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Original research

The ability of beetles to accumulate and transfer dissociative forms of *Mycobacterium bovis*

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Abstract. The issue of tuberculosis has persisted for many years due to the active spread of the infectious agent. In Ukraine, this problem has become more acute amidst hostilities, leading to increased migration of animals, both natural and artificial. This situation poses a potential threat, exacerbating the epizootic situation and activating the spread of zoonoses. Despite this, comprehensive data on all possible sources of the pathogen and ways of introducing it to previously safe areas are still lacking. Bacteriological (microscopy, isolation of mycobacterial cultures) and analytical methods were used to identify the insects as a mechanical vector of *M. bovis*. To achieve this goal, the dissociative strain of *M. bovis* 117-a passage and L-forms of mycobacteria were used. Wheat grain was contaminated with a suspension of mycobacteria at a concentration of 10 mg of mycobacterial mass in 1cm^3 of isotonic 0.9% sodium chloride solution for every 10 g of substrate. Insects were introduced into the contaminated grain, and on the 4th and 8th days, beetles were randomly selected and transferred to test tubes containing Mordovsky nutrient medium (pH 6.5) The tubes were then cultivated at a temperature of 3.0 ± 0.5°C. The study revealed the presence of experimental strains of mycobacteria in various proportions among different species of beetles. Specifically, mycobacteria were found in 100% of specimens of *Oryzaephilus surinamensis*, 87.5% of *Calathus melanocephalus*, 100% of *Armadillidium vulgare*, 62.5% of *Rhyparochromus vulgaris*, 75% of *Forficula auricularia*, 87.5% of *Sitophilus oryzae*, and 87.5% of *Tenebrio molitor*. This suggests that insects of different species and at different stages of development are capable of mechanically transferring mycobacteria. Additionally, the morphological similarity of microbial cells after passage through beetles with the original strain indicates that the organisms of these insects may provide a susceptible environment for mycobacteria.

Keywords: insects; microscopy; Mycobacterium; infection spreading.

Здатність жуків до накопичення та перенесення дисоціативних форм *Mycobacterium bovis*

Анотація. Проблема туберкульозу актуальна протягом багатьох років через активне поширення збудника інфекції. Нині вона загострилася в Україні в зв'язку з бойовими діями, які спричинюють посилену міграцію тварин, як природню, так і штучну. Така ситуація становить потенційну загрозу, загострюючи епізоотичну ситуацію та активізуючи поширення зоонозів. Незважаючи на це, досі відсутні вичерпні дані про всі можливі джерела збудника та шляхи його занесення до безпечних раніше територій. Для ідентифікації комах, як механічних переносників *M. bovis* використовували бактеріологічні (мікроскопія, виділення культур мікобактерій) та аналітичні методи. Для досягнення поставленої мети використовували дисоціативний штам пасажу *M. bovis* 117-а та L-форми мікобактерій. Зерно пшениці контамінували суспензією мікобактерій у концентрації 10 мг маси мікобактерій в 1 см3 ізотонічного 0,9 % розчину натрію хлориду на кожні 10 г субстрату. У заражене зерно вносили комах, а на 4-8 добу випадковим чином відбирали жуків і переносили в пробірки з живильним середовищем Мордовського (pH 6,5). Потім пробірки культивували при температурі 3,0 ± 0,5°C. Дослідження виявило наявність експериментальних штамів мікобактерій у різних пропорціях серед різних видів жуків. Зокрема, мікобактерії були виявлені у 100% зразків *Oryzaephilus surinamensis*, 87,5% *Calathus melanocephalus*, 100% *Armadillidium vulgare*, 62,5% *Rhyparochromus vulgaris*, 75% *Forficula auricularia*, 87,5% *Sitophilus oryzae* та 87,5% зразків *Tenebrio molitor*. Це свідчить про те, що комахи різних видів і на різних стадіях розвитку здатні до механічного перенесення мікобактерій. Крім того, морфологічна подібність мікробних клітин після проходження через жуків із вихідним штамом вказує на те, що організми цих комах є сприйнятливим середовищем для мікобактерій.

Ключові слова: мікобактерії; жуки; мікроскопія; Mycobacterium

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Introduction

Nowadays, the world is not free of tuberculosis infection with outbreaks occurring in different regions. Currently, this problem is exacerbated by the hostilities in Ukraine, as natural and artificial migration of animals increases. This situation creates a potential threat of animal morbidity and contributes to the spread of infectious zoonotic diseases, especially tuberculosis.

The main species that pose a threat are *Mycobacterium bovis* and *Mycobacterium tuberculosis*, which are also susceptible to humans. At present, tuberculosis causes significant economic and social losses (Pérez-Morote et al., 2020). In order to effectively combat this zoonotic disease, the primary task is to identify all possible ways of introducing the pathogen into previously healthy farms, to identify potential sources of infection and to develop effective anti-TB drugs. Currently, special attention is being paid to the study of the antibacterial effect of plant extracts and 1,2,4-triazole-3-thiol derivatives (Palchykov et al., 2019; Zazharskyi et al., 2020; Zazharskyi et al., 2021).

