



Atypical mycobacteria cultures isolated in different natural and geographical zones of Ukraine

A. I. Zavgorodnii*, A. P. Paliy*, V. V. Bilushko*, S. A. Pozmogova*, A. V. Ushkalov*,
K. O. Sviridova*, O. V. Pavlichenko**, R. V. Petrov***, D. O. Kisil***

**Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine*

***State Biotechnological University, Kharkiv, Ukraine*

****Sumy National Agrarian University, Sumy, Ukraine*

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

*State Biotechnological University,
Alchevskikh st., 44, Kharkiv,
61002, Ukraine. Tel.: +38-050-
760-62-84. E-mail:
pavlichenkoelena777@gmail.com*

*Sumy National Agrarian
University, H. Kondratiieva st.,
160, Sumy, 40021, Ukraine.
Tel.: +38-066-392-79-28. E-mail:
romanpetrov1978@gmail.com*

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Despite Ukraine's official status as free of tuberculosis in cattle, positive tuberculin reactions continue to be recorded in herds, complicating epizootic surveillance. One of the main reasons for these reactions is the spread of non-tuberculous (atypical) mycobacteria, which circulate widely in the environment and have zoonotic potential. This study aimed to investigate the species composition, cultural and morphological properties, and regional characteristics of atypical mycobacteria isolated from cattle in various natural and climatic zones of Ukraine. We studied 307 cultures of atypical mycobacteria isolated from 17 regions of Ukraine. Identification was based on tinctorial, cultural-morphological, and biochemical properties, as well as comparison with reference strains of mycobacteria, using standard methods. We evaluated growth characteristics at different cultivation temperatures, pigment formation, biochemical activity, and classification according to Runyon's classification. We determined that the isolated cultures belonged to 18 species of non-tuberculous mycobacteria, classified as groups II, III, and IV according to Runyon. The most common species were *Mycobacterium scrofulaceum*, *M. gordonae*, *M. intracellulare*, *M. smegmatis*, *M. phlei*, and *M. fortuitum*. Significant regional specificity was observed: numerous fast-growing species dominated the steppe zone; the forest-steppe zone had a more limited spectrum with a predominance of *M. gordonae* and *M. scrofulaceum*; and Polissya had mainly representatives of group IV. Photochromogenic mycobacteria of group I were not detected. Atypical mycobacteria are widespread among cattle in Ukraine, particularly on tuberculosis-free farms. The diversity of species and regional heterogeneity cause animals to be sensitized to tuberculin and exhibit false-positive reactions. This must be considered in epizootic surveillance and the differential diagnosis of tuberculosis within the "One Health" concept.

Keywords: tuberculosis; mycobacteria; tuberculin; diagnosis; epizootological surveillance.

Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* and related members of the *M. tuberculosis* complex (MTBC), remains one of the most important infectious diseases of animals and humans worldwide (Lorente-Leal et al., 2021; Khairullah et al., 2024). This disease is not only of veterinary importance but also has significant zoonotic implications, as it poses a potential threat to human health (Santos et al., 2020; Szacawa et al., 2025). Due to the insufficient effectiveness of modern diagnostic tests for tuberculosis and the movement of infected animals, there is a risk of uncontrolled spread of the tuberculosis pathogen (Pfeiffer, 2013; Krajewska-Wędzina et al., 2022). In Poland, microbiological studies have confirmed tuberculosis in 46 wild boars caused by *M. caprae* (Welz et al., 2023).

Interspecies transmission of the tuberculosis pathogen *M. bovis* has been established (Konold et al., 2020; Chang et al., 2023). An intradermal tuberculin test identified 15% of the sheep as reactive (4/26), and *M. bovis* was cultured from pathological material obtained from these animals. These animals had been in contact with dairy cows, 98% (45/46) of which tested positive for tuberculosis, and *M. bovis* was isolated from their pathological material after slaughter. The isolation of a common *M. bovis* genotype (SB0134) from both species of animals strongly suggests that the sheep were infected with tuberculosis from the dairy cows (Gelalcha et al., 2019).

Various methods are used to diagnose tuberculosis. The main methods are intradermal tuberculin testing, pathological studies, bacteriological studies, molecular genetic studies, and others (Lekko et al., 2020; Thomas et al., 2021; Zavgorodnii et al., 2022).

A retrospective analysis of data for 2012–2022 shows a gradual decrease in the number of infected herds and a consistently low

prevalence of tuberculosis in cattle in Europe. At the same time, the unevenness of indicators and the presence of tuberculosis foci in individual countries indicate the need to improve existing programs for monitoring and eradicating this disease (Schiller et al., 2011; Didkowska et al., 2021; Welby et al., 2022). The global infection control strategy is based on the "One Health" principle, which aims to eradicate zoonoses and break the epizootic chain between animals and humans (Pitt & Gunn, 2024; Igreja et al., 2025).

The State Service of Ukraine for Food Safety and Consumer Protection reports that Ukraine's cattle population has been free of tuberculosis since 2016. However, allergy test results show isolated positive reactions to PPD tuberculin in healthy herds. These results necessitate clear differentiation between true infection and para-allergic manifestations of the reaction, as well as enhanced epizootiological surveillance, particularly under wartime conditions (Zavgorodnii et al., 2021; Bruczyńska et al., 2023).

