DOI: 10.31073/onehealthjournal2026-II-02
UDC 351.779/504.064

ANIMAL TUBERCULOSIS IN RADIATION-EXPOSED SETTINGS: AN INTERSECTORAL "ONE HEALTH" APPROACH IN A LABORATORY ANIMAL MODEL

Kassich V. Yu.[1] (ORCID: 0000-0001-9859-8036), Ushkalov V. O.[2] (ORCID: 0000-0001-5694-632X), Kasianenko O. I.[1] (ORCID: 0000-0001-8453-1957), Plyuta L. V.[1] (ORCID: 0000-0001-8935-4873), Ushkalov A. V.*[, 3] (ORCID: 0000-0001-8317-7909)
1 – Sumy National Agrarian University, Sumy, Ukraine, e-mail: kassich_v_u@ukr.net
2 – National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, e-mail: ushkalov63@gmail.com
3 – National Scientific Center «Institute of Experimental and Clinical Veterinary Medicine», Kharkiv, Ukraine, e-mail: vetdocman@gmail.com

Abstract. *Information on the course and pathogenesis of animal tuberculosis — a dangerous zoonosis - against the background of radiation exposure, which is accompanied by a decrease in the specific reactivity of the organism and the development of progressive immunodeficiency, remains limited. At the same time, taking into account the current global and regional threats of man-made disasters, as well as the risks associated with the military aggression of the Russian Federation against Ukraine (the capture and damage to the Chernobyl NPP, the occupation of the Zaporizhzhia NPP, periodic disruptions to the stability of their operation and systematic missile attacks on energy infrastructure), the problem becomes particularly relevant within the framework of the intersectoral concept of «One Health», which combines animal and human health and the state of the environment.*

In our studies, guinea pigs irradiated with different doses of gamma radiation were infected with tuberculosis mycobacteria of different species (Mycobacterium bovis, M. tuberculosis, M. avium) and examined for 90 days using clinical, allergic, serological, pathoanatomical and histological methods to study clinical and pathological manifestations, immunological reactions and dynamics of animal death.

A dose-dependent acceleration of mortality and generalization of the tuberculous process in irradiated animals was established, especially under the influence of sublethal and lethal doses of gamma radiation (150–200 R; 0.0387–0.0516 C/kg), which was accompanied by the development of acute radiation sickness, pronounced vascular disorders and activation of exudative-necrotic processes. Pathological changes were in the nature of a confluent caseous-necrotic lesion of target organs.

The results obtained indicate that radiation damage significantly modifies the course of tuberculosis, accelerating the development of the infectious process and increasing its severity, which is of great importance for assessing epizootic and epidemiological risks in areas of potential or real radiation contamination. The study emphasizes the need for an integrated approach to the control of zoonotic infections in emergency conditions within the One Health concept, especially in conditions of military threats to nuclear and radiation safety.

Key words: mycobacteria, pathogenesis, gamma irradiation, allergic

Information on the course and pathogenesis of animal tuberculosis under radiation exposure remains limited, despite the growing global and regional risks of technogenic accidents in nuclear power, the possibility of using nuclear weapons and the associated disruptions of the ecological balance. In the face of such threats, the intersectoral concept of «One Health» becomes particularly relevant, which considers human, animal and environmental health as interconnected components of a single system.

In this context, tuberculosis - a dangerous zoonosis - serves as an illustrative model of an infectious process sensitive to changes in the immunological reactivity of the organism, in particular as a result of the action of ionizing radiation. The present study evaluates the impact of radiation damage on the course of tuberculosis in guinea pigs infected with various types of pathogenic mycobacteria. The results obtained indicate that against the background of radiation pathology, the pathogenesis of tuberculosis is significantly modified: the infectious process develops faster, and pathological reactions are more pronounced and intense.

According to the World Health Organization, the incidence of tuberculosis in the world is about 133 cases per 100,000 population, and in 2022, 10.6 million new cases and 1.3 million

deaths were registered (WHO, 2022). The extremely low infectious dose of the pathogen - from one to five bacilli with an aerosol transmission mechanism - causes a high epidemiological risk for both humans and animals (Tellier, 2019), which once again emphasizes the importance of the integrated One Health approach.

The pathogenesis of tuberculosis is determined by the complex interaction between the virulence of the pathogen and the immune response of the susceptible human or animal organism. The initial stages of the infectious process depend on the results of the primary interaction between the micro- and macroorganism (Neill, 2001; de Martino, 2019), and a deep understanding of the molecular mechanisms of this interaction is key to developing effective methods of diagnosis, treatment and prevention (Bloom, 2016). Within the One Health concept, such knowledge has applied value not only for clinical medicine, but also for veterinary practice, epidemiology and biosecurity.

Tuberculosis occupies a special place among infectious diseases of farm animals, as it causes significant economic losses and poses a direct threat to public health. The pathogenesis of bovine tuberculosis involves the recruitment and activation of immune cells under the influence of mycobacterial antigens, cytokines and chemokines in the affected lungs and regional lymph nodes with the formation of specific granulomas induced by *Mycobacterium bovis* (Palmer, 2022). The morphological and cellular characteristics of granulomas are widely used as markers of the severity of the disease and the effectiveness of immunoprophylaxis, which necessitates an in-depth study of the mechanisms of their formation and destruction, especially in conditions of immunosuppression.

Modern geopolitical conditions have significantly increased the risks of radiation exposure to biological objects. The military aggression of the Russian Federation against Ukraine was accompanied by the seizure of the Chernobyl and Zaporizhzhia nuclear power plants; as of January 2026, the Zaporizhzhia NPP remains under the control of the Russian Federation. This creates real threats of man-made accidents, as well as the potential use of tactical nuclear weapons. In such conditions, studying the impact of ionizing radiation on the pathogenesis of infectious diseases in animals and humans is a priority task in the One Health system (Cvetnić, 2020, 2021; Khan, 2008; Torres, 2019; Belay, 2021; Reil, 2022).

Despite the more than 144-year history of studying tuberculosis - from the discovery of the pathogen by Robert Koch in 1882 to the introduction of tuberculin into diagnostic practice - clinical-allergic and serological methods remain the main tools for detecting infection in both humans and animals. It is important to note that the practical value of tuberculin for allergy diagnostics was first substantiated by Clemens von Pircke in 1907 (Stevenson, 2024).