Mycobacteria can adjust to varying environmental conditions due to the ability to change microbial cells, and the peculiarities of the cell wall structure (the presence of lipids in its composition) provide additional protection for the microorganism. The phenomenon of morphological fluctuations and changes complicates the diagnosis and eradication of the disease. Changes in morphological features, biochemical and biological properties ensure the survival of mycobacteria in new habitats.

Due to their diverse properties, mycobacteria have acquired a wide range of potential biological and mechanical vectors. Many scientists have described that mycobacteria have been found in the body of cockroaches, ticks, butterflies and many other invertebrates (Fischer et al., 2004; Wallace et al., 2010 Tkachenko et al. 2021a). Allen (1987), Pavlik, & Falkinham (2009) and Guzman & Vilcinskas (2020) reported that after passing through the intestines of cockroaches, viable mycobacteria can be released with faeces into the external environment. Sarwar (2015) claims that up to 85% of infectious diseases are caused by arthropods, which are able to spread the pathogen through bites and mechanically on the surface of the body. Cano et al. (2018) suggested that insects of the genus *Hemiptera* are potential reservoirs and vectors of *M. ulcerans*, and Marsollier et al. (2002) added that water bugs from the family *Naucoridae*, hosts of *M. tuberculosis complex*, can also spread mycobacteria. Crispell et al. (2019) discovered that macro-organisms can transmit mycobacteria both within the same species and between species. For this study, we selected insect species commonly found in Ukraine. These insects were collected in the forest-steppe zone of Dnipro city, specifically under tree bark and beneath stones, in remote areas distanced from agricultural facilities

The aim of this study was to determine the epizootic significance of insects in the potential spread of tuberculosis, and to investigate their ability to mechanically transfer the pathogen and accumulate mycobacteria inside the body.

Materials and methods

The research was carried out in the training laboratory of the Department of Infectious Diseases of Animals of the Dnipro state agrarian and economic university during 2022-2023.

The subject of the study was the cultures of dissociative form of *Mycobacterium bovis* (*M. bovis*) passage №117-A (microscopy of smears was stained by the Ziehl-Nielsen method - non-acid-resistant (blue) thin sticks) and L-forms of mycobacteria (at microscopy - non-acid-resistant ovals), which were stored at a temperature of 3.0 ± 0.5 ^{0}C in the museum of the department. Insects at different stages of development (larval, adult) were also included in the research. Adult beetles of the several species such as *Suriname mealybug Oryzaephilus surinamensis* (Linnaeus, 1758), rice weevil *Sitophilus oryzae* (Linnaeus, 1763) were used in the study. Beetles of these species were selected from grain feed mixtures. The moss bugs *Calathus melanocephalus* (Linnaeus, 1758), common armadillo woodlice *Armadillidium vulgare* (Latreille, 1804), ground bugs *Rhyparochromus vulgaris* (Schilling, 1829), common earwig *Forficula auricularia* (Linnaeus, 1758) were collected in the forest-steppe zone of Dnipro city under the bark of trees and under stones (Fig. 1). It is important to note that there were no pastures in the collection sites, ensuring no contact with livestock, which could potentially be a reservoir of mycobacteria. Additionally, the larvae of the grain storage beetle, the large flour beetle *Tenebrio molitor* (Linnaeus, 1758), were obtained from affected wheat stocks that had no contact with livestock.

Prior to the experiment, the experimental strains were inoculated into 10 tubes with fresh Mordovsky nutrient medium pH=6.5 (pH was determined by a laboratory PH/mV&Temperature meter ADWA AD1030) to accumulate the required amount of mycobacterial mass. The growth of the 117-A passage culture in test tubes was observed at 4.90 ± 0.74 days, and the L-form at 5.0 ± 0.68 days.

The next step was the analysis of the experimental strains of mycobacteria by electron microscopy method. The study of *M. bovis* by scanning electron microscopy was performed on a REM-106-i electron microscope. Sampling for electron microscopy was performed by preparing a suspension of microbial cells in distilled water. To do this, one bacteriological loop of the bacterial mass was dissolved in 2 ml of distilled water previously poured into centrifuge tubes. To compact the microbial cells, the tubes were centrifuged for 15 minutes at 3000 rpm. Finally, the excess supernatant was removed with a pipette. The material was fixed with a solution contained 80% glutaraldehyde, which was added to tubes containing 0.1 ml of centrifuged bacterial precipitate of the experimental samples. The tubes were shaken to evenly suspend the precipitate and left at melting ice temperature for 30 min, shaking after 15 min. At the end of the exposure, the samples were centrifuged at 1500 rpm for 15 min, and the supernatant was removed with a pipette, leaving the precipitate. The material was fixed twice.

In order to remove excess water from bacterial cells, the experimental samples were dehydrated by treatment with alcohols of different concentrations (30-100%), which were added to the samples in a ratio of 1:10. As the concentration of the alcohol used to treat the sample increased, the exposure time also increased.

