberculous mycobacteria, in particular: *M. scrofulaceum*, *M. fortuitum*, *M. chelonae*, *M. kansasii*, *M. marinum*, *M. xenopi*, *M. vaccae*, *M. phlei*, *M. diernhoferi*, *M. smegmatis*,

M. peregrinum were isolated from biomaterial from animals (cattle, pigs) and poultry (pigeons, sparrows, chickens), and from environmental objects (soil, feed, water, manure, etc.) (Sumbul & Doymaz, 2020; Pavlik *et al.*, 2021; Ulmann *et al.*, 2021).

Lesions of the gastrointestinal tract and respiratory organs in cattle caused by *Mycobacterium caprae* were detected by Ozturk-Gurgen *et al.* (2020). Scientists believe that the changes they observed reflect a continuous primary period of infection, without the lesions typical of the secondary (post-primary) period, which is typical for humans and bovine TB.

Circulation of a significant number of NTMBs (according to the International Classification of Bacteria, ICD-10, 1993; synonyms: ACM – atypical acid-fast mycobacteria; NTM – nontuberculous mycobacteria; ACB – acid-fast bacteria; MAS – *M. avium complex*) in the environment and specifically in cattle herds results in paralleric reactions to tuberculin in animals, which can be up to 50% in herds (Zavgorodnii *et al.*, 2016; Zavgorodnii *et al.*, 2021).

Bovine mycobacteriosis and tuberculosis are diseases that are widespread in the world (Busol *et al.*, 2014; Zimpel *et al.*, 2020; Varela-Castro *et al.*, 2022). Some scientists believe that one of the factors of nonspecific reactions to tuberculin may be latent microbiism caused by human and avian mycobacteria or atypical mycobacterial species (Oleksyuk & Levkivskiy, 2009).

Despite the fact that Ukrainian livestock production is free from TB (Dyachenko *et al.*, 2005) tuberculin-reactive animals continue to be found in many TB-prone farms (Zavgorodnii *et al.*, 2016). Thus, for each specific case of detection of tuberculin-reactive animals, a comprehensive approach to timely and effective diagnosis should be professionally applied (Zavgorodnii *et al.*, 2016).

Animal tuberculosis causes significant economic losses to the livestock industry and pos-

es a threat to human health, and therefore the fight against this anthropophagous disease is of epidemiological importance (Golovko *et al.*, 2016). Epizootic control of bovine tuberculosis is conducted comprehensively. However, preference is given to timely lifetime diagnosis using the allergic method (Kassych *et al.*, 2004; Dyachenko *et al.*, 2005).

Disclosure of the mechanism of paraallergic reactions to tuberculin requires a comprehensive approach to the exploration of the infectious process in mycobacteriosis, in particular, immunological, biochemical, pathophysiological and pathohistological aspects of the interaction between the pathogen and the macroorganism. The establishment of pathological and histomorphological changes in the organs and tissues of NTBM-infected animals will allow disclosing of the mechanism of sensitisation and the nature of these reactions to tuberculin more fully. The relevance of such researches is confirmed by the fact that in recent years there have been a rather small number of reports in the scientific literature on the influence of NTMB at the organ, tissue and cellular levels in cattle.

The purpose of the research was to determine pathological and pathohistological

changes in the body of cattle with mycobacteriosis.

Materials and Methods

In one of the cattle farms in the Volyn region, more than ten cows were found to be positive for tuberculin during a routine tuberculosis test. Based on the analysis of the epizootic situation in this farm in 2017-2022, it was found that the farm's cattle herd was free of tuberculosis pathogen, cows' reactions to tuberculin were paraallergic in nature, and the dairy herd was infected with NTMB of several species, which cause latent mycobacteriosis (Boyko *et al.*, 2020a; Boyko *et al.*, 2020b). Some features of the infectious and epizootic processes of this disease have been identified (Boyko *et al.*, 2021). A comprehensive research of infectious and epizootic processes in mycobacteriosis of dairy cows has been launched in June 2017. During this time, 14 allergy studies were performed, 13 diagnostic slaughters of reactive animals were conducted, and biomaterial from 57 cows was subjected to comprehensive (pathological, histological, microbiological and biological) examination (Table 1). However, despite a significant number of studies conducted in this area, the nature of paraallergic reactions to tuberculin has not been definitively established.

Table 1. Register of diagnostic slaughter of cows that tested positive for tuberculin or allergen atypical mycobacteria, conducted during 2017-2022

No. mon	Date of slaughter	Place of slaughter	Number of animals	Inventory number of animals	Pathological changes typical for tuberculosis
1	24.07.2017	Horodok	10	8121, 6634, 1010, 6443, 1019, 5952, 6605, 1018, 4543, 3497	Not detected
2	18.08.2017	Ivanychi	2	3464, 1412	Not detected
3	10.10.2017	Horodok	3	3415, 9838 and 794504	Not detected
4	05.06.2018	V. Mosty	3	3427, 7899, 6571	Not detected
5	12.12.2018	Horodok	3	794572, 3458, 7378	Not detected
6	26.06.2019	Dubno	3	5588, 124539, 124558	Not detected
7	25.10.2019	Horodok	5	7364, 9905, 5810, 1729, 8112	Not detected
8	23.06.2020	Horodok	4	3495, 4615, 6571, 5510	Not detected
9	25.10.2020	Horodok	6	3433, 5991, 5895, 5856, 4594, 5620	Not detected

Table 1. Continued

No. mon	Date of slaughter	Place of slaughter	Number of animals	Inventory number of animals	Pathological changes typical for tuberculosis
10	26.04.2021	Horodok	4	5894, 4283, 5647, 794591	Not detected
11	03.11.2021	Horodok	4	7837, 9772, 089896, 7894	Not detected
12	27.04.2022	Horodok	4	9438, 794588, 9819, 944265	Not detected
13	02.11.2022	Horodok	6	107890, 5587, 9373, 429894, 9408, 1721	Not detected
Total			57		

Source: author's development

The epizootic situation was controlled by double allergy testing of the entire animal population with a simultaneous test using purified tuberculin for mammals in standard solution (hereinafter referred to as tuberculin) and allergen dry purified from atypical mycobacteria (NTM) with a solvent of mycobacterial allergens (Kassych *et al.*, 2004).

Cows with the most pronounced reactions to both tuberculin and NTM were selected for diagnostic slaughter. Animals were slaughtered on day 5-7 after the allergy research.