However, a number of studies indicate the possibility of false-positive tuberculin reactions in animals under the influence of adverse environmental factors, in particular ionizing radiation (Akhtar, 2019; Farhat, 2006; Huang, 2022; Didkowska, 2021; Jenkins, 2018; Conlan, 2015; Stringer, 2011; Maqsood, 2024). Responses to radiation exposure are species-specific and heterogeneous among both microorganisms and animals, which complicates the interpretation of diagnostic results and requires a comprehensive interdisciplinary analysis (Wang, 2019; Huang, 2019; Zaharie, 2020; Kassich, 2023; Barnacle, 2024).

Thus, research into the impact of radiation exposure on the course of animal tuberculosis is of great importance not only for veterinary science, but also for the public health system and environmental safety, fully consistent with the principles of the One Health concept.

Materials and methods. *Ethical statement.* The experiments with laboratory animals were performed according to the requirements of current documents on the procedure of animal experiments and protection of vertebrates, used for experimental and other scientific purposes, adopted in Ukraine and EU (European convention, 1986; Law of Ukraine, 2012) - protocol of Commission on bioethical expertise of (07.11.2023).

Laboratory animals. To study the patterns in development of infectious process and pathogenesis of tuberculosis under the effect of ionizing radiation we used 200 guinea pigs, body weight 300-350 g, selected by analog principle.

Strains of mycobacteria. Strains *M. bovis* of strain Bovinus 8, *M. tuberculosis* of strain M, *M. avium* of strain Avium 780 from the collection of the Department of epizootiology and parasitology Sumy National Agrarian University.

Irradiation of laboratory animals. For irradiation, a gamma-radiation source of 137 Cz was used with an irradiation intensity of 2.65 R/second in gamma-radiation doses of 50 R, 100 R, 150 R, and 200 R (0.0129, 0.0258, 0.0387, 0.0516 C/kg).

Experimental design. The animals were divided into 5 groups, with 40 animals in each group. To exclude natural tuberculosis the guinea pigs were previously tested (30 days before radiation exposure) with a tuberculin test (tuberculin dose of 25 IU in 0.1 cm3), manufactured by Sumy Biological Factory. We did not detect reacting animals. Four groups of animals (1, 2, 3, 4) were separately exposed to gamma-radiation doses 50 R, 100 R, 150 R, and 200 R (0.0129, 0.0258, 0.0387, 0.0516 C/kg, respectively) with radiation intensity 2.65 R/second at gamma-ray source with radiation source of 137 Cz.

The guinea pigs, irradiated by each dose and intact guinea pigs were divided into subgroups with 10 animals in each.

One day after irradiation all experimental animals underwent an allergenic test with mammalian PPD tuberculin (at a dose of 25 IU in 0.1 cm3) with negative results, and 7 days after irradiation the animals were infected with the following mycobacteria: *M. bovis* of strain Bovinus 8 (subgroup 1a, 2a, 3a 4a, 5a), *M. tuberculosis* of strain M (subgroups 1b, 2b, 3b, 4b, 5b), and *M. avium* of strain Avium 780 (subgroups 1c, 2c, 3c, 4c, 5c). The experimental design is described in Table 1.

The mycobacterial cultures were administered to guinea pigs subcutaneously in groin with 1 mg of raw bacterial mass, suspended in NaCl1 ml.

Non-irradiated animals (subgroups 5a, 5b, 5c, 5d) underwent an allergologic test with tuberculin (at a dose of 25 IU for 0.1 cm3) 7 days after radiation exposure, and then these animals were also infected with the same strains of *M. bovis, M. tuberculosis* and *M. avium*. Figure 1 presents the electronic phono of production strain *M. bovis*. For examination with electronic microscope, the preparations (mycobacterial cultures) were fixed in 1 % buffered osmium tetroxide solution for 2-3 hours at 4 C. After fixation the bacterial cells were washed in buffer solution, dehydrated in alcohols of ascending concentration and acetone, and introduced into the mixture of epoxide resins (Epon-Araldite mixture). The proteins were polymerized in a thermostat at 60 C for two days. Ultrathin slices were prepared with ultramicrotome UMTP-6 and after contrasting with lead citrate they were studied under an electronic microscope EMB-100 BR with an accelerating voltage of 75 kV. The magnifications were selected according to the study's aim.

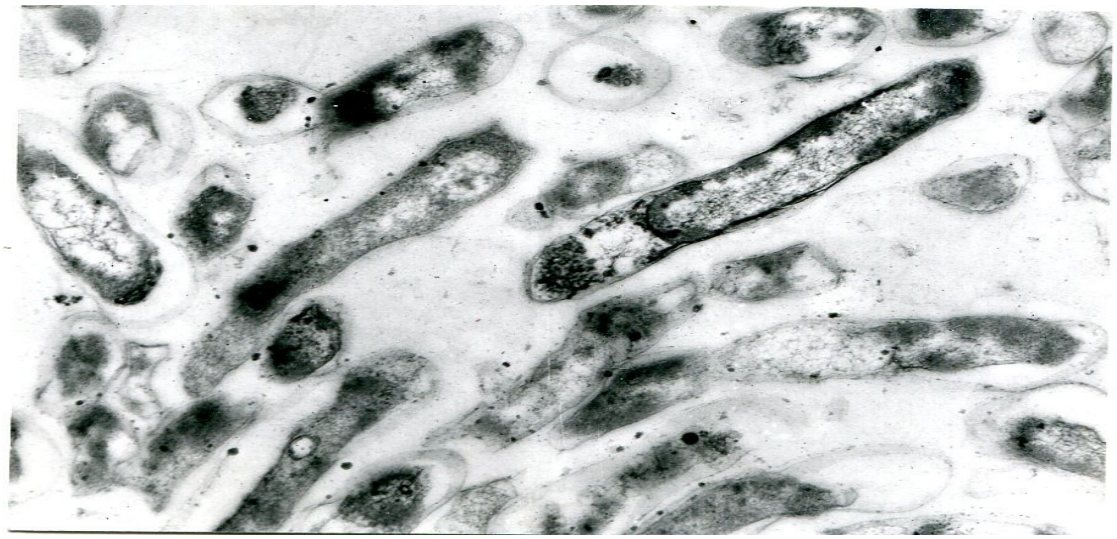

Fig. 1. Mycobacteria of production strain *M. bovis*. Conglomerate of rod-like forms (authors' photo)