Additionally, tuberculosis can be latent, and most cases of tuberculosis lesions are not observed in infected animals slaughtered for diagnostic purposes. Therefore, these farms require comprehensive diagnostic studies to determine the nature of the reactions to tuberculin and the epizootic status of the herds (Good & Duignan, 2011; Zavgorodnii et al., 2021).

Despite the low prevalence of tuberculosis among cattle, there is still a risk of sporadic cases, which requires constant monitoring. At the same time, there is an increase in the prevalence of non-tuberculous mycobacteria associated with domestic and exotic animals, including companion birds, which can be a source of a whole range of mycobacteria, including those that are potentially dangerous to humans (Slany et al., 2016; Debelu et al., 2021). The importance of zoonotic surveillance is confirmed by the first reported case of rifampin-

in-resistant tuberculosis transmission from a dog to a human (Zmak et al., 2025). Recent studies show that non-tuberculous mycobacteria are becoming increasingly important in veterinary and medical-social terms due to the growth of mycobacteriosis in humans and the sensitization of animals, which complicates the differential diagnosis of tuberculosis (Lipner et al., 2017). There is evidence of an increased risk of mycobacterial transmission between humans and animals in shared environments, as well as difficulties in laboratory differentiation between non-tuberculous mycobacteria and *M. bovis* (Luciano & Roess, 2020). The key role of the environment (soil, water, biofilms) in the circulation of non-tuberculous mycobacteria and their persistence in animals and humans has been established (Pavlík et al., 2022; Honda, 2023). The presence of antibiotic-resistant mycobacteria strains in both animals and humans suggests a shared selection process (Zmak et al., 2025). A single ecological chain “animal–human–environment” has been confirmed, especially for *M. avium* complex (Kaczmarkowska et al., 2022; Zulu et al., 2023).

It is emphasized that effective control of the spread of non-tuberculous mycobacteria is possible only within the framework of the “One Health” concept (Marianelli et al., 2024).

Thus, animals can serve not only as a reservoir but also as an early indicator of epidemiological changes. Due to the lack of systematic data on the species composition of atypical mycobacteria in tuberculosis-free farms in Ukraine, it is important to study cultures of non-tuberculous bacteria isolated from animals that react to tuberculin in different natural and climatic zones of the country.

Materials and methods

The study used 307 cultures of atypical mycobacteria, 180 of which were previously obtained from state laboratories of the State Service of Ukraine for Food Safety and Consumer Protection, and 127 were isolated by employees of the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine” on livestock farms in 17 regions located in different natural and geographical zones of the country. Field isolates were compared with reference strains stored in the microorganism collection of the Tuberculosis Research Laboratory of the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine” (Fig. 1).

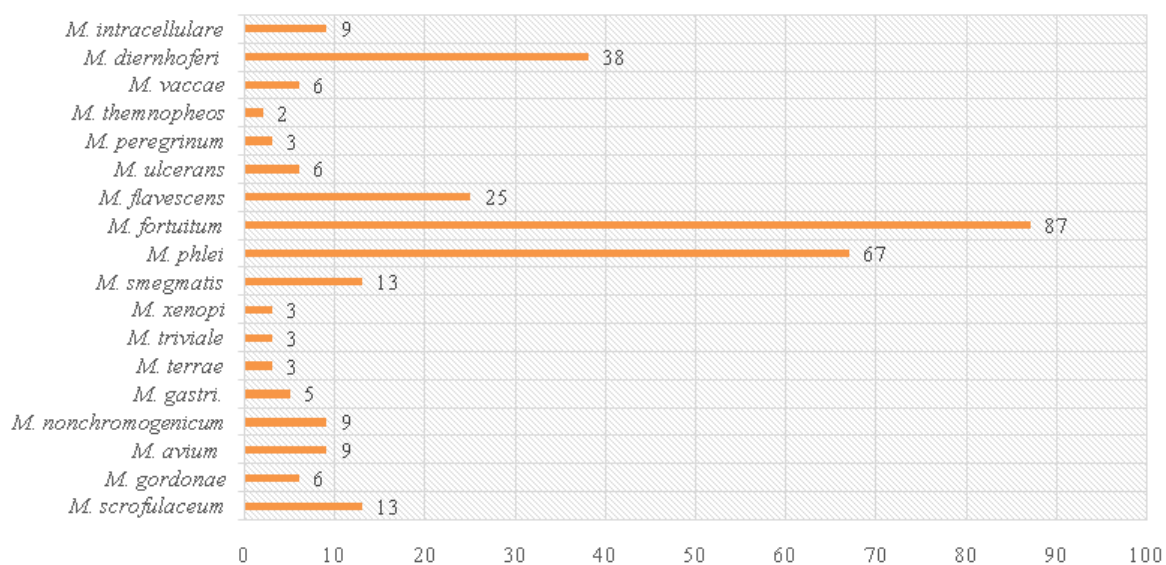


Fig. 1. Species composition of atypical mycobacteria isolated in Ukraine and their quantitative indicators

Mycobacterial cultures were examined using bacteriological tests, which included nine cultural-morphological and biochemical assays (Leisching et al., 2016). Mycobacteria were cultivated using dry egg nutrient medium and equipment in accordance with current regulatory standards. In cultural studies, the dynamics of primary colony growth, including pigmentation, morphological features, and the presence of aerial mycelium, were evaluated. The ability of isolates to grow on nutrient media at temperatures of 25, 37, and 45 °C and in a medium with 5.0% sodium chloride was also determined. In addition, the isolated cultures were studied for amidase and catalase activity, the ability to hydrolyze Tween-80, and to restore potassium tellurite. To conduct the tests, a suspension was prepared from the bacterial mass at a ratio of 1.0 mg/cm³ of sterile 0.85% sodium chloride solution, after which 0.5 cm³ of each culture was inoculated onto the nutrient media.