After dehydration of the samples in absolute alcohol and centrifugation, the test samples were placed on top of a carbon tape and dried in air.

To impart electrical conductivity, the experimental samples were sputtered with silver in VUP-5 under a vacuum of about 10-5 mm Hg. The prepared samples were placed into the set of electron microscope and microscopy was performed.

The infection of insects with experimental strains of mycobacteria.

Before the experiment, in order to exclude spontaneous carriage of *M. bovis* in bugs, 25% of the collected samples were analyzed microscopically for the presence of acid-fast bacilli (staining of smears by the Ziehl-Nielsen method). Infection was carried out by contamination with mycobacterial suspensions of the substrate, which is characteristic of eating by a particular species of insects (10 mg of bacterial mass in 1 cm^3 of 0.9% sodium chloride solution for every 10 g of substrate) (Zazharsky & Alifonova, 2022).

To achieve this goal, two series of studies were conducted. In the first series of the experiment, the imago of the all selected beetle species were infected with dissociative forms of mycobacteria (passage 117-A). In the second series, the larval stage of Tenebrio molitor was infected with L-forms of mycobacteria. In both cases, the insects were kept for 8 days on a substrate contaminated with

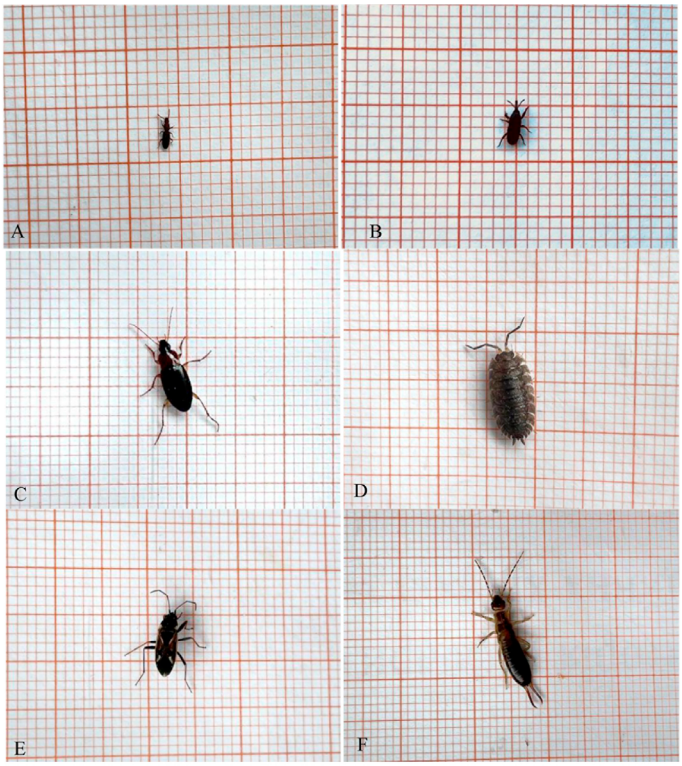

Fig. 1. Studied species of the beetles (A - *Oryzaephilus surinamensis*; B - *Sitophilus oryzae*; C - *Calathus melanocephalus*; D - *Armadillidium vulgare*; E - *Rhyparochromus vulgaris*; F - *Forficula auricularia*).

culture suspension. During this time, on days 4 and 8, four beetles/larvae of each species were randomly selected and examined for microscopic and culture studies to determine the ability to mechanically transfer and reserve the pathogen in their organisms.

Determination of the ability of insects to mechanically transfer mycobacteria

In order to determine the ability of imaginal beetles (I series of the experiment) to mechanically transfer viable mycobacteria on the body surface, on days 4 and 8 of the experiment, experimental insects were selected with sterile tweezers and transferred to a test tube with Mordovsky's nutrient medium with pH = 6.5 (1 beetle per test tube). After that, the tubes were incubated in a thermostat with a temperature of 37.0 ± 0.5 ^{0}C, and the growth was inspected daily, evaluating typical growth characteristics (time of growth emergence, nature of colonies, etc.).

The estimation of the ability to accumulate mycobacteria in the intestinal tract by beetles with different developmental stage

To isolate the samples of the intestinal contents of adult beetles (I series of the experiment) and L-form of mycobacteria from the intestine of larvae (II series), on days 4 and 8 of the experiment, insects were rinsed in 96% ethanol solution and washed in 0.9% sodium chloride solution, then placed on a slide and the intestinal tract was isolated. After that, the intestinal contents were removed and stained using the Ziehl-Nielsen method, and the prepared smears were examined under an immersion microscope system (Olympus XS). When studying beetles of the species *Oryzaephilus surinamensis* and *Sitophilus oryzae*, due to their too small size, removal of the intestine is impossible, so for the study, insects were washed in 96% ethanol solution, then washed five times in 0.9% sodium chloride solution and then rubbed on a slide. The resulting homogenized tissue was stained by the Ziehl-Nielsen method and further examined according to the same scheme with other beetle species.