Table 1

Scheme of experiment with irradiation and infection of animals

No. of group	No. of subgroup	Number of animals	Irradiated (R/C/kg)				Not irradiated	Infected with tuberculosis			Non-infected
			50/ 0.0129	100/ 0.0258	150/ 0.0387	200/ 0.0516		*M. bovis*	*M. tuberculosis*	*M. avium*	
1	1 a	10	+					+			
	1 b	10	+						+		
	1 c	10	+							+	
	1 d	10	+								+
2	2 a	10		+				+			
	2 b	10		+					+		
	2 c	10		+						+	
	2 d	10		+							+
3	3 a	10			+			+			
	3 b	10			+				+		
	3 c	10			+					+	
	3 d	10			+						+
4	4 a	10				+		+			
	4 b	10				+			+		
	4 c	10				+				+	
	4 d	10				+					+
5	5 a	10					+	+			
	5 b	10					+		+		
	5 c	10					+			+	
	5 d	10					+				+

The experimental animals were studied for 90 days. Deaths of experimental animals were registered, autopsies were performed, and pathological changes were registered.

The biological material for histological examinations was preserved in 10 % neutral formalin for 10 days, and then processed by the following scheme: -washing of the material under running water for 5 hours; -dehydration in alcohols of ascending concentration (70 -100); -deoiling in chlorophorm; -embedding in chloroform-paraffin; -cutting of the tissue pieces into tissue blocks and their installation on slides; -preparing of histological slices with microtome, their putting on object-plates and drying; -preparations staining with hematoxylin-eosin and further embedding into polisterol under microscopic cover-glasses. The histological slices were examined under an immersion system of light microscope Jenaval. The preparations were photographed by camera adapter MFN- 10 UCh.2.

The obtained results of the study and the differences between them were subjected to a one-way analysis of variance (ANOVA), (t-test), according to the Dunnett criterion with an error rate of 0.05 ($P<0.033^*$; 0.002^{**}; 0.001^{***}), using GraphPad Prism 8.3.0 for Windows.

Results. We performed pathoanatomical examination of experimental guinea pigs after their death during the experiment, and of animals euthanized three months after the infection with tuberculosis.

In the group exposed to 50 R (0.0129 C/kg) all guinea pigs, infected with *M. bovis*, died with the presentation of generalized tuberculosis. At that, the animals died 46-82 days after the infection.

The dissection of the dead animals revealed in the dissected regional groin lymph nodes the foci of caseous (cheesy) necrosis. The spleen and the liver were significantly enlarged, dense, with greyish or yellowish nodes, which conjugated and formed a sheer caseous mass. The lungs presented a lot of greyish foci (affects) in the form of humps, and nodes with caseous content.

Guiney pigs, exposed to the dose of 50 R (0.0129 C/kg), and infected with *M. tuberculosis*, were euthanized 3 months after the infection. Pathoanatomical studies established generalized tuberculosis in all animals.

In animals, exposed to 50 R (0.0129 C/kg) and infected with *M. avium*, pathoanatomical examinations did not detect notable pathological changes and lesions, which is logical and confirms the fact of non-pathogenicity of aviary tuberculosis pathogen in guinea pigs, even on the background of immunosuppressive gamma-irradiation.

The non-infected guinea pigs, exposed to 50 R (0.0129 C/kg) did not present signs of disease during the entire period of observations. After the end of the experiment, these animals were euthanized. The dissection did not detect pathoanatomical changes.

In the group, exposed to 100 R (0.0258 C/kg), 50 % of guiney pigs infected with *M. bovis* died 28-60 days after the infection, presenting general disseminated tuberculosis; 10 % of irradiated animals died 14-21 days after the irradiation with the presentation of hemorrhagic diathesis and pronounced exudative-hemorrhagic reaction (typical for radiation pathology); 40 % of animals from this group died within the same period, however, their dissection did not reveal noticeable pathoanatomical changes.

In the group of animals exposed to 100 R (0.0258 C/kg) and infected with *M. tuberculosis*, 30 % of guinea pigs died during two weeks after the irradiation with the presentation of hemorrhagic diathesis with mucous edemas and hemorrhages of skin and subcutaneous tissue. During the further observation period (90 days), 20 % more animals died. Their dissection demonstrated generalized disseminated tuberculosis. The animals, alive by the end of experiment (by day 90) were euthanized. Their dissection showed pathoanatomical changes, characteristic of tuberculosis: focal caseous lesions in the liver, spleen, lungs, and groin lymph nodes (Figure 2 and 3).

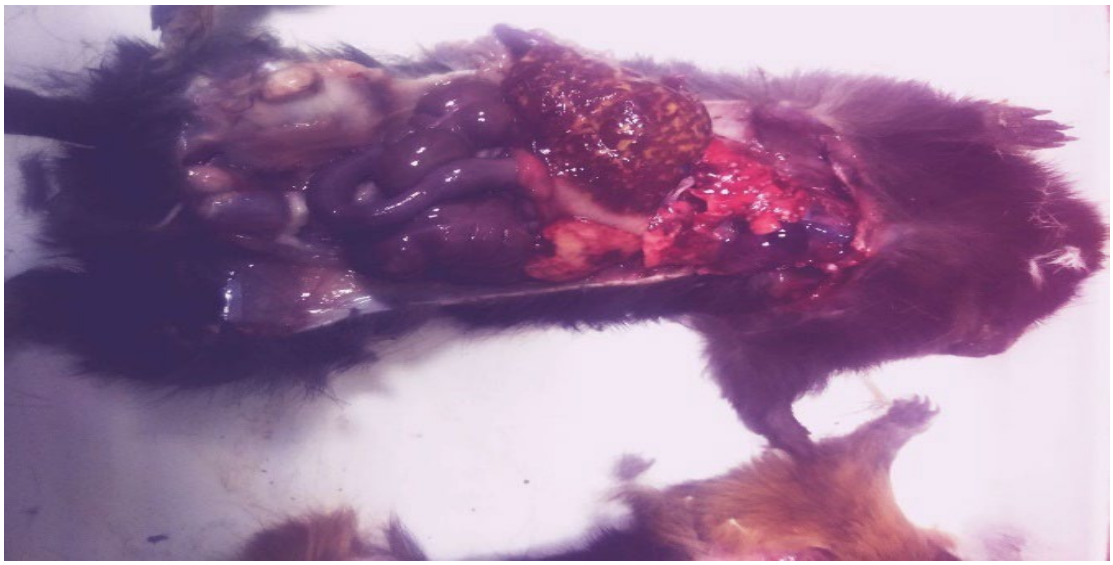

Fig. 2. Generalized miliary tuberculosis in guinea pigs

Fig. 3. Enlargement of spleen and liver, and foci of lung tuberculosis lesions in the guinea pig, exposed to 200 R (0.0516 C/kg), which died 25 days after infection with *M. tuberculosis*

In the groups, exposed to the dose of 100 R (0.0258 C/kg) among infected *M. avium* and non-infected animals, 10 % died in 18-21 days under the effect of radiation with the pronounced presentation of hemorrhagic diathesis, massive hemorrhages, haemo-extravasates, and lymphocyte extravasates. The remaining animals stayed alive and did not present clinical manifestations of disease till the end of experiment, after which they were euthanized and underwent pathoanatomical examination. We did not detect pathological changes.

