

A NEW LOOK AT THE ALIMENTARY ROUTE OF TUBERCULOSIS TRANSMISSION USING A RABBIT MODEL (EXPERIMENTAL STUDY)

**Reshetnikov M.N.^{1,2},
Plotkin D.V.^{1,3},
Vinogradova T.I.⁴,
Dogonadze M.Z.⁴,
Ivanova D.A.^{1,2},
Zuban O.N.^{1,2},
Yablonsky P.K.⁴**

¹ Moscow Research and Clinical Center for Tuberculosis Control (Stromynka Str., 10, Moscow 107014, Russian Federation)

² Russian Medical Academy of Continuous Professional Education (Barrikadnaya Str., 2/1-1, Moscow 125993, Russian Federation)

³ Pirogov Russian National Research Medical University (Ostrovityanova Str., 1/6, Moscow 117513, Russian Federation)

⁴ Saint-Petersburg State Phthisiopulmonology Research Institute (Ligovskiy Av., 2-4, Saint-Petersburg 191036, Russian Federation)

Corresponding author:
Mikhail N. Reshetnikov
e-mail: neipogen@list.ru

ABSTRACT

Background. The increase in the number of cases of generalized tuberculosis infection with intestinal damage, especially in HIV-positive patients, underscores the need for further study of this problem. Tuberculosis is transmitted by airborne droplets; however, there are known cases of intestinal tuberculosis in which infection occurred through the alimentary route. Since such cases are extremely rare, there are likely specific immunological and epidemiological conditions that allow *Mycobacterium tuberculosis* to penetrate the intestinal wall and develop primary inflammation.

The aim. To study the possibility of an alimentary route of tuberculosis infection in immunocompetent and immunocompromised individuals using a reproducible rabbit model.

Materials and Methods. The experiment involved rabbits, divided into three groups. Animals were orally administered a suspension of a standard laboratory isolate *Mycobacterium tuberculosis* H37Rv. The first group included immunocompetent rabbits immunosuppressed with infliximab, the second group had animals reinfected against the background of immunodeficiency, and the third group included rabbits with a combination of drug-induced immunosuppression and chemically induced enterocolitis. To assess the results of infection, clinical observation, detection of MTB DNA in feces, skin test with ATR, pathoanatomical, histological and molecular genetic examination of organs were used.

Results. Despite the temporary shedding of MTB DNA in feces (60–70 %), no signs of tuberculous inflammation in the organs were present in animals in groups 1 and 2. In group 3, macro- and microscopic signs of tuberculosis were detected in the lungs of 83.3 % of the animals and in the mesenteric/intrathoracic lymph nodes of 58.3 %. At the same time, no specific granulomatous intestinal lesions were found in any of the groups.

Conclusion. The development of tuberculous enterocolitis in rabbits following oral infection is unlikely. The alimentary route of infection can lead to generalized tuberculosis only when two key predisposing factors are present: systemic immunosuppression and previous damage to the intestinal barrier.

Keywords: experimental tuberculosis, alimentary route of infection, animal model, rabbit, model of tuberculous enterocolitis, erosions and ulcers of the intestine, dissemination

Received: 18.11.2025
Accepted: 29.05.2026
Published: 23.07.2026

For citation: Reshetnikov M.N., Plotkin D.V., Vinogradova T.I., Dogonadze M.Z., Ivanova D.A., Zuban O.N., Yablonsky P.K. A new look at the alimentary route of tuberculosis transmission using a rabbit model (experimental study). *Acta biomedica scientifica*. 2026; 11(3): 171-181. doi: 10.29413/ABS.2026-11.3.21

НОВЫЙ ВЗГЛЯД НА АЛИМЕНТАРНЫЙ ПУТЬ ЗАРАЖЕНИЯ ТУБЕРКУЛЕЗОМ С ИСПОЛЬЗОВАНИЕМ КРОЛИЧЬЕЙ МОДЕЛИ (ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ)

Решетников М.Н.^{1,2},
Плоткин Д.В.^{1,3},
Виноградова Т.И.⁴,
Догондзе М.З.⁴,
Иванова Д.А.^{1,2},
Зубань О.Н.^{1,2},
Яблонский П.К.⁴

¹ ГБУЗ «Московский городской научно-практический центр борьбы с туберкулезом Департамента здравоохранения города Москвы» (107014, г. Москва, Стромынка, д. 10, Россия)

² ФГБОУ ДПО «Российская медицинская академия непрерывного профессионального образования» (125993, г. Москва, ул. Баррикадная, д. 2/1, стр. 1, Россия)

³ ФГАОУ ВО «Российский национальный исследовательский медицинский университет им. Н.И. Пирогова» Минздрава РФ (117513, г. Москва, ул. Островитянова д. 1, Россия)

⁴ ФГБУ «Санкт-Петербургский научно-исследовательский институт фтизиопульмонологии» Минздрава РФ (191036, Санкт-Петербург, Лиговский пр., д. 2-4, Россия)

Автор, ответственный за переписку:
Решетников Михаил Николаевич
e-mail: neipogen@list.ru

РЕЗЮМЕ

Обоснование. Увеличение числа случаев генерализованной туберкулезной инфекции с поражением кишечника, особенно у ВИЧ-позитивных пациентов диктует необходимость дальнейшего изучения данной проблемы. Туберкулез передается воздушно-капельным путем, однако известны случаи туберкулеза кишечника, при которых инфицирование произошло алиментарным путем. Поскольку такие случаи крайне редки, вероятно, существуют особые иммунологические и эпидемиологические условия для проникновения *Mycobacterium tuberculosis* в стенку кишки и развития в ней первичного воспаления.

Целью настоящего исследования является изучение возможности алиментарного пути заражения туберкулезной инфекцией у иммунокомпетентных и иммунокомпрометированных особей на воспроизводимой кроличьей модели.

Материалы и методы. В эксперименте были использованы кролики, распределенные на три группы. Животным перорально вводили суспензию культуры стандартного лабораторного штамма *Mycobacterium tuberculosis* H37Rv. Первая группа включала иммунокомпетентных и иммуносупрессированных инфликсимабом кроликов, вторая – животных с повторным заражением на фоне иммунодефицита, третья – кроликов с комбинацией медикаментозной иммуносупрессии и химически индуцированного энтероколита. Для оценки результатов заражения использовали клиническое наблюдение, детекцию ДНК МБТ в кале, кожную пробу с АТР, патологоанатомическое, гистологическое и молекулярно-генетическое исследование органов.

