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STUDY OF BIOLOGICAL PROPERTIES OF SOME SPECIES OF ATYPICAL MYCOBACTERIA IN GUINEA PIGS

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Summary. As the eradication of tuberculosis in farm animals progresses, the importance of atypical mycobacteria (AM) and various types of mycobacteriosis is becoming more significant. These mycobacteria can sensitize animals to tuberculin and, in some cases, cause tuberculosis-like lesions, complicating the implementation of anti-tuberculosis measures. The study aimed to assess the persistence of *M. scrofulaceum*, *M. avium*, and *M. phlei* in guinea pigs after single and three oral administrations, in comparison to *M. bovis*. It also examined their ability to cause sensitization to allergens and the duration of this effect based on bacterial load and elimination rates. Results indicated that the persistence of *M. avium*, *M. scrofulaceum*, and *M. phlei* in guinea pigs was temporary following oral administration. These bacteria caused sensitization but did not lead to the development of an infectious pathological process. After three administrations, compared to a single administration, the excretion time of *M. avium* and *M. scrofulaceum* in feces increased from 15 days to 30 days (*M. phlei* remained 15 days). Additionally, the allergic response to the allergens from atypical mycobacteria extended from 60 days to 90 days (for *M. phlei*, it increased from 30 days to 60 days). The persistence of *M. bovis* was a permanent colonization, the excretion of the pathogen in the feces occurred after the dissemination of the pathological process, i. e., in the later stages of the disease, the allergic state persisted for up to 90 days. The duration of the allergic state, persistence, and elimination depended on the bacterial load and the type of mycobacteria

Keywords: persistence, sensitizing, allergic state, pathogenicity

Introduction. The main method of *in vivo* diagnosis of tuberculosis in farm animals is the intradermal tuberculin test (PPD) for mammals, which is the most informative test in the system of early diagnosis of tuberculosis. It is known that not only tuberculosis pathogens but also certain types of atypical mycobacteria (AM), which have antigens closely related to pathogenic species, are capable of sensitizing a macroorganism to tuberculin (Jenkins et al., 2018). Detection of animals with non-specific reactions to tuberculin leads to unwarranted slaughter of healthy animals, causing economic losses to livestock farms. The phenomenon of para-allergies in human and veterinary medicine is widespread and has a steady tendency to increase, as evidenced by the literature (Zavgorodnii et al., 2018, 2021; Zavgorodniy et al., 2021, 2023; Pavlik, Ulmann and Falkinham, 2022), and Ukraine is no exception in this regard. Thus, in tuberculosis-free livestock farms, 0.02–0.03% of animals reacting to tuberculin are detected annually.

According to official data, in 2024, allergic tests identified reactive animals on 14 farms in Vinnytsia, 3 — in Zhytomyr, 5 — in Kyiv, 7 — in Khmelnytskyi, 16 — in Cherkasy, 1 — in Sumy, 1 — in Volyn, and 1 — in Chernihiv regions. However, none of these animals were confirmed to have tuberculosis through necropsy or bacteriological methods.

At present, there are more than 160 species of AM recognized. AM are classified as saprophytes, symbionts, and commensals. They are isolated from the environment, water, soil, and biofilm, meaning that sensitized animals may potentially be exposed to high concentrations of bacteria (up to 10^6/mg CFU) over an extended period, which can result in the development of infection or the induction of an immune response (Falkinham, 2021). In the field of humane medicine, there has been a notable rise in the isolation and diagnosis of mycobacteriosis cases worldwide, though the reasons for this trend are not fully understood. However, the development of new methods, particularly in the areas of molecular biology and diagnostics, has played a significant role in the detection and identification of new AM species. Furthermore, it has been observed that as the number of tuberculosis cases decreases, the number of AM infections increases (Falkinham, 2021; Pavlik et al., 2022). The most common clinical manifestations of mycobacteriosis in humans include lung lesions caused by *Mycobacterium avium* complex (MAC) (Pavlik, Ulmann and Falkinham, 2022; Zhurilo, Barbova and Sladkova, 2020; Heifets, 2004; Field and Cowie, 2006; Mourad, Baker and Stout, 2021; Kim et al., 2021; Park, Kang and Choi, 2021), lymphadenitis (*M. scrofulaceum*), and skin diseases. In addition, there are cases of soft tissue and bone infections, as well as disseminated diseases caused by *M. ulcerans, M. marinum, M. fortuitum, M. abscessus*, and *M. chelonae* (Griffith, 2007; Goldstein et al., 2019; Lobo and Lun, 2021; Trčko, Plaznik and Miljković, 2021; Hendrikx et al., 2022).