In the group of animals, exposed to 150 R (0.0387 C/kg) and infected with *M. bovis*, we registered death of 80 % of animals during 20 days after the irradiation. Their dissection revealed pathoanatomical changes, characteristic of acute radiation sickness (hemorrhagic syndrome: increased bleeding, hemorrhagic diathesis, progressing anemia, gastrointestinal syndrome, as a manifestation of post-radiation gastroenteritis and sensibilization syndrome). Pathomorphological examination of the bone marrow revealed a decrease in the number of myelocariocytes, and erythroblasts, a decrease in mitotic index, increased cytolysis, and disappearance of immature cell forms.

The remaining guinea pigs died 28-55 days after the infection with the presentation of disseminated generalized tuberculosis, which was more pronounced macroscopically in the animals, irradiated with 50 R (0.0129 C/kg) and 100 R (0,0258 C/kg). In these animals, pathoanatomical examination stood out strongly pronounced vascular reaction, manifested by significant serous effusion,

presence of extravasates, and isolated hemorrhages. On the background of pronounced disorders of vascular wall permeability, the foci of caseous degeneration in the lymph nodes, liver, and spleen had caseous-necrotic character involving the entire organs.

At that, histological examination detected in the liver fatty infiltration and tissue degeneration, diffuse and nodular histiolymphocytic infiltrations, and masses of polynuclear cells of epithelial type (Figure 4). In the lungs the researchers detected primary effects in the form of foci of primary pneumonia with granuloma necrotic events in the centre, peri-focal inflammation and tuberculous swellings (nodules). In the liver and spleen, the scientists detected large-centred lesions in the form of miliary tuberculosis and tuberculous splenomegaly. In the tissue multiple foci of tuberculous inflammation are notable, forming the zones of caseous (cheesy) necrosis in the form of nodules (humps) with the periphery of numerous epithelioid cells, large polynuclear macrophages and Pirogov-Lanhgans giant cells, which are typical for infectious granulomas of tuberculous origin and suggest the specificity of tuberculosis inflammation. We noted in the lymph nodes inflammatory manifestations with the presence of epithelioid cells, polynuclear macrophages, and Pirogov-Lanhgans giant cells, typical for tuberculous granulomas.

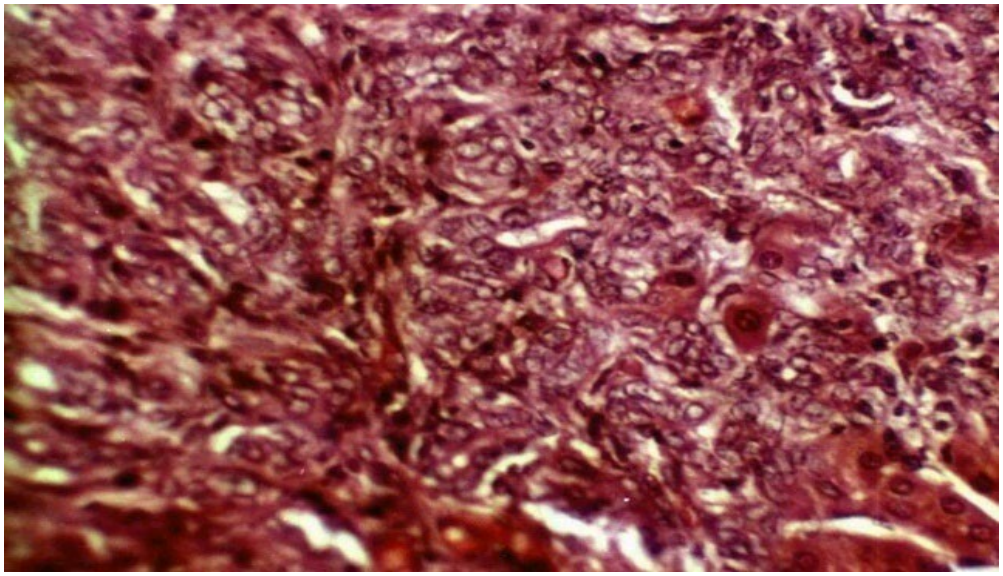

Fig. 4. The zone of guinea pig liver, exposed to 150 R. The group of polynuclear cells of epithelial type. Isolated hepatocytes are noticeable

In the group of animals, exposed to 150 R (0.0387 C/kg) and infected with *M. tuberculosis*, 60 % of experimental animals died during 2-3 weeks after radiation exposure. Their dissection revealed large hemorrhages in the subcutaneous tissue, myocardium, lungs, gastrointestinal walls, peritoneum, pleura, and other organs. The spleen had decreased volume, the lymph nodes presented serous-hemorrhagic edema, and the bone marrow was meaty, of bright red colour, and softened in some cases. The remaining animals from this group were euthanized after the end of experiment (90 days after the infection). Their dissection demonstrated caseous-necrotic focal lesions in the lungs, spleen, liver, and regional lymph nodes.

In the group of animals, exposed to 150 R (0.0387 C/kg), and infected with M. avium, 70 % of experimental animals died 2-3 weeks after irradiation with the presentation of hemorrhagic diathesis, pronounced disorders of blood and lymph circulation (venous hyperemia, plasmorrhagia, edemas), and disorders in the lymphoid tissue and the bone marrow. The guinea pigs, which stayed alive by the end of experiment, were euthanized 90 days after administration of the infectious pathogen. Their dissection did not demonstrate pronounced pathoanatomical changes.