Результаты. У животных 1-й и 2-й групп, несмотря на временное выделение ДНК МБТ с калом (60–70 %), признаки туберкулезного воспаления в органах отсутствовали. В 3-й группе макро- и микроскопические признаки туберкулеза выявлены в легких у 83,3 % животных и в мезентериальных/внутригрудных лимфатических узлах у 58,3 %. При этом специфического гранулематозного поражения кишечника не обнаружено ни в одной из групп.

Заключение. Развитие туберкулезного энтероколита у кроликов при пероральном заражении маловероятно. Алиментарный путь инфицирования может приводить к генерализованному туберкулезу только при сочетании двух ключевых предрасполагающих факторов: системной иммуносупрессии и предшествующего повреждения кишечного барьера.

Ключевые слова: экспериментальный туберкулез, алиментарный путь заражения, животная модель, кролик, модель туберкулезного энтероколита, эрозия и язвы кишечника, диссеминация

Статья поступила: 18.11.2025
Статья принята: 29.05.2026
Статья опубликована: 23.07.2026

Для цитирования: Решетников М.Н., Плоткин Д.В., Виноградова Т.И., Догондзе М.З., Иванова Д.А., Зубань О.Н., Яблонский П.К. Новый взгляд на алиментарный путь заражения туберкулезом с использованием кроличьей модели (экспериментальное исследование). *Acta biomedica scientifica*. 2026; 11(3): 171-181. doi: 10.29413/ABS.2026-11.3.21

INTRODUCTION

Despite significant progress in the fight against tuberculosis (TB), it continues to be a global infectious threat to the world's population. The HIV pandemic, the use of genetically engineered biological drugs (interleukin inhibitors) and glucocorticoids, as well as comorbid conditions such as diabetes mellitus, autoimmune and oncological diseases have created a significant number of immunocompromised patients. In these individuals, there have been an increasing number of cases of generalized and extrapulmonary TB, including abdominal involvement [1]. Among all forms of abdominal TB, enterocolitis is the most severe and can lead to complications such as perforation, bleeding, and intestinal obstruction [2]. Animal models can be used to study the routes of infection, disease pathogenesis, and test new therapeutic approaches.

Over the past several decades, extensive research has demonstrated that rabbits can be used as a suitable model to study pathological processes caused by *Mycobacterium tuberculosis* (MTB). This includes active TB and latent TB infection. The outcome of infection in rabbits is determined by the nature of the MTB strain that infects them, the inoculum dose, and any prior vaccination. Rabbits are also an excellent model to study extrapulmonary TB, as their clinical signs, disease progression patterns, and immunopathological reactions are similar to those seen in humans [3, 4]. Outbred rabbits have been shown to be relatively resistant to intravenous and aerosol infection with MTB cultures, and usually recover from infection within 4–6 months. After experimental aerosol infection, rabbits develop pulmonary granulomas and, in some cases, cavities that resemble those seen in humans [5].

In most cases, TB is transmitted via the airborne route. After the development of the primary focus in the lungs or mediastinal lymph nodes, the disease spreads to other organs and tissues through lymphohematogenous dissemination, when the immune system is weakened [6, 7]. However, there have been reports of cases of intestinal tuberculosis where infection occurred through the alimentary route, either through consumption of contaminated milk or ingestion of sputum [7]. These cases are extremely rare and specific immunological and epidemiological factors likely allow MTB to penetrate the intestinal wall and cause primary inflammation.

AIM

To study the possibility of an alimentary transmission of tuberculosis in immunocompetent and immunocompromised individuals using a reproducible rabbit model.

MATERIALS AND METHODS

The experimental study was conducted on 32 male Soviet Chinchilla rabbits, weighing 2540–3110 g [8]. All

animals were sourced from the Rappolovo Laboratory Animal Nursery at the Kurchatov Institute Research Center and were free from any visible signs of disease. Prior to the study, they underwent a two-week quarantine period at a certified animal facility located at the St. Petersburg Research Institute of Phthisiopulmonology under the Ministry of Health of the Russian Federation.

Rabbits were maintained under identical conditions, receiving a standard diet and water supply. All procedures involving animals were carried out in accordance with ethical principles outlined in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (CETS No. 170) and in compliance with GOST 33216-2014 Guidelines for Handling Laboratory Rodents and Rabbits [9]. The study was approved by the independent ethics committee of the St. Petersburg Research Institute of Phthisiopulmonology (Protocol No. 80.3, dated June 23, 2021), and fully complied with the decree of the chief sanitary physician of the Russian Federation, No. 4, dated January 28, 2021, which approved the sanitary regulations, SanPiN 3.3686-21, on Sanitary and Epidemiological Requirements for the Prevention of Infectious Diseases.

A suspension of the standard international virulent test strain *Mycobacterium tuberculosis* H37Rv was used as the infectious agent. This strain was obtained on August 7, 2013 from the Scientific Center for Expert Evaluation of Medicinal Products of the Ministry of Health of the Russian Federation. The inoculum dose used was 10^6 colony-forming units (CFU), which corresponds to 0.1 mg/0.5 mL in 2 mL of saline solution for rabbits. This mycobacterial suspension was administered orally using two enteric-coated gelatin capsules via a tablet dispenser (Neoterica Vetpro, Russia).

Depending on the experimental infection conditions, all animals were randomly divided into three groups. Group 1 consisted of 10 rabbits, which were divided into two subgroups: subgroup 1a ($n = 5$) receiving oral infection without prior immunosuppressive treatment, and subgroup 1b ($n = 5$) with prior drug-induced immunosuppression. Group 2 ($n = 10$) consisted of rabbits with secondary drug-induced immunodeficiency, who received oral infection twice with a 30-day interval. Group 3 ($n = 12$) included rabbits with both drug-induced immunodeficiency and chemical enterocolitis induction, before receiving MTB-containing capsules (Table 1).