The species affiliation of the isolated mycobacterial cultures was determined by studying their tinctorial, cultural-morphological, and biochemical characteristics in comparison with reference strains of mycobacteria of the species *M. scrofulaceum*, *M. gordonae*, *M. flavescens*, *M. thermopheos*, *M. ulcerans*, *M. peregrinum*, *M. parafinicum*, *M. diernhoferi*, *M. gastri*, *M. smegmatis*, and to the complexes: *M. avium* – intracellulare; *M. nonchromogenicum* – *M. terrae* – *M. triviale*; *M. fortuitum* – *M. chelonae*.

Bacterioscopy was performed by microscopy of smears from isolated mycobacterial cultures stained using the Ziehl-Neelsen method (Genc et al., 2026). Based on the results of tinctorial, cultural-mor-

phological, and biochemical properties, the group and species affiliation of the isolated mycobacterial cultures was determined (Runyon, 1959).

Results

A retrospective analysis of reported data on tuberculosis diagnoses in cattle from 2015 to 2025, conducted on Ukrainian farms, showed that of the 12,908,269 animals tested using the allergic method, 13,373 had a positive reaction to intradermal tuberculin administration. During diagnostic slaughter in 2015 and 2019, respectively, 22 and one carcasses with pathological lesions characteristic of tuberculosis were identified in animals from the same farm.

At the same time, according to the results of bacteriological studies conducted from 2015 to 2025 on biological material from 8,062 animals, five cultures of *M. bovis* were isolated from one tuberculosis-affected farm, one culture of *M. avium* was isolated from another unaffected farm, and 233 cultures of atypical mycobacteria were isolated from additional unaffected farms. These results indicate the need for enhanced control of the epizootic situation and clarification of the nature of allergic reactions to mycobacterial allergens, especially in herds with an uncertain epizootic status concerning tuberculosis.

The obtained data indicate that mycobacterial cultures isolated from animals and the environment differ from each other in terms of their morphological, cultural, and biochemical properties. Thus, preparations stained using the Ziehl-Neelsen method appeared as straight, short, long, granular, and non-granular bright red rods that

were either straight or slightly curved, and located singly or in groups in the field of view (Fig. 2).

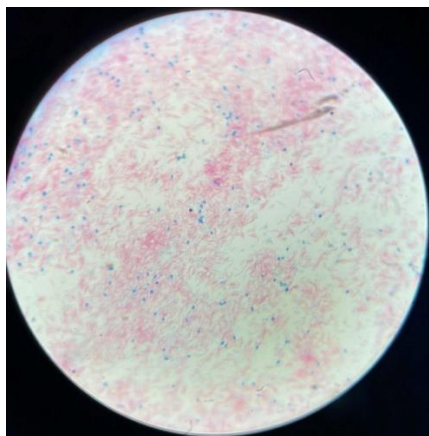


Fig. 2. Acid-fast rods of atypical mycobacteria

When grown on Pavlovsky's nutrient medium, the mycobacterial cultures exhibited uneven growth patterns. Some grew as separate colonies of various sizes, moist, smooth, shiny, matte, light gray, light yellow, orange, or with a crater-like depression in the center. Others grew as continuous mucous or dry coatings with smooth, shiny, and sometimes wrinkled surfaces. These cultures had a viscous consistency and were well suspended in a physiological solution. They grew within three to five days of the first generation after cultivation at temperatures of 25, 37, and 45 °C. These cultures were classified as fast-growing (Fig. 3).

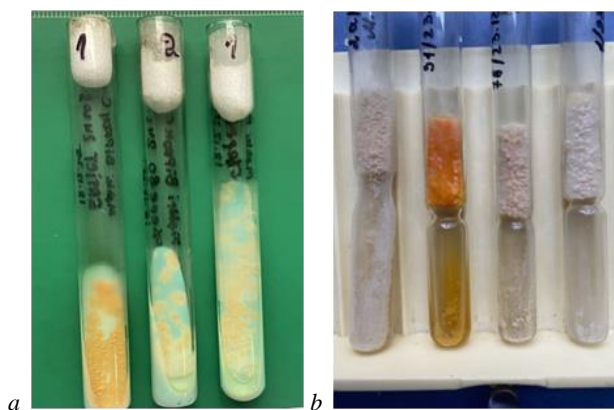


Fig. 3. Growth of atypical mycobacterial colonies: *a* – on egg medium, *b* – on Pavlovsky's potato medium

In a study of photochromogenicity in slow- and fast-growing mycobacterial cultures, it was found that some isolates formed yellow and orange pigments when grown on egg media in the dark or under light. This was the basis for classifying these cultures as scotochromogenic. According to Runyon's classification, slow-growing cultures that did not form pigment in the dark or light were classified as non-chromogenic atypical mycobacteria of the third group.