In veterinary medicine, AM are also of great clinical importance (Weese and Gomez-Nieto, 2016; Silva et al., 2019; Mönki et al., 2016; Li et al., 2023). The majority of mycobacteria pathogenic in farm animals (artiodactyls, ungulates, pigs, poultry, rabbits), as in human medicine, belong to MAC (Harris and Barletta, 2001; Hewes et al., 2005; Agdestein et al., 2012; Thorel, Huchzermeyer and Michel, 2001; Manning and Collins, 2001), and

M. genavenze is most commonly detected in exotic birds, especially parrots.

The detection of AM in biological samples from animals cannot be the basis for the diagnosis of tuberculosis. To determine the clinical significance of a particular type of AM, it is necessary to study its biological properties when repeatedly introduced into the body of animals. It is known that the development of tuberculosis is based on the intracellular persistence of mycobacteria. Long-term persistence in a macroorganism is of epidemiological importance as a mechanism of the infectious process. However, the temporary presence of mycobacteria in the body should be distinguished from their permanent colonization. The latter may indicate a possible invasion of tissues with the possibility of further development of the pathological process (Griffith et al., 2007).

Based on the foregoing, the study of the terms of persistence of AM in the macroorganism and their ability to cause sensitization to allergens, the rate of excretion, and the duration of the allergic state depending on the bacterial load is of great practical importance in determining the epizootic situation in livestock farming.

The study **aimed** to assess the persistence of *M. scrofulaceum*, *M. avium*, and *M. phlei* in guinea pigs after single and three oral administrations, in comparison to *M. bovis*. It also examined their ability to cause sensitization to allergens and the duration of this effect based on bacterial load and elimination rates.

Materials and methods. For infection of laboratory animals (guinea pigs), the most common species isolated from cattle, poultry, and environmental objects were used, namely atypical cultures of groups II and III according to the Runyon classification — *M. scrofulaceum* and *M. avium* and a fast-growing culture of group IV — *M. phlei*. In addition, *M. b*ovis was also used in the experiments for comparison. The mycobacterial cultures belong to the collection of mycobacterial cultures of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine). Suspensions of AM and *M. bovis* were administered to guinea pigs *per os*, by single and triple administration.

From healthy, previously non-reactive to allergens (PPD tuberculin for mammals and AAM) guinea pigs, seven experimental groups were formed (10 animals per group). Group I animals received a single dose of a suspension of *M. bovis* at a concentration of 1.0 mg/cm^3 of sterile isotonic solution. Animals in groups II–IV received suspensions of *M. scrofulaceum, M. avium, M. phlei* cultures at a concentration of 5.0 mg of bacterial mass in 1.0 cm^3 of sterile isotonic solution. Guinea pigs in groups V–VII were given the same AM cultures, but three times at a dose of 5.0 mg/cm^3 with an interval of two days. Thus, guinea pigs in groups V–VII received a total of 15.0 mg of bacterial mass from each AM culture. Animals in the control group (n = 3) received saline (*per os*).

Preparation of suspensions of mycobacterial cultures for inoculation. For the preparation of suspensions, mycobacterial cultures in the logarithmic growth phase grown on Pavlovskiy potato medium were used: *M. bovis* strain Vallee, *M. scrofulaceum* and *M. avium* after 30 days of cultivation, and the fast-growing group IV culture *M. phlei* after 5 days of cultivation. The suspension for infection was prepared as follows: the bacterial mass of each mycobacterial culture was added to a pre-weighed sterile vial containing beads and weighed. The difference between the first and second weighs determined the amount of bacterial mass. Sterile saline was then added to the vials containing atypical cultures at a rate of 1.0 cm^3 of solution per 5.0 mg of bacterial cells, and a concentration of 1.0 mg/cm^3 was prepared for *M. bovis*. The vials containing the bacterial mass were vortexed to a homogeneous suspension.