To model drug-induced immunodeficiency, the levels of tumor necrosis factor- α (TNF- α) were reduced by administering the TNF- α inhibitor infliximab intravenously into the marginal ear vein. Infliximab is a chimeric murine-human monoclonal antibody produced by BIOCAD, a Russian company, and was administered at a dose of 16 mg/kg of body weight [10, 11].

Infliximab was selected as the immunosuppressive agent due to its ability to exhibit sufficient

cross-reactivity and neutralization of rabbit TNF- α *in vivo* despite differences in amino acid sequences between human and rabbit TNF- α . This has been confirmed in studies using animal models of various immune-mediated inflammatory diseases [12, 13].

To induce non-specific colitis, 0.1 % and 0.5 % dextran sulfate sodium solutions (DSS) (TdB Labs, Sweden, CAS No. 9011-18-1, molecular weight 40,000 Mw) were used, based on their safer concentrations and ability to induce catarrhal and erosive-ulcerative inflammation [14, 15]. The rabbits were given water containing the DSS solution via drinking bottles for 6 days (up to 1350 mL on average). Six animals received 0.1 % DSS solution, and the other six received 0.5 % DSS solution mixed with fennel tea to mask the unpleasant taste and odor of the solution.

During the course of the experimental study, animals were monitored on a daily basis. Body weights were recorded on days 20, 60 and 90 of the study. On day 10, samples of rabbit feces were analyzed for the presence of *Mycobacterium tuberculosis* DNA. On day 35, a skin test using recombinant tuberculosis allergen (RTA) was performed (Diaskintest, Russia). The test results were assessed 72 hours after the test.

Euthanasia was carried out on day 90 after infection by anesthetic overdose: propofol 70 mg and rocuronium bromide 30 mg were administered intravenously. During the necropsy, samples were collected for morphological and bacterial examination from the greater omentum, small and large intestinal fragments, mesenteric lymph nodes, heart, liver, spleen, kidneys,

and lungs. Samples were also taken from the intestines, lungs, lymph nodes, and omentum for molecular genetic analysis.

Biological material was labeled and fixed in a 10 % neutral buffered formalin solution. Microscopic analysis was performed using a Nikon Ci-S microscope (Nikon Corporation, Japan), with video-digital image processing to archive and analyze the material. Histological sections were stained using hematoxylin and eosin as well as the Ziehl-Neelsen technique. MTB DNA in the tissues was detected using polymerase chain reaction (PCR), employing the AmpliTub-RV reagent kit from Syntol LLC (Russia).

Data collection, correction, systematization, and visualization were performed using Microsoft Office Excel 2016. Statistical analysis was carried out using Python v. 3.12 from the Python Software Foundation¹.

Qualitative data are presented in the form of absolute numbers with percentages (%). Nominal data were compared between groups using Pearson's χ^2 test or Fisher's exact test (when expected values were <10). Quantitative data (body weight) are presented as an arithmetic mean (M) $\pm$ standard deviation (SD). The Shapiro – Wilk test was used to assess the normality of the distribution. To evaluate body weight dynamics within Group 3 (comparing paired samples on days 2 and 10), a paired Student's *t*-test was used since the data distribution did not deviate from normality. Differences were considered statistically significant at $p \leq 0.05$ and a two-tailed significance level was employed.

TABLE 1
DISTRIBUTION OF ANIMALS INTO GROUPS AND TIMEFRAMES OF VARIOUS STAGES OF THE EXPERIMENT

Groups	DSS	Infliximab	MTB	Euthanasia
Subgroup 1a (n=5)	-	-	on day 1	on day 90
Subgroup 1b (n=5)	-	on day 1	on day 2	on day 90
Group 2 (n=10)	-	on days 1 and 29	on days 2 and 30	on day 90
Group 3 (n=12)	from day 1 to day 6	on day 9	on day 10	on day 90

¹ <https://www.python.org/>

RESULTS

During the entire observation period, no differences or distinctive behavioral features were noted in rabbits from Groups 1 and 2. Both groups exhibited active feeding and drinking behavior, and their average body weight increased by 142 ± 13.5 g every 30 days. In contrast, all 12 animals in Group 3 showed behavioral changes from day 2 onwards. They exhibited lethargy, apathy, hypodynamia, and decreased appetite. These symptoms completely resolved by day 16 of the experiment. Additionally, 9 out of 12 rabbits in Group 3 developed digestive disturbances (frequent loose stools) on days 2–5 due to DSS administration, accompanied by a transient body weight loss averaging 151 ± 9.3 g ($p < 0.01$, paired Student's *t*-test). By the end of the first month of the experiment, animals in Group 3 had begun to gain weight actively, and by day 30 their activity and feeding behavior did not differ from those in Groups 1 and 2. Detection of MTB DNA in fecal samples on days 7–10 showed similar positive test rates across groups: 6 out of 10 rabbits (60 %) in Group 1, 7 out of 10 (70 %) in Group 2, and 9 out of 12 (75 %) in Group 3 ($p = 0.74$). Assessment of the skin test using recombinant tuberculosis allergen (RTA) showed erythema and papule formation only in 8 rabbits from Group 3 (66.7 %).

At the time of autopsy, there were no signs of tuberculous inflammation in the major organs and tissues of the animals in Groups 1 and 2. In the myocardium of most rabbits, there was moderate hydropic degeneration of cardiomyocytes, stromal edema, and venous congestion. In the liver, the most common findings (75 %) were moderate venous congestion, hepatocyte degeneration, mild focal lymphoid infiltration of individual portal tracts, or mild fibrosis. No pathological changes were observed in the kidneys. The serous membranes of the thoracic and abdominal cavities remained unchanged

in all animals. Signs of non-specific mild to moderate enteritis were present in four animals (20 %). Notably, rabbits that received the TNF- α inhibitor showed indirect signs of immunosuppression (in the spleen and lymph nodes), such as reduced follicle size, indistinct follicle borders, a weak macrophage response, and congestion in the splenic red pulp. In the lungs of 13 animals (65 %), signs of non-specific bronchitis, pneumonia, or lung abscess were observed (Fig. 1). Bacterioscopic and molecular genetic methods did not detect acid-fast bacilli (AFB) or MTB DNA in any of the examined organs and tissues of the animals in Groups 1 and 2, while specific signs of tuberculous inflammation were detected in the rabbits in Group 3.