Twelve of the thirteen scotochromogenic subcultures grew on an egg medium for cultivating mycobacteria on day 12 at temperatures of 25 and 37 °C. These cultures formed an orange pigment in the dark and in the light, exhibited well-pronounced catalase activity, and reacted positively with urea, nicotinamide, and pyrazinamide. However, these cultures did not grow at a cultivation temperature of 45 °C on a medium with 5.0% NaCl. They also had a negative reaction with Tween-80 and potassium tellurite. This was the basis for classifying these cultures as *M. scrofulaceum*.

The other six slow-growing mycobacterial subcultures in the second generation grew well at 25 and 37 °C on day 10. They formed a light yellow pigment when cultured in light or darkness, hydrolyzed Tween-80, and had a positive urea reaction. They also exhibited high catalase activity and a slight reaction with potassium tellurite. These

cultures did not grow on a medium with 5.0% NaCl at 45 °C and had negative reactions with nicotinamide and pyrazinamide. These properties were also present in the reference culture, *M. gordonae*, allowing them to be classified as this species.

Nine non-photochromogenic mycobacterial isolates that grew poorly on a dense egg medium at 25 °C but well at 37 and 45 °C on day 15 did not form pigment. They had positive reactions with potassium tellurite, nicotinamide, and pyrazinamide. These isolates did not grow on a medium with 5.0% NaCl. They had a negative reaction with urea and showed no catalase activity. They also did not hydrolyze Tween-80. These identical properties were also found in the reference strain *M. avium*, which was the basis for classifying the cultures as this species.

Nine other non-photochromogenic subcultures in the first generation grew poorly at 25 °C and grew well at 37 °C in the form of light gray colonies with an uneven surface within 12 to 16 days, and no growth was observed at 45 °C. They hydrolyzed Tween-80, had positive catalase, nicotinamidase, and pyrazinamidase activity, did not grow on a medium with 5.0% NaCl, and had a negative reaction with potassium tellurite and urea. The same properties were determined in the reference strain *M. nonchromogenicum*, which allowed us to classify the cultures we studied as belonging to this species of atypical mycobacteria.

Five slow-growing, non-chromogenic, second-generation cultures grew in an S-shape on a dense egg medium on the 12th day of cultivation at temperatures of 25 and 37 °C. These cultures hydrolyzed Tween-80 and reacted positively with urea and nicotinamide. They did not grow on a medium with 5.0% NaCl and exhibited negative catalase and pyrazinamidase activity at a temperature of 45 °C. Additionally, they reacted with potassium tellurite. The reference strain *M. gastri* exhibited these same properties, enabling the mycobacterial cultures to be classified as *M. gastri*.

Three cultures of mycobacteria in the second generation were isolated from animals and soil samples. On day 13, they grew in an S-shape at temperatures of 25 and 37 °C. The cultures formed light gray colonies with smooth, uneven surfaces of oily consistency. They exhibited positive catalase, pyrazinamidase, and nicotinamidase activity and hydrolyzed Tween-80. These cultures did not grow in a medium containing 5.0% NaCl at a cultivation temperature of 45 °C, nor did they react with potassium tellurite or urea. The reference strain *M. terrae* exhibited identical properties, which formed the basis for classifying the field isolates as *M. terrae*.

Three mycobacterial cultures, which were isolated from bovine biomaterial during the second passage, grew well in an S-shape on day 14 at temperatures of 25 and 37 °C. The cultures grew in the form of light gray, oily colonies on a medium with 5.0% NaCl. They were catalase-positive and hydrolyzed Tween-80. The cultures reacted with potassium tellurite and did not grow at 45 °C. They had negative reactions with nicotinamide, pyrazinamide, and urea. These characteristics allowed the cultures to be classified as *M. triviale*.

Three field cultures in the second generation grew in an S-shape on day 16 of cultivation, forming light yellow colonies with an oily consistency at temperatures of 25, 37, and 45 °C. These cultures did not grow on a medium with 5.0% NaCl and had negative reactions with potassium tellurite (though some strains had positive reactions). They exhibited catalase activity and reacted positively with nicotinamide and pyrazinamide but negatively with urea and Tween-80. These characteristics allowed the cultures to be classified as *M. xenopi*.

The thirteen isolated pigmentless cultures in the second generation grew well within three to four days on a nutrient medium at temperatures of 25, 37, and 45 °C, as well as on a medium containing 5.0% NaCl. The cultures exhibited positive reactions to urea, nicotinamide, pyrazinamide, and potassium tellurite, as well as catalase activity and Tween-80 hydrolysis. These properties were also observed in the reference strain *M. smegmatis*, enabling classification of the mycobacterial cultures as *M. smegmatis*.

Sixty-seven fast-growing isolates grew well on a medium at 25, 37, and 45 °C with 5.0% NaCl within 3 to 6 days, forming yellow pigment in both light and dark conditions. These cultures showed positive reactions with urea, nicotinamide, and pyrazinamide; hydroly-

zed Tween-80; and had a positive reaction with potassium tellurite and catalase activity. Based on these results, the mycobacterial cultures were classified as *M. phlei*.

