SECTION 4. DISEASES

4.1 Etiological structure of mycobacterial infections of animals in the Dnipropetrovsk region

The problem of animal tuberculosis has existed for various reasons for hundreds of years. Purposeful study of the biological properties of the infectious agent and the development on this basis of means of prevention and control has positively influenced the tension of the epizootic situation in a number of countries and continents. However, as evidenced by literary reports, animal disease occurs with varying frequency even in tuberculosis-free countries. The reason for this may be the significant variability of the causative agent of tuberculosis, in particular the biological cycle of development of the pathogen, which was reported by Weisfeiler Yu.K. (1975), Zhurila O.A. et al. (2001), Tkachenko O.A. et al. (2010) [186, 187].

Robert Koch discovered the causative agent of tuberculosis in 1882. According to its biological properties, the causative agent is attributed to the genus *Mycobacterium*. The species M. tuberculosis, *M. avium*, *M. bovis* are of practical importance. The *M. bovis* is the causative agent of the disease for both humans and some species of mammals. At the same time, according to the modern classification, mycobacteria are distributed: Group I – *M. tuberculosis complex*; Group II – *M. leprae*; Group III – all other types of mycobacteria (atypical). The first group, based on the identity of DNA sequences, includes: *M. tuberculosis, M. bovis, M. africanum, M. microti, M. caprae, M. canettii, M. pinnipedii*. This grouping is due to the extremely high (99.9%) similarity of chromosomal DNA and sequence identity of 16S ribosomal DNA. This gives grounds to individual authors to classify mycobacteria not as species, but as subspecies. The same goes for BCG.

The diagnosis of tuberculosis is complicated by atypical mycobacteria, which by their biological nature have similarities (even at the antigenic level) with mycobacteria of tuberculosis.

According to the current decree (2009), the main methods for diagnosing tuberculosis in animals are: allergic studies, clinical examination, pathological and anatomical and bacteriological research [188]. Each of these methods has both advantages and disadvantages.

One of the most widespread and effective methods of allergic diagnosis of tuberculosis in animals is the intradermal tuberculin test. This method allows specialists of veterinary medicine to timely establish a diagnosis and carry out a set of measures aimed at combating and recovering from tuberculosis in livestock. However, recently in the literature there have been reports on the detection of paraallergic reactions in animals to the introduction of PPD-tuberculin for mammals, caused by atypical mycobacteria [189–191]. In addition, studies by many scientists indicate that the intradermal test with the introduction of PPD-tuberculin detects not all animals with tuberculosis [192–194].

The pathological and anatomical method is widely used in post-mortem research to study the epizootic situation of the tuberculosis economy and monitor the effectiveness of health-improving measures in disadvantaged areas [195, 196].

However, the changed forms of mycobacteria (dissociative and L-forms) may not cause pathological changes typical for tuberculosis, however, productive paraspecific and very rarely necrotic changes are found in different organs, characterized by inflammatory foci with the presence of giant Pirogov-Langhans cells, typical of infectious granulomas [197, 198].

Bacteriological diagnostics involves bacterioscopic and cultural studies [187, 188]. The essence of the bacterioscopic diagnostic method is to identify mycobacteria by light, phase contrast and luminescence microscopy of smears made from the biomaterial of the animals under study. It is also used to study the morphology and tinctorial properties of the resulting crops. The purpose of the cultural research method is to isolate the culture of mycobacteria on a nutrient medium and study its properties. The initial stage of the culture method is the pre-sowing treatment of the test material. For this, both traditional methods of processing biological material (Gona-

Levenshtein-Sumioshi, A.P. Alikaeva, flotation, enzymatic enrichment) and new proposed methods are used [199].

To obtain a culture of mycobacteria, special nutrient media are used: Levenshtein-Jensen, Gelberg, Petraniani, State Enterprise Veterinary Medicine, Novaya Mordovsky, Finn-2, Shkolnikova, Soton, Dorozhkov, and the like. Recently, automated systems for recording the growth of mycobacteria have been widely used in humane medicine: BACTEC, BACTEC 460 TB, BACTEC MGIT 960, BacT / Alert, MB / BacT, MB / BacT ALERT 3D, etc., are more effective than traditional egg nutrients [200–206].

The biological research method is used to isolate and identify mycobacteria from biological material. For this purpose, laboratory animals are used: guinea pigs, rabbits and chickens.