All non-infected (control) animals, exposed to 150 R (0.0387 C/kg), died during three weeks period. Their dissection demonstrated pathoanatomical changes, characteristic of acute radiation sickness with sharply pronounced vascular reaction, vascular permeability disorders, multiple hemorrhages, lymphatic extravasations, and the presentation of hemorrhagic diathesis.

In the group of animals exposed to 200 R (0.0516 C/kg) and infected with *M. bovis*, 40 % of guinea pigs died 10-18 days after irradiation with pathoanatomical changes, characteristic of acute radiation sickness. The remaining animals died in 18-47 days after infection with the presentation of disseminated

generalized tuberculosis and sharply pronounced exudative reaction. In their lungs, spleen and liver caseous degeneration involved the entire organs, with foci of confluent caseous-necrotic character.

Among the animals, exposed to 200 R (0.0516 C/kg) and infected with *M. tuberculosis* and *M. avium*, 80 % of guinea pigs died during three weeks after radiation exposure with pronounced signs of radiation sickness. The remaining animals were euthanized and underwent a pathoanatomical study. In the animals, infected with *M. tuberculosis* we noted focal caseous lesions in the lymph nodes and internal organs, characteristic of tuberculosis. In the animals, infected with *M. avium*, we did not detect pathoanatomical changes, characteristic of tuberculosis, which supported the well-known fact of non-pathogenicity of avian tuberculosis pathogen in guinea pigs.

In the control (non-infected) group all animals, exposed to 200 R (0.0516 C/kg) died during three weeks with pathoanatomical changes in their organs and tissues, characteristic of radiation sickness.

In the group of non-irradiated animals, infected with *M. bovis*, the guinea pigs died 22-56 days after the infection with the presentation of generalized tuberculosis.

In the group infected with M. tuberculosis, 40 % of experimental animals died in 71-85 days, and the remaining animals were euthanized. All the studied guinea pigs demonstrated different degrees of typical lesions in the lymph nodes and internal organs, characteristic of tuberculosis.

We did not detect internal organs lesions in euthanized animals, infected with *M. avium*.

Comes under notice the dynamics of death of animals, infected with *M. bovis* and irradiated by various doses of gamma-radiation. Thus, in the groups exposed to 150 R (0.0387 C/kg) and 200 R (0.0516 C/kg), 33-78 % of guinea pigs died with the presentation of acute radiation sickness. The remaining animals, the animals exposed to 50 R (0.0129 C/kg) and 100 R (0.0258 C/kg,) and the intact animals infected with *M. bovis*, died later with the presentation of disseminated tuberculosis. At that, the researchers noted that the infected animals died of tuberculosis in the following terms: during 47 days the guinea pigs died, exposed to 200 R (0,0516 C/kg); up to 55 days – exposed to 150 R (0.0387 C/kg); up to 56 days – non-irradiated animals, infected with *M. bovis*; up to 60 days – exposed to 100 R (0,0258 C/kg); and up to 80 days – exposed to 50 R (0.0129 C/kg).

Figure 5 demonstrates graphic representation of guinea pigs death dynamics.

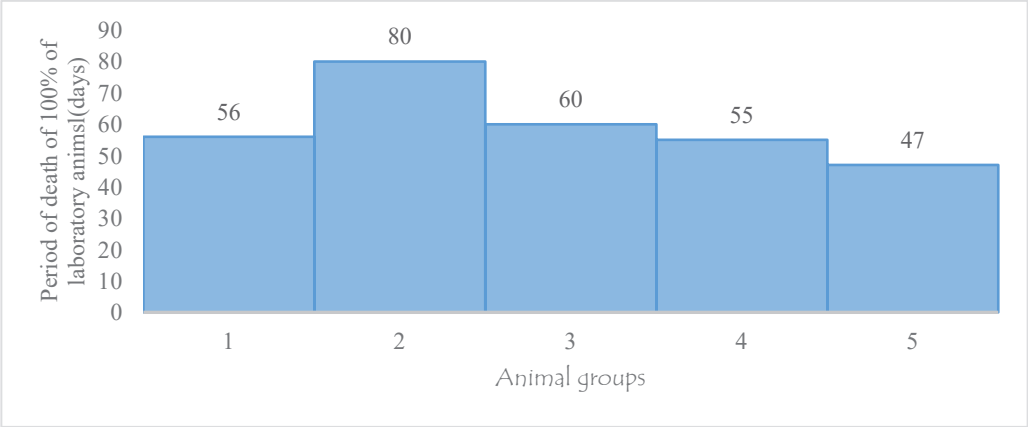

Fig. 5. Dynamics of death of guinea pigs exposed to radiation and infected with mycobacteria

Therefore, the guinea pigs, infected with *M. bovis* and exposed to radiation doses of 150 R (0.0387 C/kg) and 200 R (0.0516 C/kg) died much earlier than control (non-irradiated) animals, and the animals, exposed to 50 R (0.0129 C/kg) and 100 R (0.0258 C/kg) with the presentation of disseminated generalized tuberculosis. At that, on the background of significant disorders in vascular permeability, the tuberculous inflammation went with a very pronounced exudative reaction, which was not observed in control animals. In the lymph nodes, lungs, spleen, and liver the caseous degeneration involved the entire organs, and foci of caseous necrosis conjugated, forming sheer cheese-like mass; in other words, they had confluent caseous-necrotic character.

Therefore, on the background of developed radiation sickness the pathogenesis of tuberculosis accelerated, the infectious process developed more rapidly, and the pathological changes had a more pronounced and intense character.

Discussion. The results of our clinical and pathomorphological studies demonstrated that mortality in guinea pigs infected with tuberculosis and exposed to a radiation dose of 50 R (0.0129 C/kg) was delayed, whereas exposure to higher doses of 150 R and 200 R (0.0387 and 0.0516 C/kg, respectively) led to a significant acceleration of animal death compared with non-irradiated controls.

These findings indicate a dose-dependent interaction between radiation injury and infectious pathology, in which each factor aggravates the course of the other.

Exposure to sublethal and lethal doses of ionizing radiation markedly worsened the course of tuberculosis, while the infectious process itself intensified the manifestations of radiation sickness. The course of tuberculosis in irradiated animals depended on the combination of radiation dose, pathogen virulence, and timing of exposure and infection. Infection with pathogenic mycobacteria against the background of radiation-induced immunosuppression resulted in a predominance of alternative-destructive inflammatory reactions, with pronounced vascular permeability disorders and extensive exudative-necrotic lesions. These observations are consistent with data reported by other authors (Saeed, 2016).