The autopsy of rabbits from Group 3 showed macroscopic signs of complete tuberculous infection in the lungs in 10 animals (83.3 %), as well as enlargement of mesenteric and intrathoracic lymph nodes in 7 animals (58.3 %). The examination of small and large intestine loops revealed signs of serous edema, transient areas of visceral peritoneal hyperemia, and erosion or shallow ulceration on the mucous membrane in 75 % of cases ($n = 9$).

The histological examination of intestinal tissue did not reveal any specific granulomatous reaction in any of the animals. Instead, the observed changes were consistent with a non-specific enterocolitis, characterized by submucosal edema, leukocytic infiltration, villous atrophy, and the presence of erosions (Fig. 2). AFB were not detected in the intestinal tissues using Ziehl – Neelsen staining, and MTB DNA was not detected in any of the samples analyzed using PCR.

In individual mediastinal and mesenteric lymph nodes ($n = 7$; 58.3 %), disruption of the lymphoid tissue architecture was observed, due to the formation of epithelioid cell granulomas with caseous central masses. The lymphoid tissue was preserved in subcapsular regions. The granulomatous inflammation consisted primarily of activated macrophages with abundant

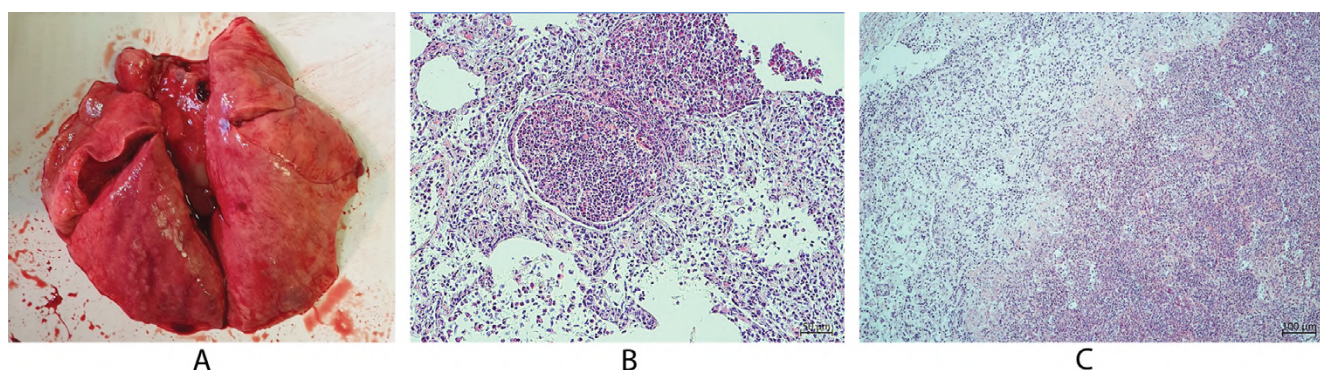


FIG. 1.

A – gross specimen: rabbit lungs with signs of pneumonia; B – lung tissue with signs of nonspecific acute purulent endobronchitis, hematoxylin and eosin staining, scale bar 50 μ m; C – acute abscess in rabbit lung tissue; hematoxylin and eosin staining, scale bar 100 μ m

cytoplasm, epithelioid cells, and occasionally Langhans giant cells. Sections stained with the Ziehl – Neelson method revealed numerous microcolonies of acid-fast bacilli (Fig. 3).

Detection of MTB DNA in lymph node tissue samples yielded positive results for 5 samples (41.7 %). The granulomatous inflammation was detected in the spleens of 3 rabbits (25 %) (Fig. 4).

Examination of lung tissue from 10 animals (83.3 %) in Group 3 revealed tuberculous inflammation of varying severity. There was a predominance of destructive

forms and the formation of small cavities. Numerous dystelectases, as well as productive and alternative epithelioid cell granulomas with central caseous necrosis, were observed in the alveolar pattern (Fig. 5). There was also moderate interstitial inflammation with an exudative component in the surrounding tissues. Single AFB was detected in sections stained with the Ziehl – Neelsen method. The kidneys, liver, and myocardium of these rabbits also showed moderate signs of intoxication, including venous congestion, hemorrhage, degenerative changes, and stromal edema in the internal

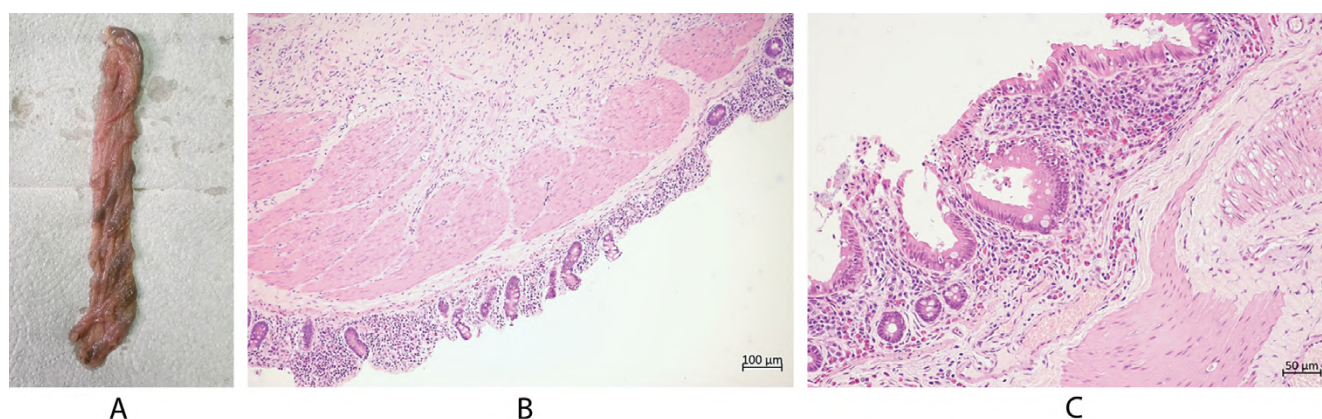


FIG. 2.