The pathological examination was conducted in a modernly equipped slaughterhouse. To identify changes typical for tuberculosis, all lymph nodes were examined: mandibular, pharyngeal, cervical, forearm, scapular, knee fold, supraorbital, mediastinal, mesenteric and portal, as well as parenchymal organs: liver, kidneys, spleen, heart, lungs and both lining of the lung pleura, udder parenchyma, mesentery, small and large intestine.

For histological examination, the pharyngeal, mandibular, mediastinal, portal, mesenteric, supraorbital, pre-orbital, scapular and knee fold lymph nodes, and altered areas of the large intestine were selected.

The sampled material was fixed in a 10% solution of neutral formalin. After thorough washing, the material was placed in a homogenised paraffin mixture. The blocks were cut serially, with a cut thickness of 5 µm. Sections were stained with hematoxylin and eosin. Photographs of histological specimens were taken using a ZEISS Primo

Star 3 microscope (Germany), a colour digital camera Axiocam 208 (Germany) and Zen ZEISS software at the Department of Histology and Medical Biology of Lesya Ukrainka Volyn National University. The research was conducted according to the ARRIVE guidelines for reporting experiments using live animals.

Results and Discussion

In previous studies, it was established that the first manifestations of par allergic reactions in heifers and in first-calf cows of the dairy farm of the examined farm were caused by sensitisation of the animals' organism to NTMB. The latter caused sensitisation of the animals' organisms and an infectious disease – mycobacteriosis (Boyko *et al.*, 2020a; Boyko *et al.*, 2020b).

The study of the infectious process became possible provided that after each scheduled allergy test of cows, a clinical examination of reactive animals was performed, a commission selection of cows for diagnostic slaughter, and post-slaughter examination of organs and tissues, sampling of biomaterial and its complete microbiological and histomorphological examination. Using such comprehensive research allowed for a more objective assessment of certain aspects of the infectious process in cows caused by NTMB.

New methods have been developed for the detection and identification of NTMBs, including molecular genetic methods (polymerase chain reaction – PCR), which are faster and easier to use than methods based on the isolation of pure cultures (Shevchenko *et al.*, 2019).

However, due to the widespread distribution of most NTMB species in the environment, the definitive diagnosis of an infectious disease caused by these mycobacteria cannot be based on culture or PCR alone. Thus, pathological and pathohistological findings are required to establish the final diagnostic result for mycobacteriosis (Prevots & Marras, 2015).

Analysing the data presented in Table 1, it can be noted that out of 57 cows with positive

reactions to allergens, no pathological changes characteristic of tuberculosis were found in any of the examined animals. However, in all cows slaughtered for diagnostic purposes, several other pathological changes were noted, in particular: granulomatous foci, atrophy of lymphoid and proliferation of epithelioid tissue, hyperplasia of varying intensity and nature, and dotted haemorrhages in the cortical zone of the lymph nodes (Fig. 1-2).

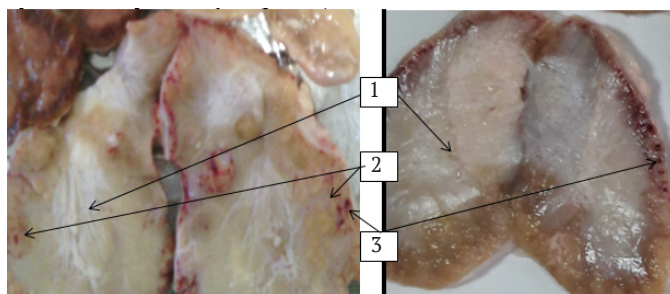


Figure 1. Pathological changes in the supraclavicular lymph nodes

Notes: 1 – degeneration with complete atrophy of the parenchyma of the cerebral layer; 2 – parenchymal remnants in the cortical layer; 3 – haemorrhages in the cortical zone

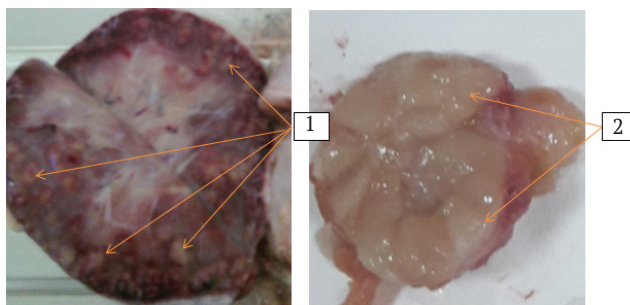


Figure 2. Pathological changes in mediastinal (1) and mandibular (2) lymph nodes

Notes: 1 – granulomatous foci in the mediastinal lymph node; 2 – parenchymal hyperplasia in the submandibular lymph node (pronounced parenchymal greasiness)

Notably, the pathological changes presented in these figures were detected only in the sagittal section of the lymph nodes examined. Therewith, there was no crunching characteristic of tuberculosis, which is caused by the calcification of tuberculous nodules, which

confirms the absence of tuberculous changes. Thus, the pathological picture presented in the figures, on the one hand, confirms the presence of an infectious process in the body of the reacting cows, which is mainly localised in the lymph nodes, and on the other hand, indicates

the absence of a tuberculosis process caused by *Mycobacterium bovis* or *M. tuberculosis* in the body of the reacting animals.

In some lymph nodes, changes in the colour of the parenchyma (reddening or dark grey discolouration), sebaceousness, and replacement of the node parenchyma with connective tissue were detected, indicating the presence of atrophic processes, as evidenced by the growth of connective tissue, which displaces lymphoid tissue, as a result of which the lymph node gradually loses its physiological function (Fig. 1). Hyperplastic processes in the lymph nodes were manifested by the growth of lymphoid tissue, and infiltration with lymphocytes, which is evidence of the presence of latent inflammatory processes in the surrounding tissues of the nodes themselves, provided that foreign agents – bacteria, fungi, etc. In the described case, these are acid-fast NTMB species that sensitised the immune system to their antigens, which manifested as allergic reactions to NTM, and to antigens of other mycobacterial species, including tuberculosis with parallergetic reactions to tuberculin.