Gamma irradiation of guinea pigs infected with mycobacteria promoted exacerbation of infection with activation of exudative-necrotic processes, which has also been described in classical experimental studies (Solovyova, 1959; Kirshina, 1986; Vulchanova, 1967, Klemparska, 1982). Although the pathoanatomical changes remained typical for tuberculosis, irradiation caused their earlier development and greater intensity, resulting in accelerated mortality.

From the perspective of the One Health concept, the obtained data have broader implications beyond experimental pathology. Radiation-induced modulation of tuberculosis pathogenesis in animals reflects potential risks in real-world scenarios where environmental radiation exposure may coincide with endemic zoonotic infections. Such conditions may arise following nuclear accidents, military actions involving nuclear facilities, or contamination of ecosystems, affecting livestock, wildlife, and human populations simultaneously.

Our findings generally agree with those of Solovyova, who reported that pathoanatomical changes in irradiated and non-irradiated animals infected with *M. bovis* at a dose of 200 R were largely similar. However, we additionally demonstrated a more intense tuberculin hypersensitivity response during the first 2–2.5 months after irradiation, followed by its decline. This observation is particularly relevant for veterinary and public health surveillance, as radiation exposure may alter the reliability of allergic diagnostic tests used in tuberculosis control programs.

Previous studies on the immunological reactivity of guinea pigs (Kassich, 2023) showed that irradiated and infected animals developed complement-fixing antituberculous antibodies within diagnostic titers. The detection of high titers of tissue autoantibodies in animals exposed to 200 R did not interfere with serological tuberculosis diagnostics, indicating preserved specificity of antituberculous immune responses. However, the occurrence of isolated nonspecific allergic reactions (e.g., to brucellin and mallein) highlights the complexity of immune dysregulation under radiation exposure and underscores the need for integrated interpretation of diagnostic results.

Within the One Health framework, these findings emphasize that radiation-induced immune alterations in animals may influence epizootic monitoring, zoonotic transmission risks, and the safety of animal-derived food products. Accelerated progression of tuberculosis and atypical inflammatory responses in livestock could compromise disease detection, increase pathogen shedding, and pose additional risks to human health through occupational exposure or food chains.

Conclusions. The results of the conducted experimental study demonstrated that exposure of guinea pigs infected with tuberculosis to ionizing radiation significantly modified the course of the infectious process in a dose-dependent manner. A radiation dose of 50 R (0.0129 C/kg) caused a delay in mortality by 10–12 days compared with non-irradiated infected animals, whereas higher doses of 150–200 R (0.0387–0.0516 C/kg) led to a pronounced acceleration of death. Ionizing radiation at sublethal and lethal doses aggravated the course of tuberculosis, while the infectious process itself intensified the manifestations of radiation sickness, indicating a mutual potentiation of pathological effects.

Tuberculosis developing under conditions of radiation-induced immunosuppression was characterized by predominance of alternative-destructive inflammatory reactions with marked exudative-necrotic changes and generalized organ involvement. Gamma irradiation of animals infected with *M. bovis* and *M. tuberculosis* resulted in earlier onset and greater severity of typical tuberculous lesions, while preserving their morphological specificity. The severity and dynamics of pathological changes depended on the radiation dose, virulence of the pathogen, and the temporal relationship between irradiation and infection.

From the perspective of the One Health concept, the obtained data indicate that combined exposure to environmental radiation and zoonotic pathogens may significantly increase epizootic and epidemiological risks. Radiation-induced modulation of tuberculosis pathogenesis in animals can

complicate the interpretation of diagnostic tests, influence disease surveillance efficiency, and potentially affect the safety of livestock products. The proposed experimental model may therefore be used to assess complex interactions between environmental factors, animal health, and public health, emphasizing the necessity of integrated interdisciplinary approaches to biological risk assessment in radiation-contaminated environments.

Acknowledgments

We would like to thank Oleksandr Assori, director of the state enterprise «SUMSKA BIOLOGICAL FACTORY» for his assistance in conducting the experiments.

Funding statement

This study was carried out within the scope of scientific research of the Faculty of Veterinary Medicine of the Sumy National Agrarian University, Sumy, Ukrainian.

Ethical statement

The experiments with laboratory animals were performed according to the requirements of current documents on the procedure of animal experiments and protection of vertebrates, used for experimental and other scientific purposes, adopted in Ukraine and EU (40) - protocol of Commission on bioethical expertise of Sumy National Agrarian University (07.11.2023).

REFERENCES

1. Akhtar R., Sadiqa M., Tipu M.Y., Khan M.R., Aslam A., Ijaz M., Mustafa G., Zahid B. Use of molecular probes for presumptive diagnosis of tuberculosis associated with *Mycobacterium tuberculosis* and *Mycobacterium bovis* infection. Pak Vet J. 2019;39(2):316–319. doi:10.29261/pakvetj/2019.067.

2. Balázs K., Kis E., Badie C., Bogdándi E.N., Candéias S., Garcia L.C., et al. Radiotherapy-induced changes in the systemic immune and inflammation parameters of head and neck cancer patients. Cancers (Basel). 2019;11(9):1324. doi:10.3390/cancers11091324.

3. Barnacle J.R., Davis A.G., Wilkinson R.J. Recent advances in understanding the human host immune response in tuberculous meningitis. Front Immunol. 2024;14:1326651. doi:10.3389/fimmu.2023.1326651.

4. Belay M., Tulu B., Younis S., Jolliffe D.A., Tayachew D., Manwandu H., et al. Detection of *Mycobacterium tuberculosis* complex DNA in CD34-positive peripheral blood mononuclear cells of asymptomatic tuberculosis contacts: an observational study. Lancet Microbe. 2021;2(6):e267–e275. doi:10.1016/S2666-5247(21)00043-4.

5. Bloom B.R., Modlin R.L. Mechanisms of defense against intracellular pathogens mediated by human macrophages. Microbiol Spectr. 2016;4(3). doi:10.1128/microbiolspec.MCHD-0006-2015.

6. Bonovska M., Tzvetkov Y., Najdenski H., Bachvarova Y. PCR for detection of *Mycobacterium tuberculosis* in experimentally infected dogs. J Vet Med B. 2005;52:165–170. doi:10.1111/j.1439-0450.2005.00839.x.