Allergic study. Mycobacterial allergens were used to determine the state of delayed-type hypersensitivity to the injected mycobacterial cultures in guinea pigs: PPD tuberculin for mammals in a standard solution and AAM, according to the 'Guidelines for the Diagnosis of Animal and Poultry Tuberculosis' (MDVMSVIMAPFU, 1994). Animals were examined 30, 60, and 90 days after administration of the cultures. Allergens were injected intradermally, tuberculin (PPD) for mammals at a dose of 25 IU/0.1 cm^3, AAM — 10 U/0.1 cm^3. The reactions were recorded after 24 h by measuring two diameters of erythema and the area (mm^2) was determined by the formula: $S = \pi r^2$.

After 30, 60, and 90 days of recording allergic reactions to the intradermal tuberculin test, three animals from each group were euthanized for pathological and bacteriological examination.

Bacterioscopic examination of feces. After 15, 30, 60, and 90 days, fecal samples were collected from each group of animals in sterile plastic containers. Sterile distilled water was added to the feces and stirred, large particles were allowed to settle, and the top layer was removed and applied to three slides. After the drops dried, the smears were stained using the Ziehl–Nielsen method.

Cultural examination of feces and pathological material. Fecal decontamination was performed using a 0.9% solution of cetylpyridinium chloride (CPC) with an exposure time of 20 h. Fecal samples were poured with distilled water, stirred, and allowed to settle for 15–20 min. From the supernatant, 10.0 cm^3 of liquid was collected into centrifuge tubes and centrifuged at 3,000 rpm for 15 min. To the precipitate, 10.0 cm^3 of 0.9% CPC solution was added, stirred, and kept at room temperature for 20 h. After exposure to the decontamination solution, the fecal samples were washed with distilled water by centrifugation at 3,000 rpm. The supernatant was discarded, and the precipitate was resuspended in a small amount of 0.85% sodium chloride solution and inoculated into 10 test tubes containing egg nutrient medium for mycobacterial culture.

The organs (liver, spleen) removed after necropsy were treated with 5.0% sulfuric acid. For this purpose, the crushed organs were rubbed with sterile sand, poured

with sterile distilled water, stirred, and allowed to settle for 15–20 min. From the supernatant, 10.0 cm^3 of liquid was taken into centrifuge tubes and centrifuged at 3,000 rpm for 15 min. To the precipitate, 10.0 cm^3 of 5.0% sulfuric acid was added, stirred, kept at room temperature for 10 min, and centrifuged at 3,000 rpm for 15 min. The precipitate was washed by centrifugation, resuspended, and inoculated into 10 tubes with nutrient medium. The cultures were incubated in a thermostat at a temperature 37.5 ± 0.5°C.

Animals that died during the experiment and euthanized after 90 days were pathologically examined for tuberculosis.

Experiments on guinea pigs were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Results and discussion. ***Bacterioscopic and cultural examination of feces.*** According to the results of microscopy (Table 1), it was found that 15 days after a single inoculation of AM (dose 5.0 mg), single (2 ± 1 per 100 fields of view) acid-fast bacilli (AFB) were detected in the feces of animals. A large number of clusters and single AFB (12 ± 3) and (20 ± 4), (13 ± 4) per 100 fields of view, respectively) were detected in animals that received a triple dose of *M. scrofulaceum, M. avium, M. phlei*). In guinea pigs infected with *M. bovis*, no mycobacteria were detected in feces collected during this period (Fig. 1).

After 30 days, no mycobacteria were detected by microscopy of feces from animals inoculated with AM at a dose of 5.0 mg. However, in the feces of guinea pigs receiving a triple dose (15 mg total), acid-fast rods *M. scrofulaceum* (5 ± 2) and *M. avium* (15 ± 3) were revealed.

The data obtained indicate that in the first two weeks, there is an active excretion of atypical mycobacteria from the macroorganism in the feces, and with an increase in the multiplicity (3 times) of the same dose (5 mg) in the body of animals, it was expected to increase the amount of excretion of AFB in the feces, which was confirmed by microscopy. It should be noted that the excretion and thus the detection of AFB in the feces decreased over time. Thus, after 30 days in the feces of guinea pigs that received 5 mg of bacterial cells once, no AFB was detected by microscopy, but with 3 times the same dose, AFBs were observed only in slow-growing cultures of *M. scrofulaceum* and *M. avium*), the saprophyte *M. phlei* was not detected by microscopy during this period.