According to Runyon, pigmentless, fast-growing cultures (87) of the fourth group grew well on a nutrient medium with 5.0% NaCl as well as at temperatures of 25 and 37 °C for 5 days. They did not grow at 45 °C. These cultures had a positive reaction with urea and potassium tellurite, as well as pronounced catalase activity. Some cultures hydrolyzed Tween 80; five others had a negative reaction and reacted positively with nicotinamide and pyrazinamide. Some did not react with these amides. This property instability was also noted in the reference strain *M. fortuitum*. Based on these findings, the isolated mycobacterial cultures were classified as *M. fortuitum*.

Among the studied isolates, 25 fast-growing cultures, as well as the reference strain *M. flavescens*, were characterized by moderate growth at 25 and 37 °C and in a medium with 5.0% NaCl and no growth at 45 °C, formation of a light yellow pigment regardless of lighting, positive reactions with urea, nicotinamide, and pyrazinamide, ability to hydrolyze Tween-80, and a negative reaction with potassium tellurite (some strains had a positive reaction). On this basis, they were classified as *M. flavescens*.

Six non-pigmented fast-growing mycobacterial cultures grew poorly on egg medium at a cultivation temperature of 25 °C, had slightly expressed catalase activity, a positive reaction with nicotinamide, pyrazinamide, and urea, did not grow at 37 and 45 °C on a medium with 5.0% NaCl, hydrolyzed Tween-80, and had a negative reaction with potassium tellurite. Identical properties were also found in the reference strain *M. ulcerans*. Considering the above, the six cultures studied were classified as *M. ulcerans*.

Three fast-growing mycobacterial cultures isolated from soil samples in the second generation grew in 3 days after inoculation and formed smooth, yellow colonies of oily consistency at 25 and 37 °C, did not grow at 45 °C, had a positive catalase reaction, Tween-80, negative nicotinamidase and pyrazinamidase activity, grew on a medium with 5.0% NaCl, and had a negative reaction with potassium tellurite (one of these cultures had a positive reaction), which was the basis for classifying these cultures as *M. peregrinum*.

Two fast-growing, pigmentless mycobacterial cultures were grown in the third passage after three days at cultivation temperatures of 25 and 37 °C on a medium containing 5.0% NaCl, hydrolyzed Tween-80, and exhibited positive amidase activity with nicotinamide, pyra-

zinamide, and urea. These cultures did not grow at 45 °C and exhibited negative catalase activity and a reaction with potassium tellurite. Based on these characteristics, the cultures were classified as *M. thermophiles*.

The reference strain, *M. vaccae*, and six other studied mycobacterial cultures formed a light yellow pigment when grown in the dark or light at 25 or 37 °C with 5.0% NaCl. No growth was observed under cultivation conditions at 45 °C. Biochemical property studies revealed that these cultures exhibited positive reactions with catalase, urea, nicotinamide, pyrazinamide, and potassium tellurite and hydrolyzed Tween-80. These cultures were found to belong to the same species as the reference strain.

Thirty-eight fast-growing mycobacterial cultures, as well as the reference strain *M. diernhoferi*, grew in an S-shape on a nutrient medium for four days. The yellow pigment formed in darkness and light at temperatures of 25 and 37 °C. The cultures did not grow at 45 °C or when 5.0% NaCl was added. They did not hydrolyze Tween-80 and exhibited positive nicotinamidase and pyrazinamidase activity. The cultures also reacted with urea and potassium tellurite. These characteristics allowed us to classify these mycobacterial cultures as *M. diernhoferi*.

Nine non-photochromogenic mycobacterial cultures grew in an S-shape within 16 days in the second generation at temperatures of 25, 37, and 45 °C. These cultures did not grow on a medium with 5.0% NaCl and had positive reactions with potassium tellurite, pyrazinamide, and nicotinamide. They also had negative properties in tests with catalase, Tween-80 hydrolysis, and urea. These cultures were classified as *M. intracellulare*. The reference strain of *M. intracellulare* exhibited identical properties. The results of the study on the species affiliation of mycobacterial cultures isolated from cattle and the environment in which the animals were kept, as well as grazing poultry, are presented in Table 1. The data in the table indicate that the isolated mycobacterial cultures varied in their morphological, cultural, and biochemical properties. It should be noted that mycobacterial cultures such as *M. avium* and *M. intracellulare* have identical characteristics in some tests. Bacteriological studies have shown that the isolated mycobacterial cultures belong to 18 species, which are classified as the second, third, and fourth groups according to Runyon's system. These species include the causative agent of avian tuberculosis (*M. avium*).

Table 1
Species composition of atypical mycobacteria isolated in different regions of Ukraine