The insects remaining after the experiment and the contaminated substrate on which they were kept were disinfected by autoclaving at 1.5 atm for 3 hours.

Results

At the first stage of the study, the morphological characteristics, tinctorial and culture properties of the 117-A passage and L-forms of mycobacteria were determined (Fig. 2).

The analysis of obtained subcultures showed that the growth of 117-A passage cultures was recorded at 4.90 ± 0.74 days, and L-forms of mycobacteria at 5.0 ± 0.68 days. Evaluating the culture properties of the experimental strains, it was found that the cultures of passage 117-A were represented by a continuous growth of orange colonies, medium-sized irregularly shaped with a smooth surface and viscous consistency. The L-form cultures looked like individual translucent greenish colonies of irregular shape with a weak growth rate with a smooth surface and mucous consistency.

The next step was to evaluate the morphology and tinctorial properties of the experimental strains in the light microscope. It was discovered that mycobacteria of the 117-A passage were represented by short, thin, straight, non-acid-resistant colonies with rounded ends and grains with unexpressed granularity. Instead, L-forms looked like non-acid-resistant grains, filamentous forms, sticks with rounded ends and pronounced graininess.

During the electron microscopy of the experimental samples, it was found that the cultures of the 117-A passage were represented mainly by short and long straight and slightly curved sticks (Fig. 3).

Studying the L-forms of mycobacteria, we have seen small spike-like formations, similar to buds, at one end of the elongated L-forms, while we observe cells that also have bud-like formations at one end, varying in size, from small to those whose size is close to the mother cell (Fig. 4). During the reproduction of L-forms by electron microscopy, it was found that at the ends of bacteria with a defective cell wall, formations appear that gradually increase in size, remaining connected to the mother cell and, only when they reach the scale of the latter, separate, forming an individual bacterial CWD cell.

Microscopic analysis of the intestinal contents of imago bugs and larvae before experimental infection with mycobacteria did

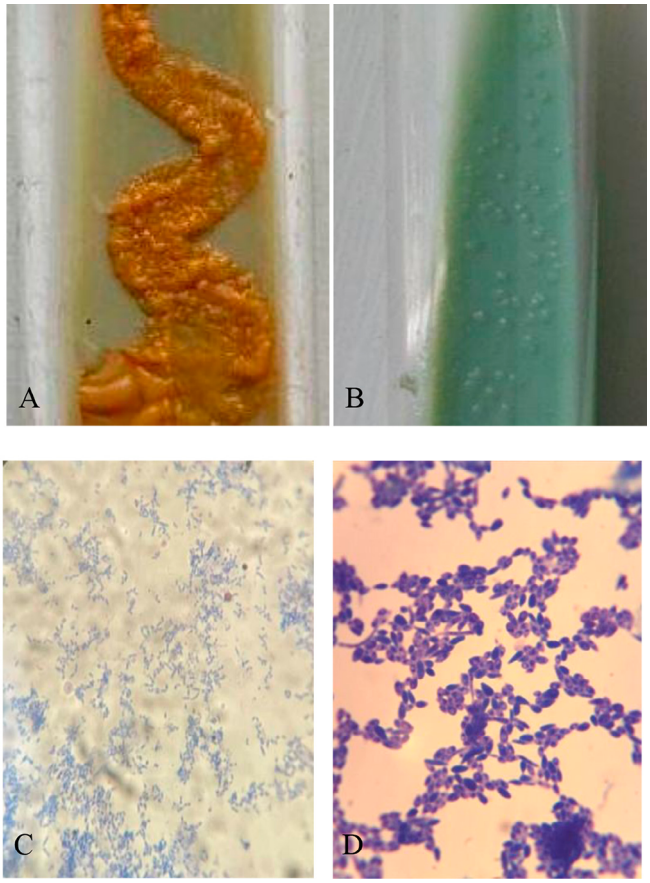

Fig. 2. The properties of mycobacteria cultured into Mordovsky's nutrient medium: A - 117-A passage; B - L-forms. Morphological features: C - 117-A passage; D - L-forms.

not reveal acid-fast microflora in any of the experimental samples, which excludes the natural carriage of *M. bovis* in them.

At the first stage of the study, four and eight days after placing the experimental beetles in the mycobacteria-contaminated substrate, the ability of the insects to carry the viable pathogen on the surface of their bodies was determined. After cultivation of tubes with Mordovsky's nutrient medium, on which the experimental bugs moved freely after infection, at a temperature of 37.0 ± 0.5°C, it was found that in the tubes in which the bugs of the species *Oryzaephilus surinamensis* (group I) moved, the growth of the experimental strain of mycobacteria (which contaminated the substrate they were kept in) was detected in 7 tubes (1 tube was overgrown with foreign microflora (mold), four of which on day 4 and three on day 8; bugs of the species *Calathus melanocephalus* (group II) - 5 tubes (3 were moldy), three on day 4 and two on day 8; *Armadillidium vulgare* (group III) - 8 tubes, four on day 4 and four on day 8; *Rhyparochromus vulgaris* (group IV) - 6 tubes (2 were overgrown with mold), three on day 4 and three on day 8; *Forficula auricularia* (group V) - 7 tubes (1 was overgrown with mold), three on day 4 and four on day 8; *Sitophilus oryzae* (group VI) - 8 tubes, four on day 4 and four on day 8 (Fig. 5).