On the 60th and 90th days after AM inoculation, no acid-fast bacilli were observed in any group of guinea pigs by fecal microscopy, but single AFB (2 ± 1/100 fields of view) were detected in the feces of some animals infected with *M. bovis*. Thus, after administration of a triple dose of *M. scrofulaceum* and *M. avium* to guinea pigs, their temporary presence in the body was observed for 30 days. In the case of *M. bovis*, to which guinea pigs are very sensitive, persistence was a permanent colonization with bacterial growth at the site of adhesion to a critical concentration that can cause pathological effects.

Differences in the timing of the excretion of AM and *M. bovis* in the feces indicate that the biological activity of mycobacteria of different species in the body of animals, particularly guinea pigs, is not the same. The results of the bacterioscopic study show that in guinea pigs infected by the alimentary route, the excretion of *M. bovis* pathogen in the feces occurred after the dissemination and spread of the pathological process, i. e., at later stages of the disease. At the same time, AM was excreted from the body in the first weeks.

All fecal samples collected were treated with cetylpyridinium chloride and inoculated onto a dense nutrient medium for mycobacterial cultivation. According to the results of the culture study (Table 2), initial cultures of AM were isolated from feces collected 15 days after inoculation from animals that received a triple dose (15 mg), with inoculated tubes accounting for 10–20% of the samples. Only *M. avium* was isolated from feces collected 30 days after triple inoculation of animals (10% of tubes) (Fig. 2). That is, a sufficiently high dose of AM infection, resulting in active shedding of mycobacteria in the feces during the first two weeks and for *M. avium* during 30 days, led to a positive cultural test result.

After 60 and 90 days of AM inoculation, no growth of cultures from fecal samples was observed in animals from any group of guinea pigs.

In the *M. bovis*-infected guinea pigs, the initial culture was isolated only from the fecal sample collected after 90 days (Table 2).

Allergic study. The results of the allergy study are presented in Table 3. Thus, in the allergy study, 30 days after a single feeding of mycobacterial cultures, all animals except those in the *M. phlei* group reacted to AAM and PPD, but some guinea pigs reacted to both allergens. In the *M. scrofulaceum* sensitized group (n = 10), one of three animals reacting to AAM reacted to PPD, in the *M. avium* group (n = 10) five animals reacted to AAM, two of them to both allergens, in the *M. phlei* group only one animal reacted to AAM. No non-specific reactions to PPD were detected after 60 and 90 days.

It should be noted that the intensity of reactions (area of erythema) in the groups of animals sensitized with *M. avium* and *M. scrofulaceum* was significantly higher for AAM (8.9 and 6.3 times, respectively) than for mammalian PPD. At the same time, in animals infected with *M. bovis*, allergic reactions were more intense to the administration of PPD (8.7 times).

Table 1 — Results of microscopy of guinea pig feces

Mycobacteria species	Fecal swabs (n = 3) after one time infection of animals, in days				Fecal swabs (n = 3) after three times infection of animals, in days			
	15	30	60	90	15	30	60	90
M. scrofulaceum	+/–/+	–/–/–	–/–/–	–/–/–	+/+/+	+/–/+	–/–/–	–/–/–
M. avium	–/+/+	–/–/–	–/–	–/–/–	+/+/+	+/+/+	–/–/–	–/–/–
M. phlei	+/+/–	–/–/–	–/–/–	–/–/–	+/+/+	–/–/–	–/–/–	–/–/–
M. bovis	–/–/–	–/–/–	+/–/+	+/+/+	Not infected			

Notes: '–' — AFB not detected; '+' — AFB detected.

Table 2 — Results of the fecal cultural examination

Mycobacteria species	Growth of initial cultures (one time infection of animals) from feces collected, in days				Growth of initial cultures (three times infection of animals) from feces collected, in days			
	15	30	60	90	15	30	60	90
M. scrofulaceum	–	–	–	–	+	–	–	–
M. avium	–	–	–	–	+	+	–	–
M. phlei	–	–	–	–	+	–	–	–
M. bovis	–	–	–	+	Not infected			

Notes: '–' — culture is not isolated; '+' — culture is isolated.