A – gross specimen: rabbit intestine (view of mucosa) with signs of enteritis; B – eroded intestinal mucosa with signs of active chronic inflammation; hematoxylin and eosin staining, scale bar 100 µm; C – intestinal mucosa with signs of chronic inflammation, superficial microerosions with translocation of microbial flora under the epithelium of the extrusion zone; hematoxylin and eosin staining, scale bar 100 µm

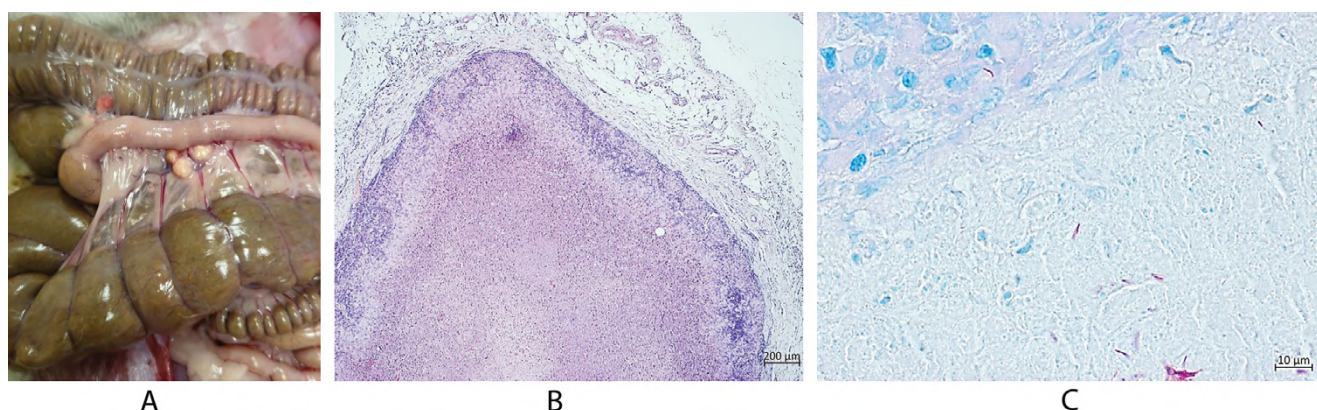


FIG. 3.

A – gross specimen: enlarged mesenteric lymph nodes of the rabbit small intestine; B – tuberculous mesenteric adenitis with subtotal involvement, formation of an extensive caseous-necrotic focus surrounded by an epithelioid cell wall with occasional Langhans-type multinucleated giant cells, stained with hematoxylin and eosin, scale bar 200 µm; C – acid-fast mycobacteria in the cytoplasm of epithelioid cells and freely present in necrotic masses of the affected lymph node, Ziehl – Neelsen staining, scale bar 10 µm

organs. Reactive changes and blurred follicular structures were noted in the immune system organs (spleen and other lymph nodes).

Therefore, in rabbits from Groups 1 and 2, even under conditions of immunosuppression and repeated oral exposure, no signs of tuberculous inflammation were detected. In contrast, the vast majority of animals in Group 3 exhibited pulmonary and lymph node foci of tuberculosis, with no granulomatous involvement of the intestines.

DISCUSSION

The alimentary route of tuberculosis transmission is considered in the literature as an additional route of infection [16, 17]. Additionally, the development of tuberculous enterocolitis has also been associated with the consumption of contaminated dairy products or the ingestion of infected sputum in the presence of a primary pulmonary tuberculosis focus [18]. Experimental evidence supporting these assertions was obtained over a century ago and was primarily descriptive. For instance, in 1868, J.A. Villemin added sputum from tuberculosis patients to the diet of several guinea pigs and euthanized the animals after three months. Upon autopsy, erosive changes were observed in the small intestine and cecum, although histological or culture confirmation of tuberculosis infection was not provided in this study [19]. Later, in 1933, M. McConkey and D.T. Smith conducted a series of similar experiments. Seventy-two adult guinea pigs were fed a diet mixed with infected sputum on a daily basis for four months. Half of the animals were maintained on a diet that was partially deficient in ascorbic acid.

Of these, 26 guinea pigs developed ulcerative intestinal tuberculosis. Among the remaining 35 animals, whose diet was supplemented with adequate vitamin C, only two were found to have intestinal tuberculosis with ulcers [20].

Commenting on the results of these experiments, one can note an undeniable methodological error in the choice of model animal. Guinea pigs are well known to be extremely sensitive and susceptible

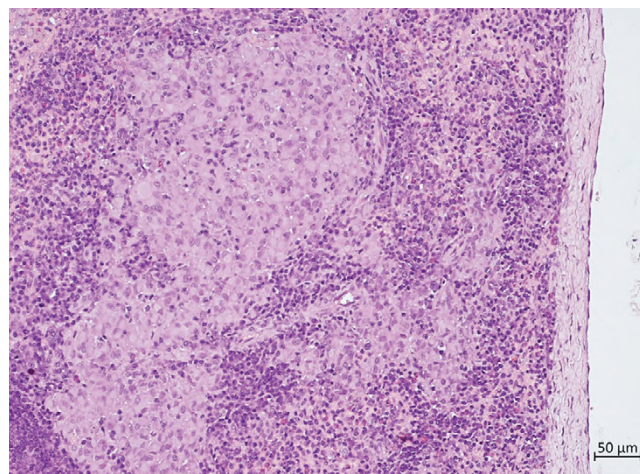


FIG. 4. Tuberculous inflammation in the spleen tissue with the formation of irregularly shaped productive epithelioid-cell granulomas containing occasional Langhans-type multinucleated giant cells, stained with hematoxylin and eosin, scale bar 50 µm