Analysing the manifestation of the infectious process in the lymph nodes depending

on their location, pathological changes were most frequently detected in mediastinal, mesenteric (especially those that collect lymph from the large intestine), supraclavicular, pharyngeal, mandibular and much less frequently in the lymph nodes of the knee fold, pre-lobar and scapular lymph nodes. The established facts suggest that the detected regional intensity of the infectious process indicates a high intensity of a load of tissues and organs of the respiratory and digestive systems with NTMB antigens. It, in turn, indicates the priority of aerogenic (hyperplasia of mediastinal lymph nodes – (Fig. 2.1) and nutritional (hyperplasia of mesenteric, pharyngeal and mandibular lymph nodes – (Fig. 2.2) ways of entering these microorganisms into the body of animals.

The severity of pathological changes decreases over time. Thus, in cows slaughtered at the first diagnostic slaughter (2017-2019), pathological changes in the lymph nodes are more pronounced than those found in cows slaughtered for diagnostic purposes in subsequent years, when the number of responding animals began to decrease markedly (Fig. 3).

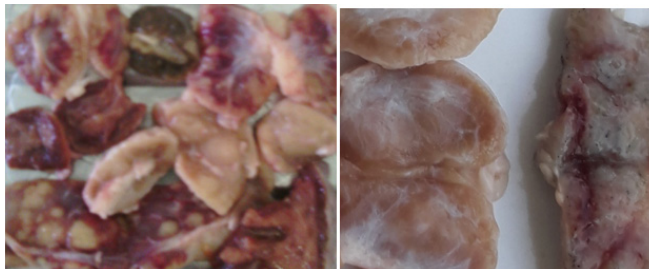


Figure 3. Pathological changes in lymph nodes in cows at the first (2017) diagnostic slaughter (A) and the last (2022) (B)

Notes: A – pronounced granulomatosis and hyperplasia of mediastinal, mandibular and pharyngeal lymph nodes at the first diagnostic slaughter of reactive cows. B – slight replacement of the parenchyma of the suprarenal node with loose connective tissue

It may indicate that at the beginning of the research, the epizootic process of mycobacteriosis in cattle in the herd was characterised by a

long and uncontrolled development. As a result, pathological reactions prevailed over physiological ones, which caused greater damage to

the structure of the organs where they occurred and, ultimately, led to more pronounced pathological changes in the lymph nodes.

At the first diagnostic slaughter, in addition to lymph nodes, pathological changes in the large intestine were found in most slaughtered cows. These changes were mainly localised in the initial part of the colon and resembled paratuberculous enteritis. In particular, the affected areas of the intestine (30 to 60 cm long) had a mild thickening on the serous membrane. In the section, the entire affected area of the intestine was characterised by a pronounced folding of the mucous membrane, which resembled the

surface of the cerebral hemispheres (Fig. 4). The detected pathological changes in the large intestine indicate local penetration of NTMB into the submucosal layer of the colon. The latter is rich in lymphoid elements in the form of solitary follicles and peyer's plaques, where the infectious process between NTMB and the animal's body occurs. This fact gives reason to believe that the nutritional route of mycobacteria entering the body is one of the main ones. Propagating in the intestinal submucosa, NTMBs provide both the entobiotic phase of their population and are both an endogenous and exogenous source of mycobacterial infection.

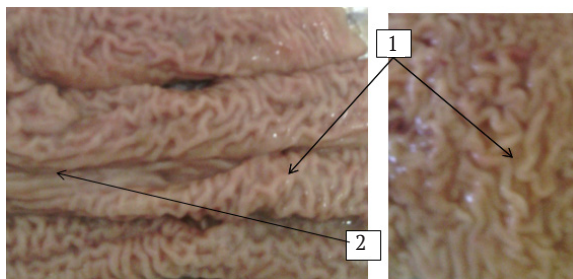


Figure 4. Pathological changes in the proximal colon

Notes: 1 – transverse pathological folding of the colon caused by an infectious process; 2 – longitudinal physiological folding of the colon.

In addition to the transverse folding of the large intestine as a local manifestation of the infectious process in mycobacteriosis caused by NTMB, most animals demonstrated local pathological changes in the colon in the form of diverticula. These diverticula can be

easily identified from the serous membrane as a bulging wall thickening in this area with a darker colour of the serous membrane. The increased folding of the mucous membrane and its thickening are clearly visible in the section (Fig. 5).

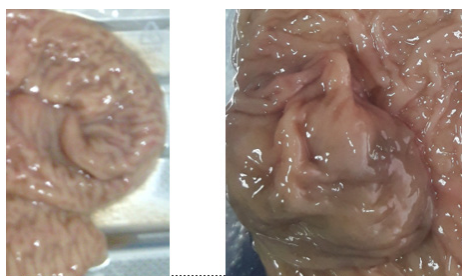


Figure 5. Pathological changes in the large intestine

Notes: A - diverticulum in the wall of the colon mucosa; B - bulging of the colon mucosa into the intestinal lumen

The detected pathological changes are not typical for tuberculosis, but they indicate the presence of local foci of the infectious process of varying intensity in the body of cows that reacted positively to tuberculin and NTM.

The isolation of acid-resistant NTMBs from lymph nodes and affected areas of the large intestine suggests that allergic reactions to tuberculin and NTM are the result of sensitisation of

the body to these microorganisms (Zavgorodnii *et al.*, 2016). To better understand the infectious process caused by NTMB, histomorphological changes in the lymph nodes taken from each animal killed for diagnostic purposes were thoroughly explored. The most characteristic histological changes found in lymph nodes from cows that were positive for PPD-tuberculin and NTM are presented below (Figs. 6-10).

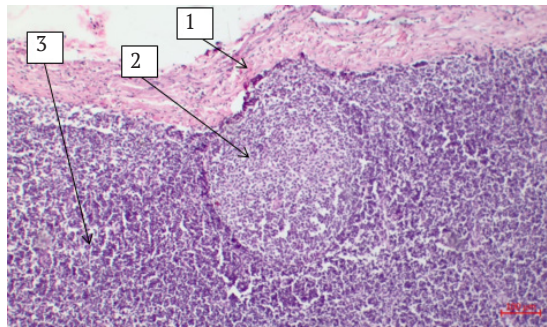


Figure 6. Structure of the lymph node (hematoxylin and eosin staining)

Notes: 1 – lymph node capsule; 2 – lymph follicle; 3 – cortical zone

Fig. 6 presents the microscopic structure of a lymph node in the physiological standard. As can be observed from Figures 7 and 8, the place of influence of NTMB pathogenicity factors on the immune system of infected animals is the lymphatic follicles, where the development of primary nodules of lymphocytes and reticular

tissue was observed at the beginning of the infectious process. Subsequently, degradation was observed in them (cytoplasm swelling, increased eosinophilia, noticeable changes in nuclei, in particular, their wrinkling (karyopyknosis), and increased basophilia), which ends in lymphocyte necrosis.