Table 3 — Results of the guinea pig allergy study

Mycobacteria species	Animals reacted, in days					
	30		60		90	
	10 animals in the group		6 animals in the group		3 animals in the group	
	PPD	AAM	PPD	AAM	PPD	AAM
	Average erythema area, mm^2/number of reacting animals					
Single sensitization						
M. scrofulaceum	3.7/1*	23.5/3	–	19.6/2	–	–
M. avium	4.2/2*	37.7/5	–	28.2/3	–	–
M. phlei	–	9.2/1	–	–	–	–
M. bovis	24.4/4	2.8/2*	185.6/6	3.4/1*	219.6/2	–
Triple sensitization						
M. scrofulaceum	5.8/5*	92.4/9	8.8/2*	153.2/6	–	10.2/2
M. avium	6.2/8*	108.8/9	10.8/5*	197.8/6	–	24.0/3
M. phlei	4.7/3*	76.6/9	3.6/2*	37.7/5	–	–
M. bovis	Not infected					

Note: * — reacted to both allergens.

After 60 days, in guinea pigs sensitized with *M. avium* (n = 6) and *M. scrofulaceum* (n = 6), the state of delayed-type hypersensitivity (DTH) to AAM persisted in three and two animals, respectively. On day 90, none of the animals sensitized with AM showed DTH.

In guinea pigs infected with *M. bovis*, responses to PPD were observed on days 30, 60, and 90, and the intensity of the immune response increased with time. For example, the average erythema area was 219.6 mm^2 on day 90 after infection, which was 9 times larger than the erythema area 30 days after infection (24.4 mm^2). In addition, some animals (1–2 individuals) reacted to AAM in the allergy studies at 30 and 60 days.

According to the results of the allergy study, it was found that three times AM feeding led to allergization of all experimental animals. This was manifested by an increase in the intensity of reactions to both allergens. Thus, after 30 days, in animals sensitized three times, the average area of erythema to AAM administration increased 3.9 times (*M. scrofulaceum*) (Fig. 3), 2.9 times (*M. avium*) (Fig. 4), 8.3 times (*M. phlei*) compared to the reactions observed with a single administration. After 60 days, reactions to AAM in animals sensitized once and three times with *M. avium* and *M. scrofulaceum* differed by almost seven times. It should be noted that the peak intensity of reactions to AAM after a single injection of *M. avium*, *M. scrofulaceum,* and *M. phlei* occurred on day 30, after three times sensitization of animals with *M. avium, M. scrofulaceum* — on day 60, in animals infected with *M. bovis* — on day 90. The lowest

intensity of reactions to allergens was observed in guinea pigs sensitized with *M. phlei* during the whole period of the experiment. In addition, it was found that the number of animals with non-specific mammalian PPD tuberculin reactions increased as the bacterial load increased. Thus, after 30 days, the number of animals reacting to PPD triple-sensitized with *M. scrofulaceum, M. avium*, and *M. phlei* increased to 5, 8, and 3, respectively, and after 60 days, their numbers decreased to 2, 5, and 2, respectively. After 90 days, no mammalian tuberculin (MTB) reaction was observed in the animals.

No reactions were observed in guinea pigs in the negative control group throughout the experiment. It should be noted that two guinea pigs infected with *M. bovis* culture died after 58 and 74 days, and another guinea pig infected with *M. avium* once died on day 63.

Pathologic examination and cultural examination of biomaterial. After 30, 60, and 90 days, three animals of each group were examined pathologically for macroscopic pathological tuberculosis changes, and the biomaterial collected from them was tested for tuberculosis by the cultural method.

There is conflicting data in the scientific literature regarding the pathogenicity of AM in guinea pigs, particularly *M. avium*. For example, Gomez-Buendia et al. (2024) found granulomatous lesions similar to tuberculosis in a postmortem examination of a guinea pig experimentally infected with *M. avium*. The lesions were observed in the pre-lobar and mediastinal lymph nodes. After seeding the pathologic material, they isolated the initial culture (Gomez-Buendia et al., 2024). In another study of three cultures of *M. avium* isolated from cattle, it was found that all three strains were pathogenic to guinea pigs and caused local lesions in the spleen and lungs with varying degrees of edema and hemorrhage. Microscopy of lung and spleen tissue sections from these infected guinea pigs showed scattered infiltration of red mycobacteria (Xin et al., 2022). In the above studies, guinea pigs were infected parenterally by intramuscular injection of *M. avium*. In our study, we considered the situation closest to the natural one, i. e., the ability of AM to cause pathological lesions in the body of guinea pigs under the alimentary method of infection with different bacterial loads.