Among the additional research methods for tuberculosis are used: thin-layer and gas-liquid chromatography, polymerase chain reaction, clinical, epizootological, serological, histostological methods, etc. [187].

Thanks to the large-scale planned anti-tuberculosis measures, the territory of the Dnipropetrovsk region is considered safe for tuberculosis in cattle. However, cultures of mycobacteria are isolated from biological material from rare animals. Therefore, the determination of the etiological structure of mycobacterial infections in animals remains relevant.

Material and methods. The experiments were performed in the Laboratory of Epizootology Department of the Dnipro State Agrarian and Economic University The study was approved by the Animal Researches Committee of Dnipro State Agrarian and Economic University, Ukraine.

In the beginning, we studied the biological properties of mycobacteria isolated from cattle from farms in the Dnipropetrovsk region, prosperous for tuberculosis (cultures №1–8), unfavorable farms (cultures №12–18 and *M. bovis* museum strains (Vallee, BCG, fast-growing).

Determination of virulence and sensitizing properties of mycobacteria was performed using a biological test [188]. To do this, we used outbred guinea pigs of

light color weighing 300–350 g. Before the experiment, the animals were tuberculinized in order to exclude spontaneous tuberculosis.

The suspension of the studied mycobacteria (concentration of 1 mg/cm^3 of saline) infected guinea pigs at a dose of 1 cm³ subcutaneously in the groin area. The animals were observed for 90 days. If guinea pigs did not die during the observation period, they were euthanized and examined by pathological and anatomical, bacteriological methods. The degree of tuberculous changes in the organs of guinea pigs was manifested according to the scheme of Trius.

Presowing processing of the biological material from the animal was carried out by the method of A.P. Alikaieva (1950). The bits of the lungs and lymph nodes were shredded with scissors to a size of 0.5 cm^3, transferred to a mortar and poured into a 6% solution of sulphuric acid. After 10–15 minutes, the acid was poured out, and the investigated material was washed several times with the sterile physiological solution. After removing the residual sulphuric acid, a small amount of the saline solution was poured into the mortar and triturated until homogeneous slurry was obtained. By means of microbial loop the obtained slurry transferred to the nutrient medium (pH = 7.0–7.2) using 6–10 tubes. Sown vials were placed in a thermostat at 37 °C. Tested tubes were reviewed within three months [187].

For histological investigations the selected material (spleen and lungs) was fixed with 10 % neutral formalin solution. Paraffin slices were made on a sledge microtome and stained with hematoxylin-eosin [207].

As a result of research it was established that the growth of colonies in culture № 1 was observed from the 17th day, № 2, 4, 7 – from 21 to 27 days, № 3, 5–8 – from 37 to 45 days. Cultures № 12–14, 17, 18 formed colonies from the end of the first to the beginning of the second week of cultivation, and № 15 and 16 on the 3–5th day. BCG mycobacteria formed colonies on the 16th day. Subsequently, during reseeding, the time of emergence of colonies of mycobacteria was reduced to the 12–14th day.

Subcultures of the Vallee strain grew on nutrient medium from 7 to 14 days, and *M. bovis* of the fast-growing strain from the 2–3rd day. When sowing a suspension prepared from pathological material of guinea pigs infected with *M. bovis* museum and

epizootic strains (Vallee, and fast-growing), the formation of colonies on a nutrient medium was observed from 23 to 28 days (Table 1).

Table 1.

Cultural properties of mycobacteria

Culture №	The emergence of growth, day	Bacterial Colony Shape	The color of the colonies	Growth on egg medium at temperature,ºC			Growth on Meat Peptone Agar	Growth on medium with sodium salicylate, мг/см³	
				22	37	45		0,5	1,0
1	17	S→R	с.к.	–	+	–	–	–	–
2	26	S→R	с.к.	–	+	–	–	±	–
3	37	S→R	с.к.	–	+	–	–	–	–
4	21	S→R	с.к.	–	+	–	–	±	–
5	45	S→R	с.к.	–	+	–	–	–	–
6	38	S→R	с.к.	–	+	–	–	–	–
7	27	S→R	с.к.	–	+	–	–	–	–
8	26[1]	S→R	с.к.	–	+	–	–	–	–
fast-growing	23[1]	S→R	с.к.	±	+	±	–	±	–
Vallee	28[1]	S→R	с.к.	–	+	–	–	–	–
BCG	16[2]	R	с.к.	–	+	–	–	–	–
12	6–10	R	gray	±	+	+	+	+	+
13	4–7	S	yellow	+	+	+	+	+	+
14	7–10	R	gray	+	+	±	+	+	+
15	3–5	S	yellow	+	+	–	–	+	+
16	3–5	S	yellow	+	+	+	+	+	+
17	6–8	R	gray	+	+	–	–	+	+
18	6–8	R	gray	+	+	–	+	+	+