7. Conlan A.J.K., Brooks-Pollock E., McKinley T.J., Mitchell A.P., Jones G.J., Vordermeier M., Wood J.L.N. Potential benefits of cattle vaccination as a supplementary control for bovine tuberculosis. PLoS Comput Biol. 2015;11(2):e1004038. doi:10.1371/journal.pcbi.1004038.

8. Cvetnić Ž., Dugac Ž. History of tuberculosis – from tuberculin to antituberculotics (Part II). Vet Stanica. 2020;51(5):535–546.

9. Cvetnić Ž. History of tuberculosis: prevalence of tuberculosis (Part III). Vet Stanica. 2020;51(6):645–658.

10. Cvetnić Ž. History of tuberculosis: tuberculosis and art (Part IV). Vet Stanica. 2021;52(1):73–84.

11. de Martino M., Lodi L., Galli L., Chiappini E. Immune response to *Mycobacterium tuberculosis*: a narrative review. Front Pediatr. 2019;7:350. doi:10.3389/fped.2019.00350.

12. Didkowska A., Krajewska-Wędzina M., Klich D., Prolejko K., Orłowska B., Anusz K. The risk of false-positive serological results for paratuberculosis in *Mycobacterium bovis*-infected cattle. Pathogens. 2021;10(8):1054. doi:10.3390/pathogens10081054.

13. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Council of Europe; 1986.

14. Farhat M., Greenaway C., Pai M., Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis. 2006;10(11):1192–1204.

15. Gaikwad U.N., Gaikwad N.R. Modalities to monitor the treatment response in tuberculosis. Indian J Tuberc. 2018;65(2):109–117. doi:10.1016/j.ijtb.2017.12.014.

16. Gebremedhn S., Gad A., Aglan H.S., Laurincik J., Prochazka R., Salilew-Wondim D., et al. Extracellular vesicles shuttle protective messages against heat stress in bovine granulosa cells. Sci Rep. 2020;10(1):15824. doi:10.1038/s41598-020-72706-z.

17. Gormley E., Corner L.A.L. Pathogenesis of *Mycobacterium bovis* infection: the badger model as a paradigm for understanding tuberculosis in animals. Front Vet Sci. 2018;4:247. doi:10.3389/fvets.2017.00247.

18. Hargitai R., Kis D., Persa E., Szatmári T., Sáfrány G., Lumniczky K. Oxidative stress and gene expression modifications mediated by extracellular vesicles: an in vivo study of the radiation-induced bystander effect. Antioxidants (Basel). 2021;10(2):156. doi:10.3390/antiox10020156.

19. Huang R., Grishagin I., Wang Y., Zhao T., Greene J., Obenauer J.C., et al. The NCATS BioPlanet – an integrated platform for exploring cellular signaling pathways. Front Pharmacol. 2019;10:445. doi:10.3389/fphar.2019.00445.

20. Huang Y., Ai L., Wang X., Sun Z., Wang F. Review and updates on the diagnosis of tuberculosis. J Clin Med. 2022;11(19):5826. doi:10.3390/jcm11195826.

21. Jenkins A.O., Gormley E., Gcebe N., Fosgate G.T., Conan A., Aagaard C., et al. Cross-reactive immune responses in cattle arising from exposure to *Mycobacterium bovis* and non-tuberculous mycobacteria. Prev Vet Med. 2018;152:16–22. doi:10.1016/j.prevetmed.2018.02.003.

22. Kaladi, M.R., Poldy, A., Mukherjee, S. *et al.* Multi-faceted approach for identifying biomarkers for radiation injury and regulatory approval of radiation medical countermeasures. *Eur. Phys. J. Spec. Top.* (2025). https://doi.org/10.1140/epjs/s11734-025-01475-5

23. Kassich V., Kasianenko O., Klishchova Zh., Kasianenko S., Mozghovyi M. Immunological reactivity of animals with tuberculosis under the influence of ionising radiation. Sci Horiz. 2023;26(3):24–35. doi:10.48077/scihor3.2023.24.

24. Khan I.A., Khan A., Mubarak A., Ali S. Factors affecting prevalence of bovine tuberculosis in Nili Ravi buffaloes. Pak Vet J. 2008;28(4):155–158.

25. Kim B.G., Choi H.S., Choe Y.H., Jeon H.M., Heo J.Y., Cheon Y.H., et al. Low-dose radiotherapy attenuates experimental autoimmune arthritis. Immune Netw. 2024;24(4):e32. doi:10.4110/in.2024.24.e32.

26. Klemparskaya, N. N. *Аутоантитела организма облученного* [Autoantibodies of an irradiated organism]. 1972, Москва: Атомиздат.279 с. (in Russian).

27. Klemparskaya, N. N., & Raeva, N. V. *Issledovanie autosensitizatsii pri luchevoi bolezni metodom Uanye [Study of autosensitization in radiation sickness by the Ouanier method]*. *Bulletin of Experimental Biology and Medicine*, 1981, 51(5), 77–81. (in Russian).

28. Law of Ukraine No. 3447-VI. On the Protection of Animals from Cruelty. 2012.

29. Lumniczky K., Impens N., Armengol G., Candéias S., Georgakilas A.G., Hornhardt S., et al. Low dose ionizing radiation effects on the immune system. Environ Int. 2021;149:106212. doi:10.1016/j.envint.2020.106212.

30. Manngo P.M., Gutschmidt A., Snyders C.I., Mutavhatsindi H., Manyelo C.M., Makhoba N.S., et al. Prospective evaluation of host biomarkers other than interferon gamma for tuberculosis diagnosis. J Infect. 2019;79(3):228–235. doi:10.1016/j.jinf.2019.07.007.

31. Maqsood R., Duffy S.C., Bin Rashid H., Gill S.S., Jabeen C., Arshad N., et al. Molecular detection and characterization of *Mycobacterium tuberculosis* complex subspecies responsible for bovine tuberculosis. Microbiol Spectr. 2024;12:e02692-23. doi:10.1128/spectrum.02692-23.

32. Neill S.D., Bryson D.G., Pollock J.M. Pathogenesis of tuberculosis in cattle. Tuberculosis (Edinb). 2001;81(1–2):79–86. doi:10.1054/tube.2000.0279.

33. Palmer M.V., Kanipe C., Boggiatto P.M. The bovine tuberculoid granuloma. Pathogens. 2022;11(1):61. doi:10.3390/pathogens11010061.