According to the results of pathological examinations 30, 60, and 90 days after infection, no macroscopic tuberculous changes in the organs were detected in any animal, regardless of the frequency of AM administration by the dietary route. Culture examination of organs from all animals receiving AM did not isolate the initial cultures.

It should be noted that one guinea pig that received *M. avium* only once died on day 63. According to the necropsy results, no macroscopic lesions characteristic of tuberculosis were found, but hyperemia and exudate were noted in the lungs. The lungs were enlarged, and some areas were dark purple-red. Bacteriologic examination of biomaterial (spleen, liver, lungs) did not reveal mycobacteria. The exact cause of death was not determined, but the animal likely died of pneumonia.

Necropsy results of guinea pigs infected with *M. bovis* showed that pathologic changes in organs were observed in animals that died during the experiment and in those that were euthanized after 60 and 90 days. The largest tuberculous lesions were observed in the liver, spleen, and inguinal lymph nodes 90 days after infection. These organs were enlarged 1.5–2 times, the liver had areas of hyperemia and multiple gray-yellowish nodules of various sizes (Fig. 5). The presence of tuberculous granulomas was also observed in the inguinal lymph node, from which a caseous-necrotic mass protruded at the incision (Fig. 5). A large granuloma with caseous-necrotic contents was found on the surface of the spleen (Fig. 6). In the intestine and abdominal cavity, the presence of fluid, thickening of the mucous membrane with single small gray nodules was observed. After decontamination and seeding of pathological material on dense mycobacterial culture medium for 16–18 days, the growth of the first colonies characteristic of *M. bovis* was detected (Fig. 7).

Thus, with a single intake of *M. scrofulaceum, M. avium, M. phlei* into the body of guinea pigs by the alimentary route, their excretion in the feces was most active in the first 15 days, which was recorded by microscopy. When the same dose was administered three times, the period of slow-growing *M. scrofulaceum* and *M. avium* excretion in the feces increased to 30 days, and the amount of mycobacteria in the feces was sufficient to isolate them on the nutrient medium. The saprophyte *M. phlei* was not detected by microscopy during this period, regardless of the dose. On the contrary, *M. bovis* was excreted in feces at later stages of generalization of the infectious process.

Single and triple sensitization with *M. scrofulaceum, M. avium, M. phlei* induced a delayed hypersensitivity state in guinea pigs, the duration of which depended on the bacterial load and the type of mycobacteria. The saprophytic fast-growing culture of *M. phlei* caused a short-organism increased the intensity of reactions to AAM by 2.9–8.3 times in 100% of animals. The duration of the allergic state in animals sensitized with *M. scrofulaceum* and *M. avium* cultures three times (separately) lasted up to 90 days, in some animals sensitized with *M. phlei* — not more than 60 days. In addition, the percentage of animals responding to the mammalian PPD increased.

Animals infected with *M. bovis* remained positive to mammalian tuberculin for 90 days.

Conclusions. The issues of para-allergy, epizootic, and clinical significance of atypical mycobacteria, and their ability to cause mycobacteriosis can be resolved with an integrated approach, using a simultaneous test, bacteriological examination with identification of isolated mycobacteria and biological test, taking into account the bacterial load and duration of exposure.

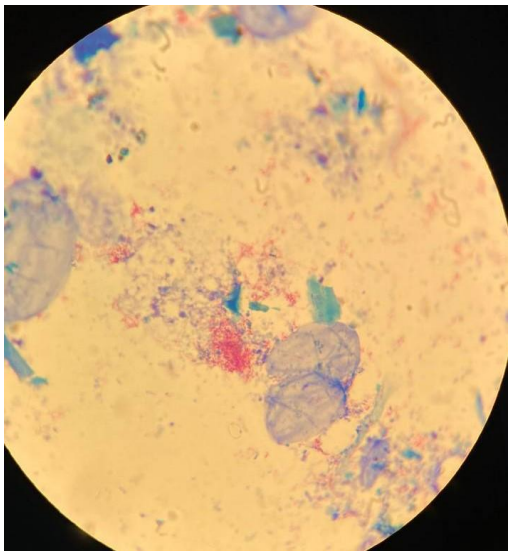

Figure 1. Acid-fast bacilli *M. avium* in feces.

Figure 2. Growth of *M. avium* from feces.