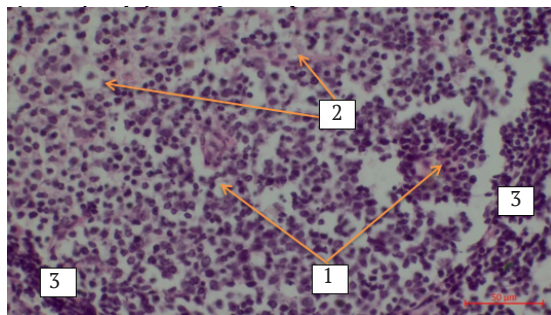


Figure 7. Reactive centres in the lymph follicle as a result of the reaction of lymphocytes to foreign antigens (haematoxylin and eosin staining)

Notes: 1 – initial stages of development of primary nodules from lymphocytes; 2 – reticulocytes under the influence of mycobacterial exotoxins; 3 – periphery of the reactive centre

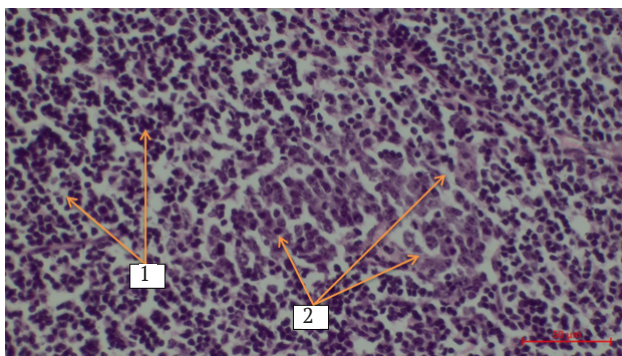


Figure 8. Reactive centres in the lymphatic follicle as a result of lymphocyte reaction to foreign antigens (haematoxylin and eosin staining)

Notes: 1 – clusters of normal lymphocytes; 2 – degraded lymphocytes with severe karyopycnosis and karyorexia under the influence of mycobacterial exotoxins in the process of primary nodule development

Cellular detritus is developed at the site of lymphocyte death (Fig. 9.1), and a pronounced primary nodule is established, surrounded by a significant layer of lymphocytes (Fig. 9.2).

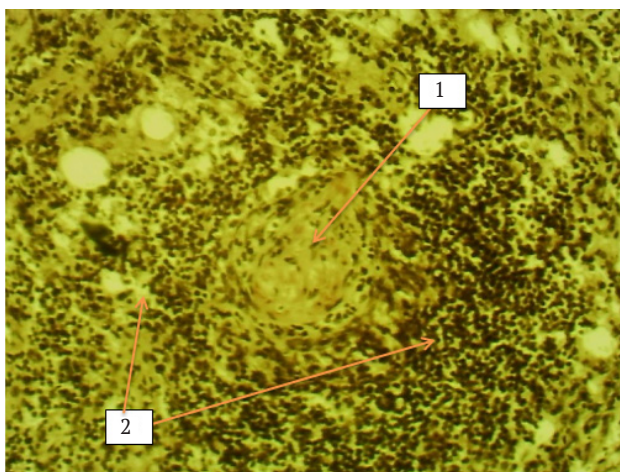


Figure 9. Development of nodules in NTBM-infected lymph nodes at later stages of the infectious process (hematoxylin and eosin staining. Magnification $\times 400$)

Notes: 1 – cellular detritus in the centre of the nodule; 2 – accumulation of lymphoid elements around the detritus

Similar morphological changes were found in histological sections of suprarenal lymph nodes (Fig. 10), where the development of primary nodules with cellular detritus in the centre (Fig. 10.1), a slight peripheral accumulation of lymphocytes (Fig. 10.2) and the establishment of a visible capsule of epithelioid elements (Fig. 10.3) were noted.

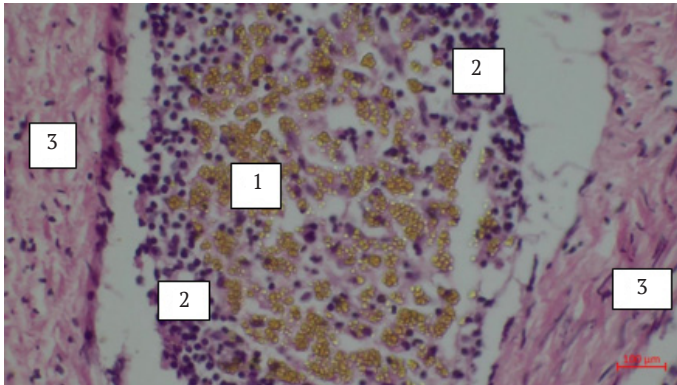


Figure 10. Development of nodules in NTMB-infected lymph nodes at later stages of the infectious process (haematoxylin-eosin staining)

Notes: 1 – cellular detritus in the centre of the nodule; 2 – accumulation of lymphoid elements around the detritus; 3 – formation of a capsule from epithelioid cells

In no case did the authors detect calcification of these capsules, as is the case with tuberculous mycobacteria (*M. bovis* or *M. tuberculosis*). Evidently, such epithelioid strands develop complete or incomplete connective tissue capsules, merging, displacing or replacing the primary nodules formed in the reactive centres and, subsequently, the lymphoid follicles themselves and, ultimately, the cortical zone (Fig. 1.2).

Morphologically, this process is accompanied by the proliferation of epithelioid tissue, which leads to atrophy of the lymphoid tissue of the lymph nodes (Fig. 11.1). In cows of 7-9 years of age, more pronounced atrophy of the cortical layer of the lymph nodes was observed compared to this pathology in younger cows. It is probable that the longer the exposure of lymphoid tissue to NTMB pathogenicity factors, the more significant the atrophy of the cortical zone of the latter.