Notes: 1 – culture isolated from pathological material of guinea pigs; 2 – when replanting mycobacteria from a freeze-dried vaccine; "–" – colony growth is absent; "±" − growth of colonies was observed on the medium of separate tubes; "+" – growth of colonies was observed on the medium of all tubes; с.к. – ivory.

As can be seen from table 1, mycobacteria of the BCG vaccine strain formed matte ivory colonies with a hilly surface and jagged edges (R-shape).

Colonies of the studied cultures № 1–8 and *M. bovis* strains, except BCG, were convex with a smooth shiny surface, smooth ivory edges and poorly emulsified in saline. 15–30 days after the appearance of growth, with the "aging" of the culture, there was a transition of the S-shape of the colonies to the R-shape: around the colonies, there was a narrow matte rim with distinct uneven edges.

Cultures № 13, 15, and 16 grew in the form of rounded S-shaped colonies of yellow color of mucous consistency. Colonies of mycobacteria in culture № 14 were gray in color, had a crater-shaped shape, and in culture № 12 – a centrally located convex part, from which departed a wide rim with a rough surface. In the latter, small spherical outgrowths appeared on the surface of the colony.

Cultures № 17 and 18 grew in the form of R-shaped colonies (uneven edges and rough surface) gray, with a diameter of 2-3 mm. The growth of mycobacterial colonies was observed in subcultures from the 2-3rd day. Colonies in cultures № 12-18 were well emulsified in saline.

In smears from culture colonies № № 1-8, after Zill-Nielsen staining, acid-resistant short thick rods, 0.5–1.0 μm long and 0.2–0.3 μm wide, without pronounced granulation were observed. In addition, cocoid forms were found in smears from cultures № 6, 7, and 16.

Mycobacterium cultures № 12–15 mycobacteria looked like red polymorphic rods with mild granulation and rarely (1–2 %) cocci.

When studying the adaptability of slow-growing crops № 1–7 to the nutrient medium, we performed five successive reseeding of the studied mycobacteria through the nutrient medium with a pH of 7.0–7.2. In most subcultures, the period of colony growth was shortened. In the second passage in cultures № 4 and 7 growth retardation from 7 to 17 days was observed. At the same time, the time of emergence of culture growth № 7 was reduced, and the term of colony formation was restored to the initial one only in the fourth passage. At the same time, the growth of colonies in culture № 4 in the third passage was observed three days earlier than in the first. In six (85.7 %) of the isolated slow-growing crops, the primary growth, starting with 3–4 generations, was observed for 8–10 days (Table 2).

Table 2.

Growth of crops № 1-7 per passage through the nutrient medium with pH of 7.0-7.2

Culture №	Passage									
	first		second		third		fourth		fifth	
	The emergence of growth, day	average number of colonies per 1 day of growth	The emergence of growth, day	average number of colonies per 1 day of growth	The emergence of growth, day	average number of colonies per 1 day of growth	The emergence of growth, day	average number of colonies per 1 day of growth	The emergence of growth, day	average number of colonies per 1 day of growth
1	17	8,25	12	1,50	10	0,28	10	1,00	12	1,33
2	26	0,50	22	2,00	14	0,33	10	0,43	10	0,25
3	37	1,71	24	0,67	8	0,25	8	1,66	10	0,75
4	21	2,00	28	4,00	18	1,50	10	0,66	10	2,00
5	45	0,42	14	2,00	10	0,67	10	0,50	14	0,50
6	38	0,75	22	1,00	14	0,40	10	0,50	10	1,33
7	27	3,28	44	0,50	36	C.p./3*	28	0,33	20	3,66

Notes. C.p. - solid growth, numerator - number of colonies, denominator - number of tubes

In the first and second passage, on average, more than one colony was found on the first day in five (71.5 %) cultures, in the third and fourth generations – in two (28.6 %) cultures and in the fifth generation – in four (57, 2 %) crops. An increase in the number of colonies on the seventh day of growth by a factor of two or more was noted in two (28.6 %) cultures – the first generation, five (71.5 %) cultures – the second generation, six (85.8 %) cultures – the third generation, seven (100 %) cultures – in the fourth and fifth generations. The appearance of continuous growth – multiple small colonies, which cannot be counted, in cultures № 2-5 and 7 (71.5 % of cultures) in three to four generations, possibly indicates a high level of adaptive ability of mycobacteria to the nutrient medium.