Figure 7. Growth of *M. bovis.*

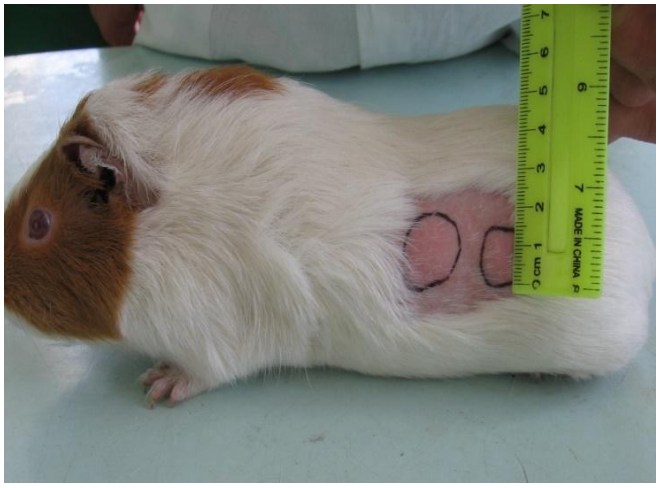

Figure 3. Triple sensitization (*M. scrofulaceum*): left erythema — reaction to AAM, right — to PPD.

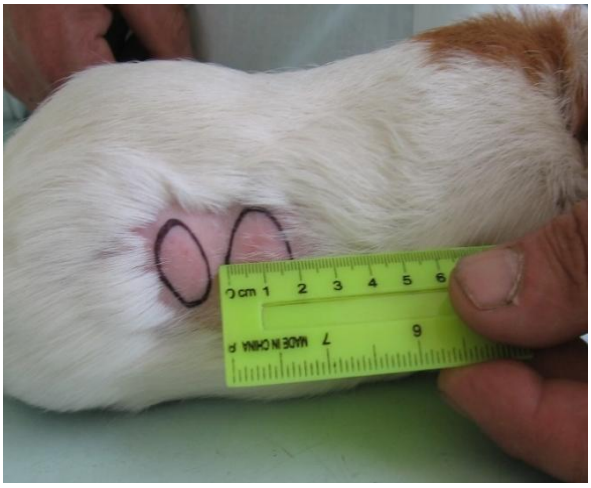

Figure 4. Triple sensitization (*M. avium*): left erythema — reaction to PPD, right — to AAM.

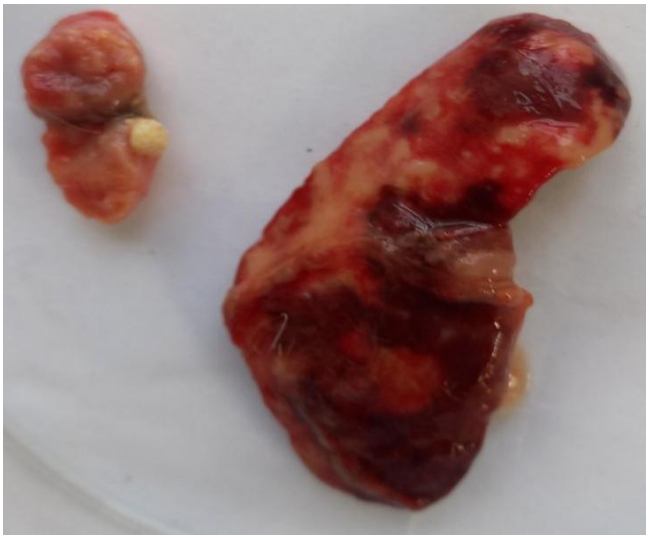

Figure 5. Lymph node and a piece of liver.

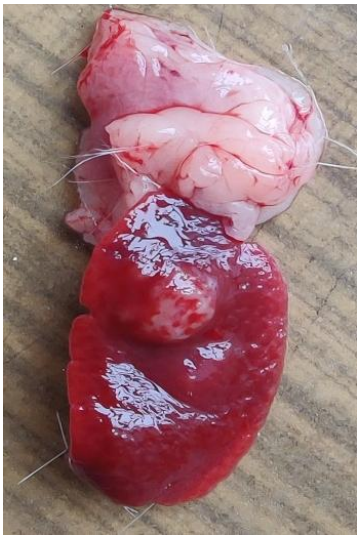

Figure 6. Spleen.

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