Test	The group, according to Runyon																	
	II			III									IV					
	Mycobacteria species																	
	<i>M. scrofulaceum</i>	<i>M. goodii</i>	<i>M. avium</i>	<i>M. intracellulare</i>	<i>M. nonchromogenicum</i>	<i>M. gastri</i>	<i>M. terrae</i>	<i>M. triviale</i>	<i>M. xenopi</i>	<i>M. smegmatis</i>	<i>M. phlei</i>	<i>M. fortuitum</i>	<i>M. vaccae</i>	<i>M. flavescens</i>	<i>M. thermophiles</i>	<i>M. ulcerans</i>	<i>M. peregrinum</i>	<i>M. diernhoferi</i>
Bacterioscopy using the Ziehl-Neelsen method	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth rate	12	10	15	16	16	12	13	14	16	4	3	5	3	5	3	5	3	4
Pigment formation	S	S	N	N	N	N	N	N	N	F	S	F	S	S	F	F	S	S
Colony growth at temperatures:																		
25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
45°C	-	-	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-
Resistance to 5.0% NaCl	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-	+	-
Catalase activity	+	+	-	-	+	-	+	+	-	+	+	+	+	+	-	+	+	-
Potassium tellurite reduction	-	+/-	+	+	-	-	+	+	+/-	+	+	+	+	+/-	-	-	+/-	+
Tween-80 hydrolysis	-	+	-	-	+	+	+	+	+	+	+/-	+	+	+	+	-	+	-
Reaction with:																		
urea	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+
Nicotinamide	+	-	+	+	+	+	+	-	+	+	+	+/-	+	+	+	+	-	+
Pyrazinamide	+	-	+	+	+	-	+	-	+	+	+	+/-	+	+	+	+	-	+

Notes: S – scotochromogenic; N – non-chromogenic; F – fast-growing; “+” – positive reaction; “-” – negative reaction; “+/-” – some strains have a positive reaction, some have a negative reaction.

Photochromogenic mycobacterial cultures (Runyon group I) were not detected in any of Ukraine's regions, indicating their limited or absent distribution in the country.

Among the cultures studied, the most common species of mycobacteria in Ukraine were representatives of the second and third groups according to Runyon, in particular: *M. scrofulaceum*, *M. gordonae* (group II), *M. avium*, *M. intracellulare*, *M. triviale* (group III), as well as fast-growing representatives of group IV – *M. smegmatis*, *M. phlei*, *M. fortuitum*, *M. vaccae*, *M. diernhoferi*.

It has also been established that on the farms of the Kyiv, Poltava, Sumy, and Cherkasy (Forest-Steppe) regions, the following mycobacteria species are widespread in cattle herds: *M. scrofulaceum*, *M. gordonae*, *M. phlei*, *M. fortuitum*, *M. vaccae*, *M. diernhoferi*, *M. flavescens*, and, less frequently, *M. terrae*, *M. intracellulare*, *M. gastri*, *M. triviale*, *M. xenopi*, *M. smegmatis*, *M. peregrinum*, *M. themnopheos*, *M. nonchromogenicum*, *M. ulcerans* (Fig. 4).

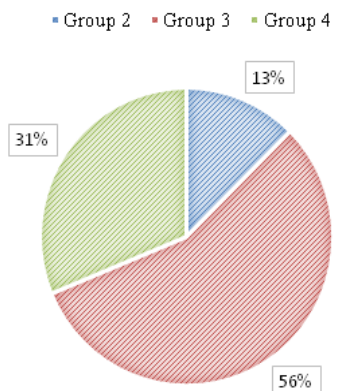


Fig. 4. Percentage ratio of different types of mycobacterial cultures according to Runyon's groups in the Forest-Steppe zone

On farms in Donetsk, Dnipropetrovsk, Zaporizhzhia, Kirovohrad, Mykolaiv, and Kherson regions (Steppe), *M. terrae*, *M. intracellulare*, *M. gordonae*, *M. smegmatis*, *M. phlei*, *M. fortuitum*, *M. vaccae*, *M. diernhoferi*, *M. flavescens*, *M. themnopheos*, *M. avium*, *M. triviale*, *M. peregrinum*, and isolated *M. nonchromogenicum*, *M. gastri*, *M. xenopi*, *M. ulcerans*, and *M. scrofulaceum* were isolated from cattle (Fig. 5).

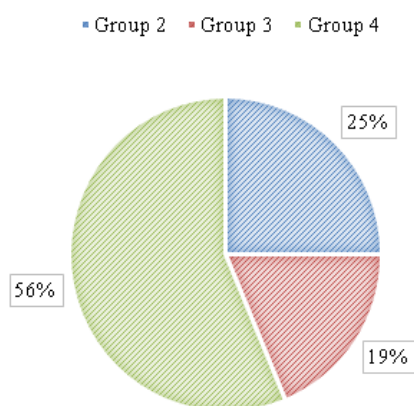


Fig. 5. Percentage ratio of different species of mycobacterial cultures according to Runyon's groups in the Steppe zone

Sensitization to tuberculin in cattle farms in Zakarpattia, Zhytomyr, Khmelnytskyi, and Rivne regions (Polissya) was caused by atypical mycobacteria of the species *M. smegmatis*, *M. phlei*, *M. fortuitum*, *M. vaccae*, *M. themnopheos*, *M. flavescens*, and occasionally *M. scrofulaceum*, *M. gordonae*, *M. intracellulare*, *M. diernhoferi*, *M. peregrinum*, and *M. ulcerans* (Fig. 6).

Analysis of the research results revealed that mycobacteria from groups II–IV are the most prevalent in Ukraine. The Steppe zone has numerous species, including rare fast-growing cultures. The Forest-Steppe zone has less diversity, dominated by *M. gordonae* and *M. scrofulaceum*. In Polissya, fast-growing species of group IV were mainly found, while representatives of groups II–III were found spo-

radically. These findings suggest a regional specificity in the distribution of atypical mycobacteria in Ukraine.