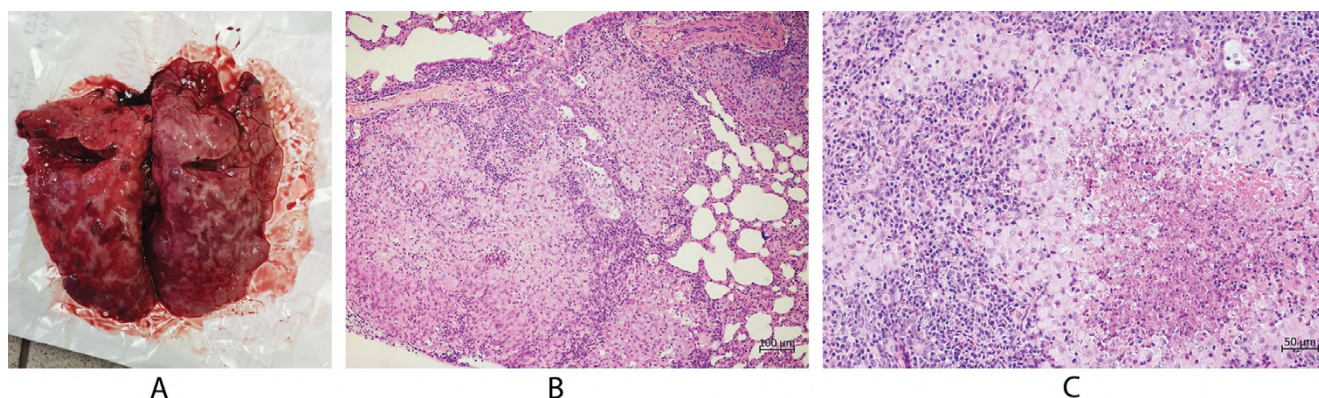


FIG. 5. A – gross specimen: lungs of a rabbit with caseous pneumonia. B, C – Tuberculous inflammation in lung tissues: subpleural acinous-nodular confluent productive epithelioid-cell granulomas (B) with Langhans-type giant multinucleated cells and caseous-necrotic foci (C), stained with hematoxylin and eosin, scale bar 100 µm (B), 50 µm (C)

to *Mycobacterium tuberculosis* infection through any route of transmission, including the alimentary route [21]. The digestive tract of guinea pigs does not constitute a significant barrier to mycobacteria, but in addition to the intestine, the lungs and immune system organs are likely to be affected as well [22].

The most reliable information regarding the alimentary route of infection in guinea pigs was obtained from an experiment conducted by J. Gao et al. in 2018 [23]. In the experimental group, animals were administered 100 mL of drinking water containing 2×10^7 CFU of the standard MTB strain H37Rv via drinking bottles for 8 hours. Euthanasia was performed at the end of the 4-month experiment. Granulomatous lesions were found in the lungs, spleen, and lymph nodes of the animals, and culture of homogenates from these tissues demonstrated the growth of MTB colonies on solid media after 1.5 months. At the same time, no specific changes indicative of tuberculous enterocolitis were detected in histological sections from various intestinal segments. It should be noted that the experimental animals were fed commercial feed that contained sufficient ascorbic acid. This may represent one of the factors in the immune defense and enteroprotective effects that prevent the development of inflammation in the intestinal wall [23].

Using the PubMed and Scopus databases, no publications were found on the alimentary infection of rabbits with MTB or on the modeling tuberculous enterocolitis in this species. The only exception is the study conducted by V.G. Shtefko (1941), who administered adrenaline intraperitoneally to rats and rabbits followed by intravenous infection with *M. tuberculosis* emulsion [24]. According to the author, this combination led to the development of ulcerative intestinal tuberculosis in 70–80 % of the surviving animals. However, most of the rabbits and rats died due to adrenaline shock. It is important to note that the reliability of these data is questionable, as the study lacks evidence that the ulcers were of a tuberculous origin. It is more likely that “stress-induced” adrenal ulcers of the intestine were described [25].

In the current study, we were also unable to replicate either infiltrative or erosively-ulcerative tubercular enterocolitis. However, we obtain reliable evidence of alimentary infection in laboratory animals, resulting in the development of generalized forms of tuberculosis. Despite the vulnerability of the rabbit’s intestine (due to its weak motility and physiological stasis), the immune system of these animals adequately responds to the presence of MTB in the food bolus [26, 27]. According to our observations, when immunocompetent rabbits are orally infected with mycobacterial suspensions, tuberculous inflammation does not develop due to the natural immune barrier provided by intestinal epithelial cells, immunocompetent agranulocytes, and immune proteins prevents it. Most likely, the majority of the pathogen is destroyed

in the intestine and undergoes temporary migration through the gastrointestinal tract, as seen in rabbits from Groups 1 and 2, where positive MTB DNA was detected in fecal samples. Drug-induced immunosuppression and repeated exposure to MTB suspension did not significantly contribute to the development of localized or systemic tuberculous inflammation in rabbits in Groups 1 and 2, as demonstrated by skin testing with RTA and post-mortem examination.

In contrast, data obtained from the autopsy of rabbits in Group 3 indicate that under conditions of systemic immunosuppression and disruption of the intestinal immune barrier (DSS-induced enterocolitis), MTB penetrates the intestinal wall. However, specific inflammation in the mucosal and submucosal layers does not develop, or it is abortive. Nevertheless, involvement of the mesenteric lymph nodes in approximately half of the cases (58 %; $p = 0.001$) may indirectly indicate MTB penetration from the intestine to these nodes, with formation of a so-called primary tuberculous complex, and subsequent persistence of MTB in the lymphoid tissue. This conclusion is supported by a positive RTA skin testing in 76 % of cases. The autopsy findings of rabbits in Group 3 suggest that, against the background of immunodeficiency, generalization of the infection occurs with lymphohematogenous involvement of the spleen (25 %; $p = 0.063$) and lungs (83 %; $p < 0.001$). Therefore, it is only when two predisposing factors are present in combination (immunosuppression and inflammation-induced damage) that favorable conditions are created for the entry of *Mycobacterium tuberculosis* into the intestinal wall and subsequent circulation, contributing to the development of tuberculous lymphadenitis, splenitis, and pulmonary tuberculosis, without typical intestinal involvement.

RESUME

Oral administration of *M. tuberculosis* to immunocompetent rabbits does not lead to the development of tuberculous enterocolitis or generalized tuberculosis, despite the temporary shedding of MTB DNA in the feces. This indicates a transient presence of mycobacteria in the intestine. Neither drug-induced immunosuppression nor repeated oral challenges are sufficient to overcome the intestinal barrier and cause specific tuberculous inflammation in the intestines or other organs.

The combined effect of immunosuppression and chemical damage to the intestinal mucosa (DSS-induced enterocolitis) creates conditions for MTB to penetrate into the systemic circulation, leading to the subsequent development of tuberculous lymphadenitis (58.3 %) and destructive pulmonary tuberculosis (83.3 %).