The overgrowth of foreign microflora is explained by its presence on the surface of the bugs' bodies before the experiment, since contact with any bacteria other than the experimental strain was excluded during the experiment (Fig. 6).

The obtained results prove the ability of insects to mechanically transfer mycobacteria and contaminate environmental objects. At the same time, evaluating the rate of culture growth on the nutrient medium, the following results were obtained: growth in tubes in which beetles of group I moved was observed on day 4 (1 pc), 5 (2 pc), 6 (3 pc) and 7 (1 pc); in group II: on day 5 (3), 6 (1), 7 (1); in group III: on day 4 (2), day 5 (3), day 6 (3); in group IV on day 5 (1), day 6 (2), day 7 (2), day 8 (1); in group V on day 5 (4), day 6 (3); in group VI on day 5 (3), and day 6 (5).

Analyzing the obtained data, it was determined that, on average, the growth of mycobacteria on the surface of the beetle body was 15.3% longer compared to direct culture. In particular, the growth of culture from beetles of group I was recorded for 5.57 ± 0.98 days, group II - 5.60 ± 0.89, group III - 5.13 ± 0.83, group IV - 6.50 ± 1.05, group V - 5.43 ± 0.53, group VI - 5.67 ± 0.52 days. That in percentage terms was longer by 13.7%, 14.3%, 4.7%, 32.7%, 10.8%, 15.7%, respectively (Table 1).

Part I. The potential of imago beetles to transfer mycobacteria

The study of the insects' ability to accumulate mycobacteria showed that during microscopy of smears made from the intestinal tract of beetles and homogenized (for beetles of the species *Oryzaephilus surinamensis* and *Sitophilus oryzae*), revealed the presence of microorganisms identical in morphological

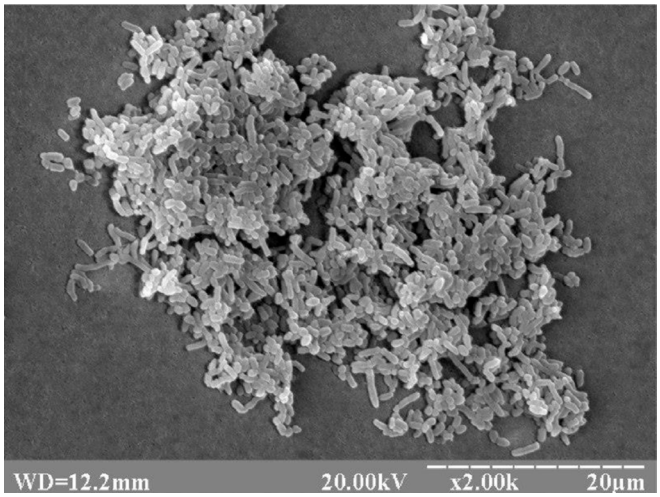

Fig. 3. Scanning electron microscopy of dissociative forms of mycobacteria (117-A passage).

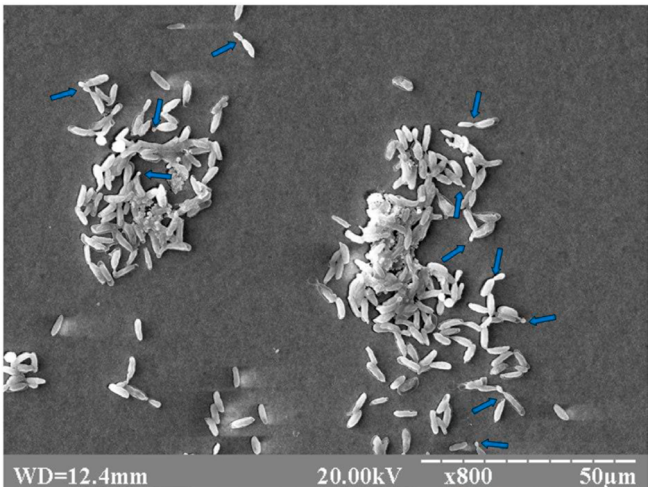

Fig. 4. Scanning electron microscopy of L-forms of mycobacteria. Arrows point to kidney-like outgrowths with various volume.