Cultures № 1–7 did not grow on meat-peptone agar and on a medium with sodium salicylate at a concentration of 1.0 mg/cm.

Mycobacteria of a fast-growing strain and cultures № 2 and 4 formed separate colonies on a medium with sodium salicylate at a concentration of 0.5 mg/cm.

At room temperature and 45 °C, cultures № 1–8 and *M. bovis* of the Vallee, BCG strains did not grow on an egg nutrient medium. At the same time, mycobacteria of cultures № 12–14, 16 and *M. bovis* of a fast-growing strain formed colonies on a nutrient medium with different intensities.

Cultures № 12–14 and 16 were grown on a nutrient medium at room temperature and 45 °C, on meat-peptone agar, on a medium with sodium salicylate at various concentrations. Cultures № 15, 17 and 18 grew well on an egg medium at room temperature and on a medium with sodium salicylate, but only mycobacteria of culture № 18 formed colonies on meat-peptone agar. Microscopy of smears from colonies formed on a simple medium showed filamentous mycobacteria up to 40 microns and a thickness of 0.5-1.0 microns.

Biochemical properties of cultures № 1-8 and *M. bovis* in terms of nitrate reduction, hydrolysis of TWIN-80, and tolerance to 5 % sodium chloride were identical. Catalase activity of isolated mycobacteria, according to the method of G.N. Pershin and Zykova T.N. (1958), was weakly expressed (+) or completely absent (-). The same activity, according to the method of Kubica G. et al. (1960) was negative. *Mycobacterium bovine* species had a high ability to ferment hydrogen peroxide (++). Mycobacterium BCG strain and culture № 3, 4 had a high level of peroxidase activity (++ and +++).

When studying the biochemical properties, it was found that cultures № 12–16, isolated from the biomaterial of cows on farms unfavorable for tuberculosis, had different biochemical properties (Table 3).

As you can see from the table. 3, cultures № 13 and 16 were resistant to 5 % sodium chloride, had a positive catalase activity, reduced nitrates, hydrolyzed TWIN-80 and accumulated citric-ammonium iron. Cultures № 14 and 15 had nitroreductase and catalase activity. However, culture № 14, in contrast to № 15, hydrolyzed TWIN-80, and did not precipitate citric-ammonia iron, but was not tolerant to 5 % sodium chloride.

Table 3.

Biochemical properties of mycobacteria

Culture №	properties						
	reduction of nitrates	catalase [1]	peroxidase [1]	catalase [2]	hydrolysis TWIN-80	tolerance to 5 % NaCl	accumulation of lemon-ammonia iron
1, 5, 8, Vallee,	–	+	+	–	–	–	nr
2, 6, 7, fast-growing	–	+	–	–	–	–	nr
3	–	–	++	–	–	–	nr
4	–	–	+++	–	–	–	nr
BCG	–	++	++	+	–	–	nr
12	–	++	++	+	+	–	–
13	+	+++	++	+	+	+	+
14	+	++	+	+	+	–	–
15	+	++	+	+	–	+	+
16	+	+++	+	+	+	+	+
17	–	+++	+	–	+	+	–
18	–	+++	+	++	+	+	+

Notes:
1– Research by the method of Pershin GN and Zykova TN. (1958).
Follows-up for the reaction of catalase activity:
"+++" – intense bubbling in the first minute;
"++" – moderate bubbling in the first minute;
"+" – single bubbles in the first minute;
"–" – no bubbles in the first minute.
Follows-up for the reaction of peroxidase activity:
"+++" – dark brown colonies;
"++" – brown colonies;
"+" – pale brown colonies;
"–" – the color of the colonies did not change.
2 – Research by the method of Kubica G et al. (1960);
nr– not researched.