The absence of granulomatous inflammation in the intestine following alimentary infection,

in conjunction with lesions in the lungs and lymphatic system, suggests that the intestine acts as a barrier that is overcome by mycobacteria only when its protective properties are severely compromised, with subsequent infection progressing via lymphohematogenous dissemination.

The rabbit is an adequate model for studying the alimentary route of tuberculosis infection, particularly for analyzing the conditions that promote systemic dissemination of the infection without the formation of a primary intestinal focus.

Detection of MTB DNA or AFB in feces does not serve as reliable evidence of either tuberculous enterocolitis or tuberculosis infection in general.

CONCLUSION

The present study demonstrates that the alimentary route of tuberculosis infection requires not only the presence of the pathogen in the intestinal lumen, but also a significant disruption of local and systemic immune defense mechanisms. These findings are significant for comprehending the pathogenesis of rare cases of abdominal tuberculosis in humans, and they confirm the role of the intestine as a potential route of entry for the infection under specific circumstances.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Hatgoankar K, Hatgaonkar A, Dawande P. Revisiting the Era of Intestinal Tuberculosis: A Case Presenting as Small Bowel Obstruction with Classical Imaging and Histopathological Appearances. *Cureus*. 2024; 16(1): e51836. doi: 10.7759/cureus.51836
2. Reshetnikov MN, Plotkin DV, Sinitsyn MV, Gafarov UO, Belentseva OV. Surgical complications of abdominal tuberculosis in patients with different immune status. *Experimental and Clinical Gastroenterology*. 2019; (4): 46–53. (In Russ.). [Решетников М.Н., Плоткин Д.В., Синицын М.В., Гафаров У.О., Беленцева О.В. Хирургические осложнения абдоминального туберкулеза у пациентов с различным иммунным статусом. *Экспериментальная и клиническая гастроэнтерология*. 2019; (4): 46–53.]. doi: 10.31146/1682-8658-ecg-164-4-46-53
3. Corleis B, Bastian M, Hoffmann D, Beer M, Dörhöi A. Animal models for COVID-19 and tuberculosis. *Front Immunol*. 2023; 14: 1223260. doi: 10.3389/fimmu.2023.1223260
4. Subbian S, Tsenova L, O'Brien P, Yang G, Kushner NL, Parsons S, et al. Spontaneous latency in a rabbit model of pulmonary tuberculosis. *Am J Pathol*. 2012; 181(5): 1711–24. doi: 10.1016/j.ajpath.2012.07.019
5. Elkington P, Lerm M, Kapoor N, Mahon R, Piekaar E, Huh D, et al. *In Vitro* Granuloma Models of Tuberculosis: Potential and Challenges. *J Infect Dis*. 2019; 219(12): 1858–1866. doi: 10.1093/infdis/jiz020
6. Gómez-Piña JJ. Tuberculosis peritoneal. *Med Int Méx*. 2018; 34(3): 490–496. doi: 10.24245/mim.v34i3.2171
7. Al-Zanbagi AB, Shariff MK. Gastrointestinal tuberculosis: A systematic review of epidemiology, presentation, diagnosis and treatment. *Saudi J Gastroenterol*. 2021; 27(5): 261–274. doi: 10.4103/sjg.sjg_148_21
8. Plotkin DV, Reshetnikov MN, Vinogradova TI, Zyuzya YuR, Gafarov UO, Zhuravlev VY, et al. Choosing a model object for reproducing abdominal tuberculosis. *Tuberculosis and socially significant diseases*. 2021; 9(3): 18–25. (In Russ.). [Плоткин Д.В., Решетников М.Н., Виноградова Т.И., Зюзя Ю.Р., Гафаров У.О., Журавлев В.Ю., и др. Выбор модельного объекта для воспроизведения абдоминального туберкулеза. *Туберкулёз и социально значимые заболевания*. 2021; 9(3): 18–25.]. doi: 10.54921/2413-0346-2021-12-3-18-25
9. GOST 33216–2014. *Rules of work with laboratory rodents and rabbits*. Moscow, Standartinform Publ., 2016. (In Russ.). [ГОСТ 33216–2014. *Правила работы с лабораторными грызунами и кроликами*. – М.: Стандартинформ, 2016.].
10. Plotkin DV, Vinogradova TI, Reshetnikov MN, Ariel BM, Zyuzya YR, Zhuravlev VY, et al. Features of the pathogenetic mechanisms of tuberculous peritonitis in an experiment. *Bulletin of RSMU*. 2021; (4): 20–7. doi: 10.24075/brsmu.2021.036
11. Shekunova EV, Kovaleva MA, Makarova MN, Makarov VG. Dose Selection in Preclinical Studies: Cross-Species Dose Conversion. *The Bulletin of the Scientific Centre for Expert Evaluation of Medicinal Products*. 2020; 10(1): 19–28. (In Russ.). [Шекунова Е.В., Ковалева М.А., Макарова М.Н., Макаров В.Г. Выбор дозы препарата для доклинического исследования: межвидовой перенос доз. *Ведомости Научного центра экспертизы средств медицинского применения*. 2020; 10(1): 19–28.]. doi: 10.30895/1991-2919-2020-10-1-19-28
12. Zhang Q, Lv Hh, Chen A, Liu F, Wu X. Efficacy of infliximab in a rabbit model of osteoarthritis. *Connect Tissue Res*. 2012; 53(5): 355–358. doi: 10.3109/03008207.2012.661001
13. Henriksen ML, Teisner A, Kjeldsen J, Kallio-koski O, Hau J, Hansen SW. The Effect of Induced Antibodies with Respect to Neutralization, Clearance Rate and Functional Activity in a Rabbit/Infliximab Model. *In Vivo*. 2016; 30(5): 557–565.
14. Leonardi I, Nicholls F, Atrott K, Cee A, Tewes B, Greinwald R, et al. Oral administration of dextran sodium sulphate induces a caecum-localized colitis in rabbits. *Int J Exp Pathol*. 2015; 96(3): 151–62. doi: 10.1111/iep.12117
15. Reshetnikov MN, Vinogradova TI, Zyuzya YuR, Plotkin DV, Volkov AA, Dogonadze MZ, et al. Choosing the optimal concentration of dextran sulfate sodium

for reproducing chemically induced colitis in rabbits. *Experimental and Clinical Gastroenterology*. 2023; (9): 125–130. (In Russ.). [Решетников М.Н., Виноградова Т.И., Зюзя Ю.Р., Плоткин Д.В., Волков А.А., Догондзе М.З., и др. Выбор оптимальной концентрации декстрана сульфата натрия для воспроизведения химически индуцированного колита у кроликов. *Экспериментальная и клиническая гастроэнтерология*. 2023; (9): 125–130.]. doi: 10.31146/1682-8658-ecg-217-9-125-130