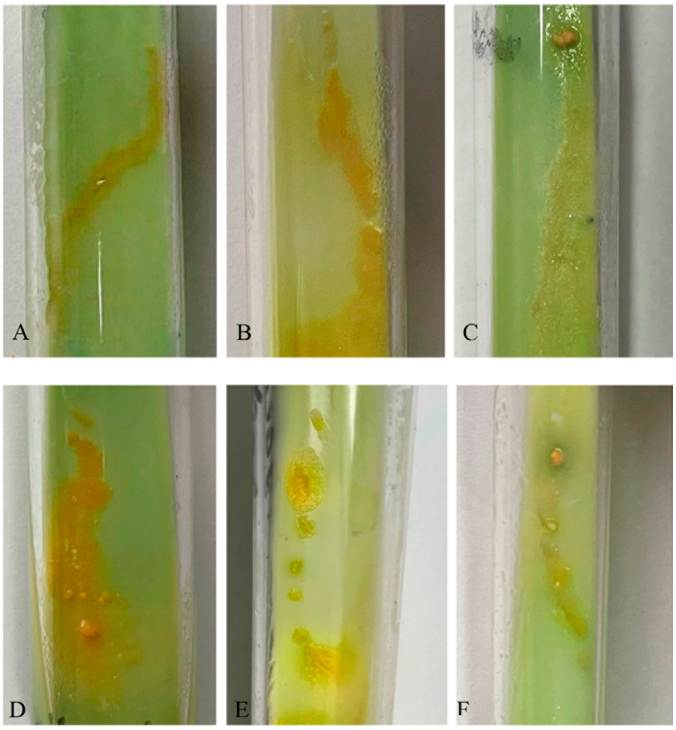

Fig. 5. Growth of mycobacteria cultured into the medium.

characteristics to the original culture of dissociants in each of the experimental groups.

In beetles of the species *Oryzaephilus surinamensis* - in 8 specimens (100%), four of which on day 4 and four on day 8; *Calathus melanocephalus* - in 7 beetles (87.5%), three on day 4 and four on day 8; *Armadillidium vulgare* - in 8 specimens (100%), four on day 4 and four on day 8; *Rhyparochromus vulgaris* - in 5 beetles (62.5%), two on day 4 and three on day 8; *Forficula auricularia* - in 6 specimens (75%), three on day 4 and three on day 8; *Sitophilus oryzae* - in 7 beetles (87.5%), three on day 4 and four on day 8 (Fig. 7).

It should be noted that the morphological characteristics of microbial cells did not change after passage through the beetles, which may indicate that the beetle's body is a susceptible environment for mycobacteria. There is also a pattern of a higher percentage of mycobacteria excretion from the intestinal tract of beetles on day 8, i.e. after a longer contact of insects with the pathogen. The obtained data suggest that beetles of different species are able to accumulate mycobacteria in their intestines, however, the percentage of bacterial excretion is not absolute and may be subjective, since we cannot state how many microbial cells were captured by each beetle. We attribute the absence of the test microorganisms in the intestinal tract of some specimens to the fact that these particular insects didn't eat the contaminated substrate.

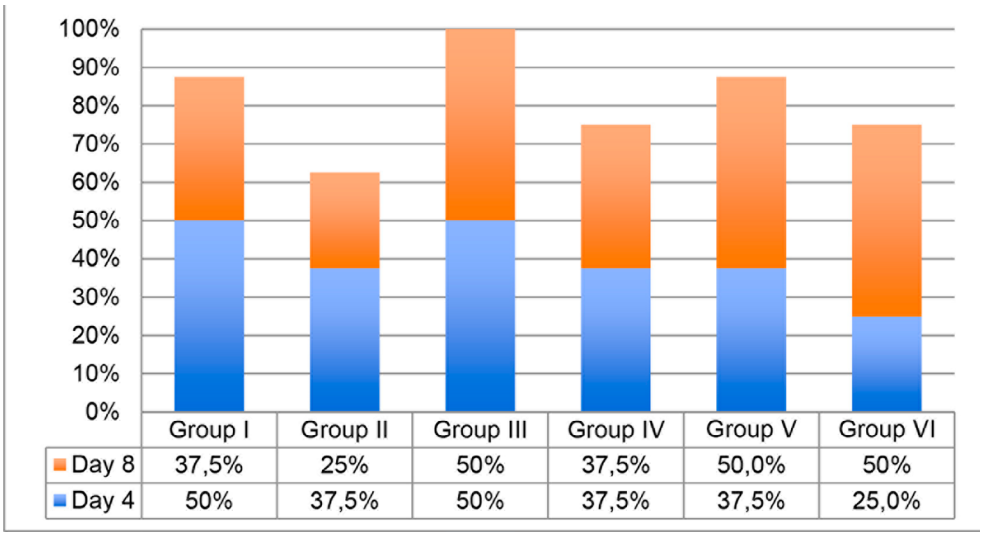

Fig. 6. Percentage ratio of adult beetles' ability to mechanically transfer mycobacteria at different periods of the study.

Table 1. Time of crop growth appearance after free movement of the beetles in test-tubes.