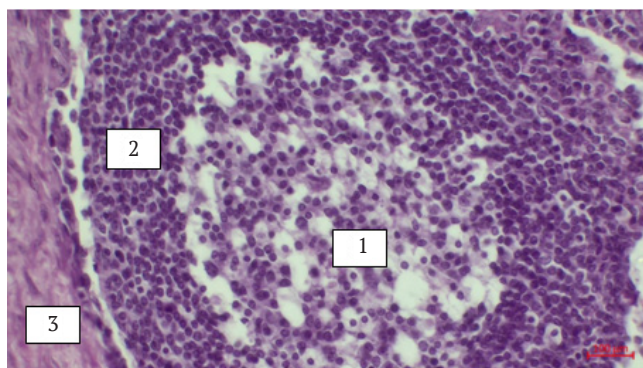


Figure 11. Development of nodules in lymph nodes infected with NTMB

Notes: 1 – necrosis of lymphocytes in the centre of the nodule, fragmentation of nuclei and condensation of chromatin, loss of whole areas of dead cells; 2 – a zone of lymphocytes around detritus; 3 – capsule of epithelioid cells

The detected pathohistomorphological changes in the lymph nodes and in the submucosal layer of the large intestine indicate an active infectious process in the body of cows caused by NTMB. Although NTMBs were isolated from all lymph nodes taken for bacteriological examination (Boyko *et al.*, 2020), pathological changes were detected only in the supraclavicular, mediastinal, mesenteric, occasionally in the submandibular and pharyngeal lymph nodes and very rarely in the anterior and posterior phrenic and knee lymph nodes. These changes, along with cortical haemorrhages, can be detected visually when the nodes are sagittally incised. Developed

primary nodules, which are noted during the histological examination, remain invisible visually and are not detected by palpation.

It can be assumed that primary nodules arise as a result of a powerful, both in terms of quantity and duration, exposure to antigenic irritation of the immune system, in particular the lymph nodes. As a result, cellular reactions occurring in the reactive zones lead to the necrosis of lymphocytes and their replacement by epithelioid cells (Figs. 9-12), which penetrate the cortical layer in the form of strands, starting from the cerebral layer, displacing lymphoid tissue and causing atrophy of the latter.

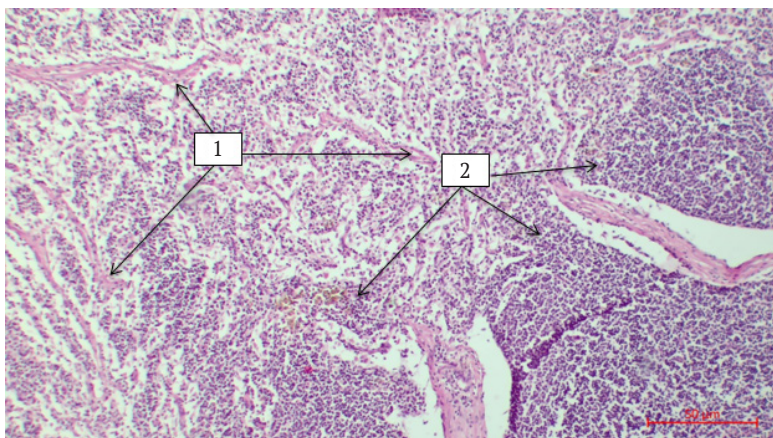


Figure 12. Replacement of lymphoid tissue with epithelioid elements in lymph nodes infected with NTMB as a result of the infectious process (hematoxylin and eosin staining)

Notes: 1 – cords of epithelioid cells; 2 – remnants of lymphoid tissue

The establishment of granulomas in the lymph nodes (Fig. 3 A) confirms the prolonged effect of mycobacterial toxins on the immune system of animals, which leads to the development of a specific granulomatous inflammation. The mechanism of its development is based on primary perivascular infiltration by CD4+ T-lymphocytes, which are gradually (within 2-3 weeks) replaced by macrophages, and the latter, due to their activation by cytokines, increase in size and resemble squamous epithelium in

shape, their cytoplasm becomes eosinophilic, and they gradually transform into epithelioid cells. Some cytokines, such as IFN- γ , acting on transformed epithelioid cells, transform them into multinucleated giant cells (Kumar *et al.*, 2019). Foci of mycobacterial penetration and proliferation lead to the accumulation of epithelioid cells, including giant cells. And these clusters, in turn, are surrounded by a thick shaft of lymphocytes, resulting in the development of granulomas (Fig. 13).

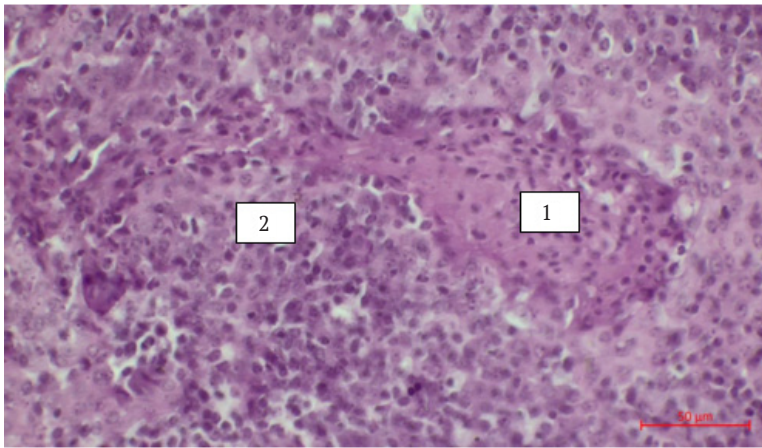


Figure 13. Granuloma development (haematoxylin and eosin staining)

Notes: 1 – clusters of epithelioid cells surrounded by a thick shaft of lymphocytes (2)

T-cell-mediated inflammation underlies the mechanism of tissue damage in many autoimmune diseases or those caused by uncontrolled reactions to commensal bacteria or atypical acid-fast mycobacteria when they are constantly and in large volumes ingested by animals (inflammatory bowel disease in humans (Kumar *et al.*, 2019). In the present research, the source of NTMB in the organs and tissues of cows was chronic local interstitial colitis (Figs. 4, 5).

The pathological changes presented in Figs. 7-13, pathomorphological changes occur at the cellular and tissue levels and are characterised by irreversibility. These pictures demonstrate the development of primary nodules from lymphocytes and reticular tissue, lymphocyte degradation – the cytoplasm swells, its eosinophilia increases; changes in the nuclei, resulting in their wrinkling (karyopyknosis), which in turn is manifested by increased basophilia, and karyorexis (nucleus fragmentation), which ends with karyolysis of the nuclei and necrosis of lymphocytes. The affected lymphoid tissue is replaced by epithelioid tissue, which leads to atrophy of the lymph node parenchyma.