Studies of the biochemical properties of cultures № 17 and 18 have established: high catalase activity, the ability to accumulate iron, a positive hydrolysis reaction of TWIN-80, and the absence of nitroreductase. Culture № 18, in contrast to № 17, was tolerant to 5% sodium chloride.

Guinea pigs infected with cultures № 1–8 and *M. bovis* of epizootic strains and Vallee developed an ulcer after 7-10 days, and progressive exhaustion was noted. During the experiment, the animals reacted to the introduction of PPD-tuberculin for mammals, and autopsy revealed lesions of internal organs specific for tuberculosis (Table 4).

Table 4.

Virulence and sensitizing properties of mycobacteria

Culture №	Animal No.	Allergic research*, days		Period of death, days	Euthanized	The presence of specific organ damage
		30	60			
1	1, 2	+	nr	37–44	–	+
2	3, 4	+	nr	38–43	–	+
3	5, 6	+	nr	36–42	–	+
4	7, 8	+	nr	38–42	–	+
5	10, 11	+	nr	37–39	–	+
6	12, 13	+	nr	35–39	–	+
7	14, 15	+	+	–	+	+
8	16, 17	+	+	–	–	+
fast-growing	18, 19	+	nr	49–57	–	+
Vallee	20, 21	+	nr	41–44	–	+
BCG	22, 23	+	+	–	+	–
12	24, 25	+	+	–	+	–
13	26	+	+	–	+	–
	27	+	–	–	+	–
14	28, 29	+	+	–	+	–
15	30, 31	+	+	–	+	–
16	32, 33	+	+	–	+	–
17	48, 49	+	–	–	+	–
18	50, 51	+	+	–	+	–
intact animals	34, 35	–	–	–	–	–

Notes. Result: "+" - positive, "-" - negative, nr– not researched.

The life expectancy of infected guinea pigs № 1–8, 10–13, 20–21 was 35–44 days, pigs № 18–19 – 49–57, respectively. Laboratory animals № 14–17 and 22–23 infected with cultures № 7, 8 and BCG were euthanized at the end of the experiment. However, after autopsy of gilts № 22–23, in contrast to № 14–17, no lesions of internal organs specific for tuberculosis were observed.

Guinea pigs, which were infected with cultures № 12–18, reacted to the introduction of PPD-tuberculin for mammals during the entire experiment. The state of allergy to tuberculin was both permanent and disappeared on the 60th day. Mycobacteria isolated from the biomaterial of animals free from tuberculosis farms did not cause death and development of the infectious process characteristic of tuberculosis in laboratory animals.

Among guinea pigs with specific lesions of internal organs, the highest lesion index was in animal's № 14–15 and 18–19 and was 21–25 points, and the lowest – in pigs № 10 and 11, respectively 15 and 17 points (Table 5).

According to the histological investigations, it was found that in the spleen tissue there are drainage foci and sites of caseous necrosis with granulomatous reaction around. The granulomas had the specific character and the characteristic structure for the tuberculous process. The caseous lymphadenitis was noted in lymph nodes.

There was a total caseous necrosis of lymphoid tissue, granulation tissue on the inner surface of the fibrous capsule contained a large number of the epithelioid cells, macrophages with admixture of lymphocytes, fibroblasts, multi-core cells, including giant multinucleated cells of the Pirogov-Langhans type.

The tissue of the lungs was with reduced airiness at the expense of the expansion of fibrous and granulation tissue with the presence of foci of caseous necrosis, which are surrounded by a layer of epithelial cells, macrophages with admixture of lymphocytes and plasmatic cells. Among the epithelioid cells were gigantic multinucleated cells of the Pirogov-Langhans type. In the outer parts of cell infiltrates there were polynuclear leukocytes and fibroblasts.

Table 5.

The degree of damage to the organs of guinea pigs infected with mycobacteria of epizootic and museum strains (by MV Trius)

Culture №	Animal No	Specific damage				The index of damage
		lymph. nodes	spleen	liver	lungs	
1	1	+	++	++	++	19
	2	+	++	++	++	19
2	3	+++	+	+++	+	18
	4	++	+	+++	+	17
3	5	+	+++	+	++	18
	6	++	++	+	+++	21
4	7	++	++	+	+++	21
	8	+++	++	++	++	19
5	10	+++	+++	–	++	17
	11	+++	–	–	+++	15
6	12	++	++	+	++	17
	13	++	+++	+	+++	19
7	14	+++	++	++	+++	25
	15	+++	+++	++	++	23
8	16	++	+++	–	+++	16
	17	+++	+++	+	++	20
fast-growing	18	+++	++	++	+++	25
	19	++	++	+	+++	21
Vallee	20	++	++	+	++	17
	21	+++	+++	+	++	20
BCG, 12–18	22– 33	–	–	–	–	0
intact animals	34, 35	–	–	–	–	0

Notes:
1. “+” – single nodules;
2. “++” – several nodules;
3. “+++” – numerous nodules.