The groups of beetles	Day of culture growth in test tubes								M ± m
	№1*	№2	№3	№4	№5	№6	№7	№8	
I	5	4	6	6	-	5	7	6	5.57 ± 0.98
II	7	5	-	6	5	-	-	5	5.60 ± 0.89
III	4	5	6	6	5	5	4	6	5.13 ± 0.83
IV	7	7	5	-	6	8	-	6	6.50 ± 1.05
V	5	-	6	6	5	5	6	5	5.43 ± 0.53
VI	-	6	6	-	5	6	6	5	5.67 ± 0.52

Note: * is the number of the contaminated test-tubes.

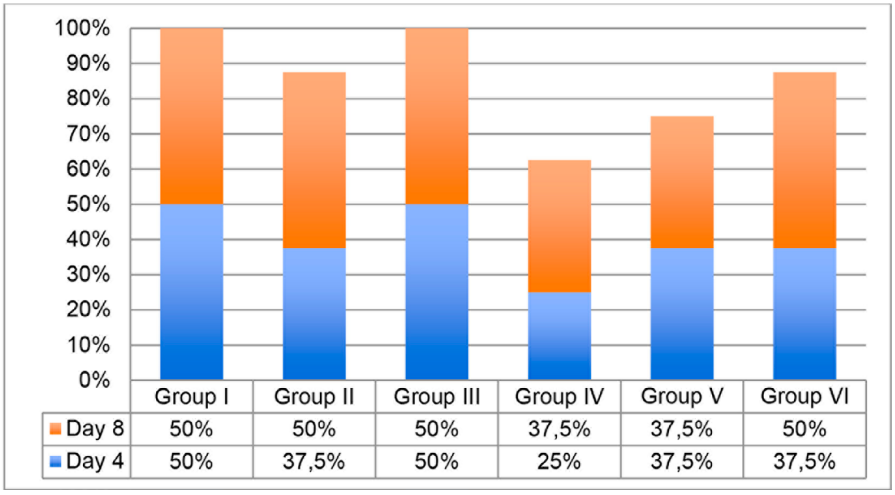

Fig. 7. Percentage ratio of the ability of adult beetles to reserve mycobacteria in the intestine at different periods of the study.

Part II. The potential of the larva of beetles to transmit mycobacteria

The second series of studies, which focused on the larval stage of the large flour beetle, showed similar results. The experiment was carried out according to an identical scheme, examining the intestinal contents of larvae on days 4 and 8 after infection (Fig. 8).

The experimental form of *M. bovis* (L-form) was detected by microscopy of smears from the intestinal tract of 7 larvae (87.5%), three of which were found on day 4 and four on day 8 (Table 2). The obtained data indicate that insects at different stages of their development are able to capture and accumulate the pathogen in the intestinal tract.

Upon analyzing the results of our own research, it was found that beetles at different stages of their development can be a reservoir of mycobacteria, a mechanical vector and potentially participate in the active spread of tuberculosis or mycobacterial infections

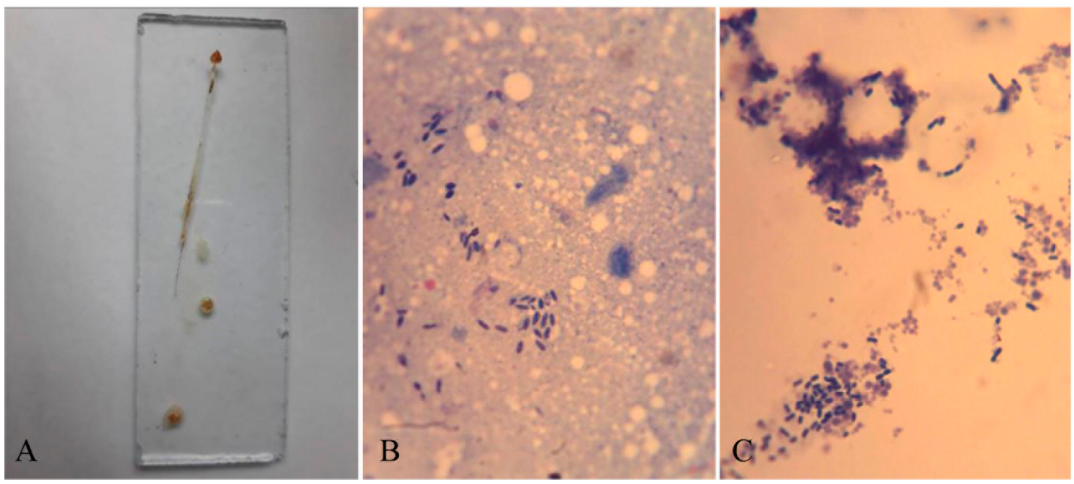

Fig. 8. Tenebrio molitor larva: A, isolated intestine; B, intestinal contents on day 4; C, day 8 after infection.

Table 2. The ability of the larval stage of Tenebrio molitor to reserve mycobacteria in the intestine.