Evidently, the body's sensitisation to NTMB antigens begins at the stage of primary nodule development in the reactive centres of the lymph nodes and is permanently maintained by each subsequent ingestion of a fresh portion of NTMB from the environment.

Finally, the possibility of permanent ingestion of NTMBs from the digestive tract, where mycobacteria can be in the entobiotic phase of existence, colonising the submucosal layer of the large intestine wall, cannot be excluded (Boyko *et al.*, 2020a; Boyko *et al.*, 2020b). However, it is assumed that this mechanism of maintaining the sensitisation of the cow's organism is not decisive, as then the permanence of paraallergic reactions remains unexplained.

It can be argued that the permanence of paraallergic reactions in animals is a consequence of a significant amount of NTMB entering the body of cows over a long period (according to observations, at least 2-3 months) from the outside (alimentary or aerogenic).

Exploring the epizootological features of cow mycobacteriosis in a particular farm, it was found that the number of cows reacting to both allergens was significantly (2-3 times) higher

in the allergic test after winter-stall housing compared to that in summer-exercise housing. It proves that keeping animals indoors for a long period (more than 2-3 months) leads to constant ingestion of NTMBs, which cause the pathomorphological changes identified and described and manifest themselves as a permanent allergy to tuberculin and NTM.

According to the authors, a significant reduction in the antigenic load on the body of NTMB cows would reduce the infectious process in the body of infected animals and, thus, sensitise the body of cows to allergens of atypical mycobacteria. The assumptions made are fully consistent with the findings published earlier by some researchers (Falkinham, 2013). They argue that any changes in human health or animal husbandry conditions could lead to the emergence of new infections through NTMB as a result of the widespread distribution of these mycobacteria in most ecological niches (water, soil, sphagnum and wildlife) (Schroder *et al.*, 1992).

For this purpose, it is necessary to explore more thoroughly the ecology of NTMBs on the territory of the dairy farm. It will allow for a more targeted and effective implementation of several preventive measures aimed primarily at breaking the epizootic chain by reducing NTMB on the premises and the farm. It will have an objective impact on the welfare of the dairy herd and reduce or even eliminate the risk of spreading NTMB through dairy products, which will be of great medical and epidemiological importance for the prevention of human mycobacteriosis.

Conclusions

According to the results obtained, it was proved that the epizootic process in cattle herds is caused by the infection of animals with acid-fast mycobacteria. This phenomenon is quite common and therefore should be constantly monitored by qualified specialists of the state veterinary service.

Nontuberculous mycobacteria colonise mainly the digestive and respiratory organs, which indicates their alimentary and aerogenic routes of entry into the animal body. They linger for the longest time in the regional lymph nodes of these organs, where they cause profound and often irreversible pathological changes at the cellular and tissue levels, which were identified through detailed pathological and histological examinations. The pathomorphological changes in the lymph nodes and large intestine of the reacting animals can be used to determine the presence of an intense infectious process caused by NTMB in their bodies and the functioning of the epizootic process – mycobacteriosis – in the dairy cow herd. Thus, reactive animals should be considered as an active source of NTBM, which is important both epizootologically and epidemiologically.

More detailed research on the species composition of NTMBs infecting animals and the ecology of NTMBs identified on the farm and their potential pathogenic properties will be of both economic and epidemiological and medical epidemiological importance. Considering the significant role of NTBMs in human infectious pathology, each case of establishing the functioning of the epizootic process of mycobacteriosis in herds of productive animals should be considered as a source of potential biohazards to human health in the food chain. Therefore, further research will focus on an in-depth study of other aspects of the mycobacteriosis infection process, the ecology of NTMBs on the territory of a dairy farm, and ways to minimise the biohazards of human infection with NTMBs that cause dangerous meat and dairy food products.

Acknowledgements

None.

Conflict of Interest

None.