34. Penn-Nicholson A., Mbandi S.K., Thompson E., et al. RISK6, a 6-gene transcriptomic signature of tuberculosis disease risk. Sci Rep. 2020;10:8629. doi:10.1038/s41598-020-65043-8.

35. Reil I., Rubin M., Cvetnić Ž., Zdelar-Tuk M., Duvnjak S., Miškić T., et al. Suspect and positive cases of bovine tuberculosis in Croatia. Vet Stanica. 2022;53(1):11–16. doi:10.46419/vs.53.1.10.

36. Savova-Lalkovska T., Valcheva V., Dimitrova A., Najdenski H., Bonovska M. Advantages of real-time PCR and RD4-PCR for detection of bovine tuberculosis. Proc Bulg Acad Sci. 2022;75(11):1696–1704. doi:10.7546/CRABS.2022.11.18.

37. Smirnova, O. A. (2017). *Environmental radiation effects on mammals: A dynamical modeling approach*. Springer. https://doi.org/10.1007/978-3-319-45761-1

38. Stevenson L.G. Robert Koch. Encyclopaedia Britannica. Accessed 11 Sep 2024. https://www.britannica.com/biography/Robert-Koch

39. Stringer L.A., Wilson P.R., Heuer C., Hunnam J.C., Mackintosh C.G. Effect of vaccination and natural infection with *Mycobacterium avium* subsp. *paratuberculosis*. N Z Vet J. 2011;59(5):218–224. doi:10.1080/00480169.2011.596182.

40. Szatmári T., Persa E., Kis E., Benedek A., Hargitai R., Sáfrány G., Lumniczky K. Extracellular vesicles mediate low-dose radiation-induced immune responses. Int J Radiat Biol. 2019;95(1):12–22. doi:10.1080/09553002.2018.1450533.

41. Tellier R., Li Y., Cowling B.J., Tang J.W. Recognition of aerosol transmission of infectious agents. BMC Infect Dis. 2019;19:101. doi:10.1186/s12879-019-3707-y.

42. Torres N.M.C., Rodríguez J.J.Q., Andrade P.S.P., Arriaga M.B., Netto E.M. Factors predictive of tuberculosis treatment success. PLoS One. 2019;14(12):e0226507. doi:10.1371/journal.pone.0226507.

43. Wang M.G., Luo L., Zhang Y., Liu X., Liu L., He J.Q. Treatment outcomes of tuberculous meningitis in adults. BMC Pulm Med. 2019;19:200. doi:10.1186/s12890-019-0966-8.

44. World Health Organization. Global tuberculosis report 2022. Geneva: WHO; 2022.

45. Zaharie S.D., Franken D.J., van der Kuip M., et al. Immunological architecture of granulomatous inflammation in central nervous system tuberculosis. Tuberculosis (Edinb). 2020;125:102016. doi:10.1016/j.tube.2020.102016.

ТУБЕРКУЛЬОЗ ТВАРИН В УМОВАХ РАДІАЦІЙНОГО ВПЛИВУ: МІЖСЕКТОРАЛЬНИЙ ПІДХІД «ЄДИНОГО ЗДОРОВ'Я» НА МОДЕЛІ ЛАБОРАТОРНИХ ТВАРИН

Кассіч В. Ю.[1] (ORCID: 0000-0001-9859-8036), Ушкалов В. О.[2] (ORCID: 0000-0001-5694-632X), Касяненко О. І.[1] (ORCID: 0000-0001-8453-1957), Плюта Л. В.[1] (ORCID: 0000-0001-8935-4873), Ушкалов А. В.[*,3] (ORCID: 0000-0001-8317-7909)

1 – Сумський національний аграрний університет, Суми, Україна, e-mail: kassich_v_u@ukr.net

2 – Національний університет біоресурсів і природокористування України, Київ, Україна, e-mail: ushkalov63@gmail.com

3 – Національний науковий центр «Інститут експериментальної та клінічної ветеринарної медицини», Харків, Україна, e-mail: vetdocman@gmail.com

***Резюме**. Інформація про перебіг та патогенез туберкульозу тварин - небезпечного зоонозу - на тлі радіаційного опромінення, що супроводжується зниженням питомої реактивності організму та розвитком прогресуючого імунодефіциту, залишається обмеженою. Водночас, з урахуванням сучасних глобальних і регіональних загроз техногенних катастроф, а також ризиків, пов'язаних із воєнною агресією російської федерації проти України (захоплення та пошкодження Чорнобильської АЕС, окупація Запорізької АЕС, періодичні порушення стабільності їх роботи та систематичні ракетні обстріли енергетичної інфраструктури), проблема набуває особливої актуальності в межах міжсекторальної концепції «Єдине здоров'я» (One Health), яка об'єднує здоров'я тварин, людини та стан довкілля.*

У наших дослідженнях морських свинок, опромінених різними дозами гамма-випромінювання, інфікували мікобактеріями туберкульозу різних видів (Mycobacterium bovis, M. tuberculosis, M. avium) та протягом 90 днів обстежували із застосуванням клінічних, алергічних, серологічних, патологоанатомічних і гістологічних методів з метою вивчення клінічних і патологічних проявів, імунологічних реакцій та динаміки загибелі тварин.

Встановлено дозозалежне прискорення летальності та генералізації туберкульозного процесу в опромінених тварин, особливо за дії сублетальних і летальних доз гамма-випромінювання (150–200 Р; 0,0387–0,0516 Кл/кг), що супроводжувалося розвитком гострої променевої хвороби, вираженими судинними порушеннями та активацією ексудативно-некротичних процесів. Патологоанатомічні зміни мали характер зливного казеозно-некротичного ураження органів-мішеней.

Отримані результати свідчать, що радіаційне ураження суттєво модифікує перебіг туберкульозу, прискорюючи розвиток інфекційного процесу та посилюючи його тяжкість, що має важливе значення для оцінки епізоотичних і епідеміологічних ризиків у зонах потенційного

або реального радіаційного забруднення. Дослідження підкреслює необхідність інтегрованого підходу до контролю зоонозних інфекцій у надзвичайних умовах у межах концепції «One Health», особливо в умовах воєнних загроз ядерній та радіаційній безпеці.
Ключові слова: мікобактерії, патогенез, гамма-опромінення, алергія
DOI: 10.31073/onehealthjournal2026-II-02