Intact animals during the experiment did not respond to the introduction of PPD-tuberculin for mammals, and after euthanasia and dissection of the animals, there were no visible pathological changes.

Culture study of material collected from guinea pigs infected with cultures № 1–8, fast-growing and Vallee cultures of mycobacteria were isolated. At the same time, the result of bacteriological examination of samples taken from gilts № 22–33 was negative. Bacteriological examination of samples taken from euthanized uninfected gilts, a negative result was obtained.

Study of the species of mycobacteria. According to the results of the study of the biological properties of mycobacteria, cultures № 1–8 were assigned to the bovine species. When comparing the properties of crops № 12–16, isolated from cattle, tuberculosis-free farms, it was established that they belong to three types of atypical mycobacteria.

Among the atypical mycobacterium species classified [199], culture № 15 was 100 % similar to *M. vaccae*. Cultures № 13 and 16 corresponded to *M. phlei* in biological properties. Culture № 12 from *M. xenopi* had the highest similarity index. The only difference was in the growth rate.

Culture № 14 had the highest similarity index (81.8 %) with *M. nonchromogenicum, M. terrae, M. smegmatis, M. phlei,* and *M. flavescens*. The differences were in the ability of *M. nonchromogenicum, M. terrae* to grow at 45 °C and growth rate, in *M. smegmatis, M. phlei* – to accumulate citric ammonium iron and tolerance to 5 % sodium chloride, *M. flavescens* to grow at 45 °C and tolerance to 5 % sodium chloride.

Among the atypical mycobacterium species that were classified, culture № 18 was 100 % similar to *M. vaccae*. Culture № 17 had the least amount of differences with *M. vaccae, M. thamnopheos*, and *M. diernhoferi* (similarity index 90.9 %). The differences were in tolerance to 5 % sodium chloride (*M. vaccae*), hydrolysis of TWIN-80 (*M. diernhoferi*), and the ability to grow during cultivation at 37 °C (*M. thamnopheos*).

Investigating the biological properties of mycobacteria isolated after bacteriological examination of material from cattle it was found that seven cultures

belonged to *M. bovis*, two to *M. phlei*, one to *M. xenopi*, one to *M. vaccae*, and one could not be identified. In the literature, there are also reports of a small number of cultures that remain unidentified [208, 209].

The studied cultures of *M. bovis* slightly differed from each other in biological properties, in particular, growth on a medium with sodium salicylate, at different temperatures, peroxidase activity, and virulence. Other authors point to similar facts [210, 211]. On the one hand, this may be due to wide polymorphism and variability of mycobacteria [212, 213]. On the other hand, this can be explained by the different effectiveness of a wide range of differential diagnostic tests that are used to determine biological properties [214, 215].

Carrying out five consecutive passages of *M. bovis* epizootic strains (№ 1–7) through a dense egg nutrient medium, showed the fact of a reduction in the time of growth and an increase in the intensity of colony formation, which may indicate a high level of adaptive ability of mycobacteria.

Therefore, the biological properties of the studied mycobacteria are different, even within the same species (in particular, *M. bovis*). Significant differences of the above type include growth on a medium with sodium salicylate, at different temperatures, the rate of formation of colonies (especially in subcultures), virulence, and peroxidase activity. The atypical mycobacteria of five cultures isolated from cattle differed from *M. bovis* in a large number of properties: virulence, morphology, cultural and biochemical properties. At the same time, by almost all indicators, four cultures were classified as atypical mycobacteria of the species *M. xenopi*, *M. vaccae*, *M. phlei*. In another culture, differences were revealed among the properties, which did not allow attributing it to one or another type of mycobacteria.

Thus, when carrying out the identification of 14 cultures of mycobacteria, their belonging to four species was established – *Mycobacterium bovis, Mycobacterium rhlei, Mycobacterium xenopi*, and *Mycobacterium vacca*.