References

- [1] Adjemian, J., Daniel-Wayman, S., Ricotta, E., & Prevots, R. (2018). Epidemiology of Nontuberculous Mycobacteriosis. *Seminars in Respiratory and Critical Care Medicine*, 39, 325-335. doi: [10.1055/s-0038-1651491](https://doi.org/10.1055/s-0038-1651491).
- [2] Adjemian, J., Frankland, T.B., Daida, Y.G., Honda, J.R., Olivier, K.N., Zelazny, A., Honda, S., & Prevots, D.R. (2017). Epidemiology of nontuberculous mycobacterial lung disease and tuberculosis, Hawaii, USA. *Emerging Infectious Diseases*, 23(03), 439-447. doi: [10.3201/eid2303.161827](https://doi.org/10.3201/eid2303.161827).
- [3] Armstrong, D.T., Eisemann, E., & Parrish, N.A. (2023). Brief Update on Mycobacterial Taxonomy, 2020 to 2022. *Journal of Clinical Microbiology*, 61(4), article number e0033122. doi: [10.1128/jcm.00331-22](https://doi.org/10.1128/jcm.00331-22).
- [4] Boyko, P.K., Boyko, O.P., Nychyk, S.A., Sytnik, V.A., & Mazur, V.M. (2020). Formation of infection of cattle with atypical mycobacteria in a separate herd. *Veterinary Biotechnology Bulletin*, 36, 9-21. doi: [10.31073/vet_biotech36-01](https://doi.org/10.31073/vet_biotech36-01).
- [5] Boyko, P.K., Nychyk, S.A., Boyko, O.P., Tytyuk, O.V., & Shevchuk, V.M. (2021). Mycobacteriosis of cattle - a spontaneous infection in a separate farm or a complex epidemiological problem? *Veterinary Biotechnology Bulletin*, 39, 18-28. doi: [10.31073/vet_biotech39-02](https://doi.org/10.31073/vet_biotech39-02).
- [6] Busol, V.O., Shevchuk, V.M., Mazur, V.M., & Kovalenko, L.V. (2014). [Evolution of intensity of the epizootic process of tuberculosis of cattle in the countries of the world](#). *Scientific Bulletin of Veterinary Medicine (Collection of Scientific Papers)*, 14(114), 74-77.
- [7] Cvetnić, Ž., Tuk, M.Z., Duvnjak, S., Reil, I., Mikulić, M., Pavlinec, Ž., & Špičić, S. (2018). Tuberculous and nontuberculous mycobacteria in human and animal infection. *Veterinary Journal of Republic of Srpska (Banja Luka)*, 18(2), 342-369. doi: [10.7251/VETJEN1802342C](https://doi.org/10.7251/VETJEN1802342C).
- [8] Dahl, V.N., Fløe, A., & Wejse, Ch. (2023). Nontuberculous mycobacterial infections in a Danish region between 2011 and 2021: Evaluation of trends in diagnostic codes. *Infectious Diseases*, 55(6), 439-443. doi: [10.1080/23744235.2023.2194411](https://doi.org/10.1080/23744235.2023.2194411).
- [9] Dyachenko, H.M., Kravchenko, N.O., & Romanenko, V.P. (2005). The problem of diagnosing tuberculosis of farm animals in modern conditions. *Veterinary Medicine: Interdepartmental Thematic Scientific Collection*, 85, 1236-1240.
- [10] Echeverria, G., Rueda, V., Espinoza, W., Rosero, C., Zumárraga, M.J., & de Waard, J.H. (2023). First Case reports of nontuberculous mycobacterial (NTM) lung disease in Ecuador: Important lessons to learn. *Pathogens*, 12(4), article number 507. doi: [10.3390/pathogens12040507](https://doi.org/10.3390/pathogens12040507).
- [11] Falkinham, J.O. (2013). Ecology of nontuberculous mycobacteria – where do human infections come from? *Seminars in Respiratory and Critical Care Medicine*, 34(1), 95-102. doi: [10.1055/s-0033-1333568](https://doi.org/10.1055/s-0033-1333568).
- [12] Golovko, V.O., Kassich, O.V., & Kassich, V.Yu. (2016). [Modern problems of infectious pathology in Ukraine](#). *Sumy NAU Herald. Veterinary Medicine*, 6(38), 119-124.
- [13] Haworth, C.S., Banks, J., Capstick, T., Fisher, A.J., Gorsuch, T., Laurenson, I.F., Leitch, A., Loebinger, M.R., Milburn, H.J., Nightingale, M., Ormerod, P., Shingadia, D., Smith, D., Whitehead, N., Wilson, R., & Floto, R.A. (2017). British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). *Thorax*, 72(2), ii1-ii64. doi: [10.1136/thoraxjnl-2017-210927](https://doi.org/10.1136/thoraxjnl-2017-210927).
- [14] Kok, N.A., Peker, N., Schuele, L., de Beer, J.L., Rossen, J.W.A., Sinha, B., & Couto, N. (2022). *Host DNA depletion can increase the sensitivity of Mycobacterium spp. detection through shotgun metagenomics in sputum*. Retrieved from <https://www.frontiersin.org/articles/10.3389/fmicb.2022.949328/full>.

- [15] Kumar, V., Abbas, A.K., & Aster, J.K. (2019). *Basics of pathology according to Robinson: Translation of the 10th English edition*. Kyiv: VSV "Medicine".
- [16] Madzinga, D., Tshitangano, T.G., Raliphaswa, N.S., & Razwiedani, L. (2022). Healthcare workers perception of measures to reduce the risk of new tuberculosis infections: A qualitative study report. *Nursing Reports*, 12(4), 873-883. doi: [10.3390/nursrep12040084](https://doi.org/10.3390/nursrep12040084).
- [17] Oleksyuk, I.I., & Levkivskiy, D.M. (2009). Latent microbism and its role in the epizootology of bovine tuberculosis. *Scientific Bulletin of the LNUVMBT named after S.Z. Gzhitskiy*, 11, 2(41), 225-229.
- [18] Ose, N., Takeuchi, Y., Kitahara, N., Matumura, A., Kodama, K., Shiono, H., Susaki, Y., Funakoshi, Y., Takabatake, H., & Shintani, Y. (2021). Analysis of pulmonary nodules caused by nontuberculous mycobacteriosis in 101 resected cases: multi-center retrospective study. *The Journal of Thoracic Disease*, 13(2), 977-985. doi: [10.21037/jtd-20-3108](https://doi.org/10.21037/jtd-20-3108).
- [19] Ozturk-Gurgen, H., Rieseberg, B., Leipzig-Rudolph, M., Straubinger, R.K., & Hermanns, W. (2020). Morphology of naturally-occurring tuberculosis in cattle caused by *Mycobacterium caprae*. *Journal of Comparative Pathology*, 174, 120-139. doi: [10.1016/j.jcpa.2019.11.010](https://doi.org/10.1016/j.jcpa.2019.11.010).
- [20] Pavlik, I., Ulmann, V., & Weston, R.T., (2021). Clinical relevance and environmental prevalence of *Mycobacterium fortuitum* group members. Comment on mugetti *et al.* Gene sequencing and phylogenetic analysis: Powerful tools for an improved diagnosis of fish mycobacteriosis caused by *Mycobacterium fortuitum* group members. *Microorganisms*, 9(11), article number 2345. doi: [10.3390/microorganisms9112345](https://doi.org/10.3390/microorganisms9112345).
- [21] Prevots, R., & Marras, T.K. (2015). Epidemiology of human pulmonary infection with non-tuberculous mycobacteria: A review. *Clinics in Chest Medicine*, 36(1), 13-34. doi: [10.1016/j.ccm.2014.10.002](https://doi.org/10.1016/j.ccm.2014.10.002).
- [22] Schroder, K.H., Kazda, J., Muller, K., & Muller, H.J. (1992). Isolation of *Mycobacterium simiae* from the environment. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*, 277(4), 561-564. doi: [10.1016/s0934-8840\(11\)80482-2](https://doi.org/10.1016/s0934-8840(11)80482-2).
- [23] Shevchenko, O.S., Todoriko, L.D., Poteyko, P.I., & Pogorelova, O.A. (2019). Questions of diagnosis and treatment of nontuberculous mycobacteriosis. *East European Journal of Internal and Family Medicine*, 1, 36-53.
- [24] Sumbul, B., & Doymaz, M.Z. (2020). A current microbiological picture of mycobacterium isolates from Istanbul, Turkey. *Polish Journal of Microbiology*, 69(2), 1-7. doi: [10.33073/pjm-2020-021](https://doi.org/10.33073/pjm-2020-021).
- [25] Taira, N., Kawasaki, H., Takahara, S., Chibana, K., Atsumi, E., & Kawabata, T. (2018). The presence of coexisting lung cancer and non-tuberculous mycobacterium in a solitary mass. *American Journal of Case Reports*, 19, 748-751. doi: [10.12659/AJCR.908090](https://doi.org/10.12659/AJCR.908090).
- [26] Thomas, A.R., Prasad, V.P., Sethi, S., & Maturu, V.N. (2023). Atypical mycobacterial infection masquerading as an endobronchial growth in an immunocompromised host. *BMJ Case Reports*, 16(4), article number e255317. doi: [10.1136/bcr-2023-255317](https://doi.org/10.1136/bcr-2023-255317).
- [27] Ulmann, V., Modrá, H., Babak, V., Weston, R.T., & Pavlik, I. (2021). Recovery of mycobacteria from heavily contaminated environmental matrices. *Microorganisms*, 9(10), article number 2178. doi: [10.3390/microorganisms9102178](https://doi.org/10.3390/microorganisms9102178).
- [28] Varela-Castro, L., Barral, M., Arnal, M.C., Fernández de Luco, D., Gortázar, C., Garrido, J.M., & Sevilla, I.A. (2022). Beyond tuberculosis: Diversity and implications of non-tuberculous mycobacteria at the wildlife-livestock interface. *Transboundary and Emerging Diseases*, 69(5), e2978-e2993. doi: [10.1111/tbed.14649](https://doi.org/10.1111/tbed.14649).