16. Long R, Divangahi M, Schwartzman K. Chapter 2: Transmission and pathogenesis of tuberculosis. *Canadian Journal of Respiratory, Critical Care, and Sleep Medicine*. 2022; 6(sup 1): 22–32. doi: 10.1080/24745332.2022.2035540

17. Perelman MI. Phthysiology. *National Leadership*. Moscow: Publishing house GEOTAR-Media, 2007, 512 p. (National Guidelines Series). (In Russ.). [Перельман М.И. *Фтизиатрия. Национальное руководство*. М.: ГЭОТАР-Медна, 2007. 512 с. (Серия «Национальные руководства»)].

18. Tobin EH, Khatri AM. *Abdominal Tuberculosis*. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. URL: <https://www.statpearls.com/point-of-care/30658>. [date of access: November 16, 2025].

19. Villemin JA. *Études sur la tuberculose*. Paris: Baillière, 1868: 241 p. (In French).

20. McConkey M, Smith DT. The relation of vitamin c deficiency to intestinal tuberculosis in the guinea pig. *J Exp Med*. 1933; 58(4): 503–12. doi: 10.1084/jem.58.4.503

21. Clark S, Hall Y, Williams A. Animal models of tuberculosis: Guinea pigs. *Cold Spring Harb Perspect Med*. 2014; 5(5): a018572. doi: 10.1101/cshperspect.a018572

22. Larenas-Muñoz F, Ruedas-Torres I, Hunter L, Bird A, Agulló-Ros I, Winsbury R, et al. Characterisation and development of histopathological lesions in a guinea pig model of *Mycobacterium tuberculosis* infection. *Front Vet Sci*. 2023; 10: 1264200. doi: 10.3389/fvets.2023.1264200

23. Gao J, Guo M, Teng L, Bao R, Xian Q, Wang X, et al. Guinea pig infected with *Mycobacterium tuberculosis* via oral consumption. *Journal of Applied Animal Research*. 2018; 46(1): 1323–1328. doi: 10.1080/09712119.2018.1505622

24. Shtefko VG. Experimental intestinal tuberculosis and the method of its production. *Problems of tuberculosis*. 1941; 5: 15–20 (In Russ.). [Штефко В.Г. Экспериментальный туберкулёз кишечника и методика его получения. *Проблемы туберкулёза*. 1941; 5: 15–20.].

25. Belel O, Basaca DG, Olariu L, Pantea M, Bozgan D, Nanu A, et al. The Interaction between Stress and Inflammatory Bowel Disease in Pediatric and Adult Patients. *J Clin Med*. 2024; 13(5): 1361. doi: 10.3390/jcm13051361

26. Casto-Rebollo C, Argente MJ, García ML, Blasco A, Ibáñez-Escriche N. Effect of environmental variance-based resilience selection on the gut metabolome of rabbits. *Genet Sel Evol*. 2023; 55(1): 15. doi: 10.1186/s12711-023-00791-5

27. Chassaing B, Kumar M, Baker MT, Singh V, Vijay-Kumar M. Mammalian gut immunity. *Biomed J*. 2014; 37(5): 246–58. doi: 10.4103/2319-4170.130922

Information about author

Mikhail N. Reshetnikov – Cand. Sc. (Med.), surgeon, leading researcher of the Moscow Research and Clinical Center for Tuberculosis Control of the Moscow Healthcare Department; associate professor of the Department of Phthysiology of the Russian Medical Academy of Continuous Professional Education of the Ministry of Healthcare of the Russian Federation; e-mail: taxol@bk.ru, <https://orcid.org/0000-0002-4418-4601>

Dmitriy V. Plotkin – Dr. Sc. (Med.), associate professor, surgeon, leading researcher of the Moscow Research and Clinical Center for Tuberculosis Control of the Moscow Healthcare Department; professor of Buyanov General Surgery Department, Institute of Surgery, Pirogov Russian National Research Medical University of the Ministry of Health of the Russian Federation; e-mail: kn13@list.ru, <https://orcid.org/0000-0002-6659-7888>

Tatyana I. Vinogradova – Dr. Sc. (Med.), professor, chief research officer at the Saint-Petersburg State Research Institute of Phthysiopulmonology of the Ministry of Healthcare of the Russian Federation; head of the Laboratory of Experimental Tuberculosis; e-mail: vinogradova@spbniif.ru, <https://orcid.org/0000-0002-5234-349X>

Marine Z. Dogonadze – Cand. Sc. (Biol.), senior research at the Saint-Petersburg State Research Institute of Phthysiopulmonology of the Ministry of Healthcare of the Russian Federation; e-mail: marine-md@mail.ru, <https://orcid.org/0000-0002-9161-466X>

Diana A. Ivanova – Dr. Sc. (Med.), scientific secretary of the Moscow Research and Clinical Center for Tuberculosis Control of the Moscow Healthcare Department; professor of the Department of Phthysiology of the Russian Medical Academy of Continuous Professional Education of the Ministry of Healthcare of the Russian Federation; e-mail: d-ivanova@list.ru, <https://orcid.org/0000-0001-5686-536X>

Oleg N. Zuban – Dr. Sc. (Med.), professor, deputy director for Research and Clinical Activities of the Moscow Research and Clinical Center for Tuberculosis Control of the Moscow Healthcare Department; professor of the Department of Phthysiology of the Russian Medical Academy of Continuous Professional Education of the Ministry of Healthcare of the Russian Federation; e-mail: pan_zuban@msn.com, <https://orcid.org/0000-0003-4459-0244>

Petr K. Yablonsky – Dr. Sc. (Med.), professor, director of the Saint-Petersburg State Research Institute of Phthisiopulmonology of the Ministry of Healthcare of the Russian Federation; e-mail: glhirurg2@mail.ru, <https://orcid.org/0000-0003-4385-9643>