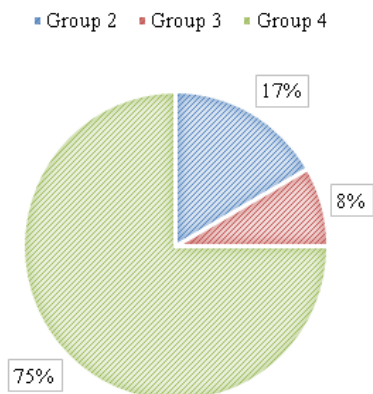


Fig. 6. Percentage ratio of different species of mycobacteria according to Runyon's groups in the Polissya region

The results indicate a widespread presence of atypical mycobacteria among cattle that react to tuberculin, even on farms officially declared tuberculosis-free in all geographical regions of Ukraine. This may be due to the animals' sensitization to non-tuberculous mycobacteria, which are widespread in the environment, including in soil, water, and feed. Animals can also encounter sources of infection in their environment.

Discussion

According to annual reports from 2012 to 2022 by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), the number of infected cattle herds in the European Union (EU) has gradually decreased by 47%. The overall prevalence of tuberculosis pathogens has decreased by 4–5%. However, the level of epizootic tension varies significantly between EU countries (Allen et al., 2018; Bezos et al., 2023; Giusti et al., 2024).

According to data from the Visavet Health Surveillance Center, a center for animal health and biosafety at the Complutense University of Madrid, the number of herds infected with tuberculosis in the EU increased from 0.54% (9,384 herds) in 2021 to 0.61% (9,845 herds) in 2022. In eradication zones, the incidence rate increased from 1.3% to 1.5%. In free zones, the infection was sporadically recorded in 149 herds (0.015%). The highest tuberculosis prevalence is observed in countries without disease-free status: Ireland (4.6%), Spain (2.5%), and Northern Ireland (11.3%). Other EU member states, including Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Poland, Slovakia, and Sweden, reported only isolated cases of tuberculosis infection among cattle.

According to data from the European Food Safety Authority (EFSA) and the European Center for Disease Prevention and Control (ECDC), 111 cases of human tuberculosis caused by *M. bovis* or *M. caprae* were reported in the EU in 2021 (32 of them in Spain), equaling 0.03 cases per 100,000 people. Despite the decline in human incidence, the pathogen continues to circulate significantly among animals in some European regions. *M. bovis* has been detected in Bulgaria, Ireland, Italy, Romania, and Northern Ireland; *M. caprae* has been detected in Austria, Germany, and Romania. However, no cases of *M. tuberculosis* infection in cattle have been reported. Sporadic cases have also been reported in France, Germany, and Poland. Bovine tuberculosis caused by *M. bovis* remains one of the most serious problems in livestock farming in the United Kingdom. According to the Royal Veterinary College in London, approximately 40,000 cattle test positive for tuberculosis each year and are slaughtered, causing significant economic losses to the livestock industry. The UK government is implementing a strategy to eradicate bovine tuberculosis in England by 2038. This strategy requires an in-depth understanding of risk factors at the individual and herd levels.

Numerous studies (Schmidt et al., 2022; Neto, 2024) confirm that mycobacteriosis continues to cause significant economic losses to livestock farms and poses a potential threat to human and animal

health. At the same time, treating and controlling mycobacteriosis requires significant health sector resources, creating an additional financial burden on public health systems. Non-tuberculous mycobacteria are particularly complex because they are present in human and animal biotopes, as well as abiotic objects such as soil, water, feed, and aerosols (Paliy et al., 2024; Shevchuk et al., 2024). Their ability to survive in the external environment and adapt to various conditions highlights the need for effective monitoring and control methods.

According to WHO data, as of January 1, 2024, there were approximately 10.8 million registered cases of tuberculosis worldwide, equating to 134 cases per 100,000 people. Despite the war, data from the Public Health Center of the Ministry of Health of Ukraine show an increase in morbidity in the central regions, particularly in the Dnipropetrovsk (86.1 per 100,000) and Kirovohrad (92.1 per 100,000) regions. In contrast, tuberculosis rates have declined in the eastern regions, likely due to population migration and weakened epidemiological surveillance. In 2022, there were 887 cases of tuberculosis reported among Ukrainian migrants in European countries, which is 4.3 times more than in 2021. Of these cases, 26% were caused by drug-resistant forms of the pathogen. The overall incidence of tuberculosis in Ukraine decreased by 8.7% in 2024 compared to 2023. However, an increase in indicators was observed in several regions, including Kherson, Ternopil, Kyiv, Khmelnytskyi, and Poltava (Public Health Center of the Ministry of Health of Ukraine, 2025). The proportion of bacteriologically confirmed pulmonary cases increased from 72.5% in 2020 to 76.1% in 2024. Meanwhile, the confirmation rate for extrapulmonary cases increased to 30%, reflecting an improvement in the effectiveness of laboratory diagnostic methods.

The data obtained in our study are consistent with current understanding of the circulation and persistence of mycobacteria in natural and anthropogenic ecosystems. A summary of global data shows the growing epidemiological significance of non-tuberculous mycobacteria. These bacteria are widely distributed in the environment and can cause various clinical forms of infection in people with chronic lung diseases or immune disorders. They can also cause para-allergic reactions in mammals and diseases in birds. The authors emphasize that the morphological similarity of non-tuberculous mycobacteria to *M. tuberculosis*, coupled with significant species diversity, complicates traditional diagnostics and necessitates the use of molecular methods for accurate identification (Maleki & Moaddab, 2025).