- [29] Vonasek, B.J., Gusland, D., Hash, K.P., Wiese, A.L., Brad, J.T.-K., Astor, C., Gibbons-Burgener, S.N., & Misch, E.A., (2023). *Nontuberculous mycobacterial infection in wisconsin adults and its relationship to race and social disadvantage*. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/36812384/>.
- [30] Zavgorodnii, A.I., Bilushko, V.V., Zagrebelnyi, V.O., & Balym, Yu.P. (2016). Study of sensitizing properties of atypical mycobacteria isolated from biomaterial from cattle and from environmental objects. *Veterinary Medicine: Interdepartmental Thematic Scientific Collection*, 102, 84-87.
- [31] Zavgorodnii, A.I., Pozmogova, S.A., Kalashnyk, M.V., Paliy, A.P., Plyuta, L.V., & Paliy, A.P. (2021). Etiological factors in triggering non-specific allergic reactions to tuberculin in cattle. *Regulatory Mechanisms in Biosystems*, 12(2), 228-233. doi: [10.15421/022131](https://doi.org/10.15421/022131).
- [32] Zimpel, C.K., Patané, J.S.L., Guedes, A.C.P., de Souza, R.F., Silva-Pereira, T.T., Camargo, N.C.S., de Souza Filho, A.F., Ikuta, C.Y., Neto, J.S.F., Setubal, J.C., Heinemann, M.B., & Guimaraes, A.M.S. (2020). Global distribution and evolution of *Mycobacterium bovis* lineages. *Frontiers in Microbiology*, 11, article number 843. doi: [10.3389/fmicb.2020.00843](https://doi.org/10.3389/fmicb.2020.00843).

Патоморфологічні зміни в органах периферичної імунної системи за мікобактеріозів великої рогатої худоби

Володимир Олександрович Бусол

Доктор ветеринарних наук, професор
Національний науковий центр «Інститут експериментальної і клінічної ветеринарної медицини»
61023, вул. Пушкінська 83, м. Харків, Україна
<https://orcid.org/0000-0002-9618-0493>

Петро Костянтинович Бойко

Доктор ветеринарних наук, доцент
Волинський національний університет імені Лесі Українки
43025, проспект Волі, 13, м. Луцьк, Україна
<https://orcid.org/0000-0003-1418-1159>

Міхал Беднарські

Доктор філософії, кандидат ветеринарних наук
Вроцлавський університет наук про навколишнє середовище та життя
50-375, вул. Норвіда, 25, м. Вроцлав, Польща
<https://orcid.org/0000-0003-0292-7929>

Віктор Миколайович Шевчук

Кандидат ветеринарних наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-5937-3333>

Валерій Миколайович Мазур

Кандидат ветеринарних наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-1311-9882>

Анотація. Проведення планових алергічних досліджень великої рогатої худоби симультанною пробю в господарствах благополучних щодо туберкульозу є необхідним для підтвердження стадії епізоотичного процесу. Мета роботи – дослідити патоморфологічні зміни у корів за виявлення параалергічних реакцій під час діагностичного дослідження на туберкульоз. Діагностику туберкульозу в тварин здійснювали комплексно з використанням усіх передбачених інструкцією методів щодо боротьби з туберкульозом – епізоотологічного, клінічного, алергічного, патологоанатомічного, гістоморфологічного, бактеріологічного і біологічного. Завдяки цьому вдалося встановити, що реакції на туберкулін у корів мали параалергічну природу, викликані нетуберкульозними мікобактеріями або, так званими, атипovими мікобактеріями. Встановлено, що у корів, які позитивно реагували на туберкулін і на алерген атипovих мікобактерій та були відправлені на забій з діагностичною метою, не виявлено патологоанатомічних змін, характерних для туберкульозу. Проте, у багатьох лімфатичних вузлах, зокрема медіастинальних, мезентеріальних, заглиткових

і нижньощелепних, відмічали патологічні зміни (грануломатозні вогнища, атрофію лімфоїдної і розростання епітеліоїдної тканин, гіперплазію різної інтенсивності та характеру, крапчасті крововиливи у кірковій зоні), які вказують на активний інфекційний процес в організмі тварин, спричинений нетуберкульозними мікобактеріями. Водночас при проведенні гістоморфологічних досліджень у лімфатичних вузлах відзначали низку змін: формування первинних вузликів із лімфоцитів та ретикулярної тканини у реактивних центрах, деградацію та некроз лімфоцитів, утворення клітинного детриту у центрі вузликів та капсул із епітеліоїдних елементів навколо вузликів тощо, за якими можна підтвердити закономірності розвитку патології і, опосередковано, – механізм сенсibiliзації організму корів, а отже і природу параалергічних реакції у тварин. Отримані результати мають важливе значення для своєчасного виявлення та ідентифікації на гістоморфологічному рівні комплексу змін в органах і тканинах корів за проникнення нетуберкульозних мікобактерій, що є запорукою успішної діагностики первинних вогнищ мікобактеріозів та ефективної їх профілактики

Ключові слова: туберкульоз; туберкулін; антиген атипових мікобактерій; інфекційний процес; атипові мікобактерії; алергічні реакції; діагностика