Research group	Experimental culture	The day of the study	Presence of test mycobacteria in the intestine of the larva	Total percentage of larvae with mycobacteria isolated in the intestine
Tenebrio molitor	L-forms of mycobacteria	4	37.5% (3 samples)	87.5%
		8	50% (4 samples)	

and the transmission of the pathogen to susceptible animals. Beetles can consume the pathogen with food and accumulate it in their organisms. It is likely that insects are a significant cause of the outbreak in previously safe areas, as they are able to move quite long distances (especially flying insects). In addition, given that pathogenic mycobacteria are predominantly acid-resistant microorganisms, it can be assumed that infected insects can be taken over by susceptible macro-organisms especially animals or birds. In their gastrointestinal tract, insects are digested by gastric juice, but the acid-resistant tuberculosis pathogen remains viable and can persist in the animal's body and/or be released into the environment, contaminating its objects.

Thus, the results of microscopic and culture studies indicate the potential threat of arthropods in the emergence of new areas of disease. However, at the present time, methods of arthropod control have not been assessed as a risk factor for tuberculosis.

Discussion

The rapid spread of tuberculosis among animals and humans has been an urgent problem for many years around the world, including in Ukraine. Mycobacteria are extremely resistant microorganisms to physical and chemical factors, which is ensured by the presence of lipids in the cell wall and the ability to change, so that the bacterium adapts to an unfavorable environment and resists the body's defenses (Bañuls et al., 2015).

An important role in the spread of tuberculosis is played by the maintenance of mycobacteria in the environment. Despite many years of fruitful work by scientists around the world, there is still no comprehensive data on all possible ways of introducing pathogens into a safe area. In order to completely eradicate the disease, the primary task is to expand and improve knowledge on the prevention and control of tuberculosis infection.

Identification of possible reservoirs of mycobacteria that pose a potential threat to outbreaks of infection deserves special attention. Many scientists believe that insects are carriers of opportunistic and pathogenic microorganisms, including the tuberculosis pathogen. In particular, *Mycobacterium intracellulare, Mycobacterium avium ssp., Mycobacterium fortuitum, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella spp., Shigella spp., Pseudomonas aeruginosa* and other bacterial species have been isolated from various arthropods (Nwankwo et al., 2016; Memona et al., 2017; Zarei et al., 2018).

Existing data indicate the ability of insects to accumulate and release mycobacteria into the environment, i.e. to be a potential cause of active disease spread (Fischer et al., 2006; Faulde & Spiesberger 2013; Erfan et al., 2015; Arif et al., 2017). Due to the structure of the cell wall, mycobacteria are resistant to the action of insect gastric enzymes, as a result of which they can be excreted in saliva and excrement. In the results of the study by Durnez et al. (2008) and Fisher et al. (2000), we find reports of mycobacteria isolation from insectivorous animals. The ingestion of mycobacteria into their bodies was probably due to eating infected insects. The available data suggest that insects infected with mycobacteria can be ingested by TB-susceptible animals or birds and digested. In turn, acid-resistant mycobacteria remain viable, persisting in the organism and being released into the external environment.

After analyzing the literature and our data, we modeled the infection of rice weevils by keeping them in wheat grain contaminated with mycobacterial suspensions. We needed to determine the ability of the beetles to accumulate different types of mycobacteria.

To determine the ability of the insects to mechanically transfer viable mycobacteria, after being in the contaminated substrate, the beetles were transferred to a test tube with a nutrient medium where they could move freely. After removing the beetles, the tubes were incubated and observed for growth. A microscopic examination of the morphology of the resulting microbial colonies revealed that they were identical to those of the original cultures.

We found that insects are capable of reserving and releasing viable mycobacteria into the environment, and the beetle's organism does not lead to significant changes in the morphological characteristics and cultural properties of the pathogen. Thus, it is likely that the insects of the test species are a favorable environment for mycobacteria.

Conclusion

It was discovered that adult beetles of different species are capable of mechanical transfer of mycobacteria on the surface of their bodies and contamination of environmental objects. Consequently, they pose a potential threat of infection spread and outbreaks of the disease in previously safe areas. However, there is no consistent correlation between the duration of the beetle's contact with the infected substrate and the growth of colonies on nutrient agar plates after the transfer of experimental insects into them.

Beetles at different stages of development are capable of accumulating mycobacterium tuberculosis in their intestinal tract. In the study of beetles of the species *Oryzaephilus surinamensis*, experimental strains of mycobacteria were found in 100% of specimens; *Calathus melanocephalus* - in 87.5%; *Armadillidium vulgare* - 100%; *Rhyparochromus vulgaris* - 62.5%; *Forficula auricularia* - 75%; *Sitophilus oryzae* - 87.5%; *Tenebrio molitor* - 87.5%. It was noted that the number of beetles in whose intestines the test microorganisms were found was higher after eight days of contact with the infected substrate than after four days. The absence of experimental mycobacteria in smears from the gut content of some specimens is attributed to the non-consumption of the contaminated substrate by the beetles.

Furthermore, the results showed that the morphological characteristics of mycobacteria remain unchanged after passage through the beetles' bodies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships with respect to this paper.

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