International trends also confirm the expected regional differences in the species composition and clinical significance of non-tuberculous mycobacteria. The study notes that different geographical areas are characterized by the dominance of different subspecies: *M. avium* complex is more common in Western Europe and North America, while *M. abscessus* is more common in Asia. The spread of non-tuberculous bacteria is largely due to improved diagnostic methods, as well as the aging and immunodeficiency of human and animal populations, leading to an increase in the number of reported cases (Prevots et al., 2023).

The increased frequency of atypical mycobacteria isolation observed in our study reflects the growing global prevalence of non-tuberculous bacteria (Mohanty et al., 2024). This underscores the importance of accurately interpreting the results of bacteriological and allergy tests, as the clinical significance of different isolated strains varies. However, their presence can affect the accuracy of tuberculosis diagnoses, particularly by creating a risk of false-positive reactions to tuberculin.

Our study of the biological characteristics of atypical mycobacteria allowed us to evaluate parameters such as growth on dense media, pigmentation, colony formation rate, and tolerance to various cultivation conditions. According to international data (Conyers & Saunders, 2024), the high level of natural resistance of nontuberculous mycobacteria to many anti-tuberculosis drugs underscores the importance of these characteristics for practical epizootiology and clinical applications. These characteristics enable us to predict the potential role of different strains in the persistence of infections and their impact on diagnostic test results.

The uneven distribution of mycobacterial species in Ukraine is associated with varying natural and climatic conditions conducive to

their persistence. However, climate change can significantly impact the ecology of microorganisms and alter their habitat (Bogach et al., 2020; Naga et al., 2025).

The results of our study demonstrate a significant proportion of non-tuberculous mycobacteria among isolates from animals suspected of having tuberculosis. This finding is consistent with international meta-analyses, which report a frequency of such cases reaching 3–4% (Daka et al., 2025). These results emphasize the necessity of accurately interpreting diagnostic data and distinguishing between tuberculosis cases and those caused by atypical mycobacteria persistence. This distinction is crucial for the effective planning and implementation of anti-epizootic measures. Given the plasticity and resistance of mycobacterial properties, only proven, highly effective antimicrobial agents should be used (Paliy, 2018; Tyski et al., 2024).

Because of the close relationship between animal and human tuberculosis, especially in the context of “One Health”, a thorough analysis of tuberculosis diagnostic data in cattle is essential for veterinary, medical, and social reasons. This allows timely detection of potential epizootic foci, assessment of the effectiveness of preventive measures, and identification of areas for improvement in state tuberculosis diagnosis and control programs.

In summary, it has been established that atypical mycobacteria isolated in different regions of Ukraine exhibit significant species diversity and variations in properties. These differences are due to natural environmental conditions and the level of anthropogenic pressure. The obtained data are consistent with global trends of the increasing prevalence of non-tuberculous mycobacteria and their ability to persist in animals, humans, and the external environment for long periods. The growth characteristics and cultivation properties of these mycobacteria underscore the importance of considering their impact on the results of allergic and bacteriological tuberculosis tests. They also stress the importance of molecular methods for precise identification. This is essential for properly diagnosing tuberculosis, assessing epizootic risks, and creating effective anti-epizootic strategies in animal husbandry and veterinary practice.

Conclusion

Analysis of the results revealed that mycobacterial groups II–IV are the most prevalent in Ukraine. In the Steppe zone, which includes Donetsk, Dnipropetrovsk, Zaporizhzhia, Kirovohrad, Mykolaiv, and Kherson regions, 67 fast-growing cultures (group IV) were isolated. These included *M. smegmatis* (12 cultures), *M. phlei* (10 cultures), *M. fortuitum* (nine cultures), *M. vaccae* (eight cultures), and *M. diernhoferi* (seven cultures), as well as three cultures of *M. terrae*, three cultures of *M. intracellulare*, and one culture of *M. avium*.

In the Forest-Steppe zone, which includes the Kyiv, Poltava, Sumy, and Cherkasy regions, *M. gordonae* and *M. scrofulaceum* were predominant, with 25 and 23 cultures, respectively. *M. phlei*, *M. fortuitum*, *M. vaccae*, *M. diernhoferi*, and other species were present in lower numbers, with two to 12 cultures per species.

Among 78 isolated cultures in Polissya (Zakarpattia, Zhytomyr, Khmelnytskyi, and Rivne regions), *M. smegmatis* (15 cultures), *M. phlei* (12 cultures), *M. fortuitum* (10 cultures), *M. vaccae* (eight cultures), *M. thermophloeos* (six cultures), and *M. flavescens* (five cultures) were found. *M. scrofulaceum*, *M. gordonae*, *M. intracellulare*, and *M. diernhoferi* were found in one to three cultures.

No photochromogenic mycobacteria of group I were found in any regional zone of Ukraine.

Atypical mycobacteria are widespread among cattle in Ukraine. Of the 307 cultures studied, 18 species were represented by groups II–IV according to Runyon's classification, as well as *M. avium*. The high species diversity and regional specificity, especially the presence of fast-growing species, create conditions that sensitize animals to tuberculin and result in false-positive reactions. This complicates the differential diagnosis of tuberculosis.

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