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Influence of pathogenic and atypical mycobacteria on immune status of guinea pigs

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Abstract. In recent years a diversity of researchings both native and foreign scientists has been devoted to the investigation of animal tuberculosis. However, there are a number of questions required more detailed studying and scientific justification. Among involved questions specific place is occupied by the problem of animal immunity in cases of infection by pathogenic and atypical mycobacteria. The aim of our work was to study the influence of pathogenic and atypical mycobacteria on the immune status of laboratory animals. The influence of pathogenic and atypical mycobacteria on the immune status of guinea pigs was studied by infecting them with a suspension from the bacterial mass of each individual mycobacterial culture (*M. bovis, M. avium* and *M. fortuitum*). The suspension was prepared at the rate of 1.0 mg / ml of sterile NaCl and injected subcutaneously into animals at a dose of 1.0 ml. Blood samples were taken on the 7th, 14th, 30th and 60th days after the injection of mycobacterial cultures to determine the most informative indicators of the immunological status of animals. Amount of neutrophils and phagocytic activity were determined in the blood of experimental animals. The total protein level was determined spectrophotometrically in the serum of guinea pigs, the level of circulating immune complexes (CIC) of average molecular weight – by the method of Grinevich & Alferov, seromukoida – by Weimer & Moshin. The data obtained that *M. bovis* caused the biggest growth in some cellular and humoral immunity indicate (the percentage of neutrophils by 28.6% and their phagocytic activity by 15.8%, the level of total protein is 76.13 g/l, circulating immune complexes 0.190 mg/ml and seromukoids per day 7 (0.099 mg/ml) on the 30th day after infection in comparison with animals of the control group. Animals infected with the culture of atypical mycobacteria *M. fortuitum* showed less noticeable changes in immunity, compared to animals infected with the pathogen of tuberculosis.

Keywords: mycobacteria; *M. bovis*; *M. avium*; *M. fortuitum*; immunobiological parameters; guinea pigs.

Уплив патогенних і атипових мікобактерій на імунний статус мурчаків

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Анотація. Вивченню туберкульозу у тварин присвячено цілий ряд наукових робіт як вітчизняних, так і закордонних учених. Проте нині залишається низка питань, які потребують більш детального вивчення та наукового обгрунтування. Серед них особливе місце посідає проблема імунітету тварин за інфікування патогенними та атиповими мікобактеріями. Метою роботи було вивчити вплив патогенних і атипових мікобактерій на імунний стан лабораторних тварин. Уплив патогенних і атипових мікобактерій на імунний статус мурчаків вивчали методом інфікування їх зависсю із бактеріальної маси кожної окремо культури мікобактерій (*M. bovis, M. avium* і *M. fortuitum*). Завись готували з розрахунку 1,0 мг/мл стерильного фізіологічного розчину та вводили тваринам підшкірно в дозі 1,0 мл. Для дослідження найбільш інформативних показників імунологічного стану тварин на 7, 14, 30 і 60 добу після введення культур мікобактерій відбирали проби крові. В крові дослідних тварин визначали кількість нейтрофілів і фагоцитарну активність згідно методичних рекомендацій "Методи досліджень маркерів функціонального стану клітин периферичної крові та кісткового мозку тварин" (2013). У сироватці крові мурчаків спектрофотометрично визначали рівень загального білка; методом Гриневича та Алферова (1985) – рівень циркулюючих імунних комплексів середньої молекулярної маси; методом Веймера та Мошина (1952) – серомукоїдів. Результати вивчення впливу культур *M. bovis, M. avium* і *M. fortuitum* на імунний статус мурчаків свідчать про те, що збудник туберкульозу *M. bovis* на 30 добу після зараження викликав у тварин найбільше підвищення деяких показників клітинного та гуморального імунітету (відсоток нейтрофілів на 28,6%, їх фагоцитарна активність на 15,8%, рівень загального білку до 76,13 г/л, циркулюючих імунних комплексів до 0,190 мг/мл, серомукоїдів на 7 добу до 0,099 мг/мл) порівняно з тваринами контрольної групи. У тварин, інфікованих культурою атипових мікобактерій *M. fortuitum*, відмічали менш виражені зміни показників імунітету, порівняно з тваринами, зараженими збудником туберкульозу.

Ключові слова: мікобактерії; *M. bovis*; *M. avium*; *M. fortuitum*; імунобіологічні показники; мурчаки.

Introduction

Despite the achieved success in the control of animals' infection diseases today the danger is represented by infectious diseases of bacterial etiology, which are characterized by high contagiousness. Tuberculosis – it is the main economically significant bacterial disease (LoBue et al., 2010; Paliy et al., 2015; Bouchez-Zacria et al., 2018; Borja et al., 2018).

Directly under production conditions, questions arise about the non-specific response of animals to tuberculin injection. This is due to many factors that directly affect the immune status of the animal (Jenkins et al., 2018).

The genus *Mycobacterium* includes more than one hundred and fifty recognized species, most of which are in the environment, and many of which may be pathogenic for mammals (King et al., 2017).

A *Mycobacterium bovis* causes animals and sometimes humans tuberculosis. In epidemiological terms, the disease can be recorded in some species of wild animals, creating a reservoir of infection (Palmer, 2013). There are exceptional strains of *M. bovis*, which have some features of *M. tuberculosis*. Strains were exposed from 8 patients who lived in Kazakhstan. Though molecular markers were typical for *M. bovis*, patterns of growth and biochemical test results were interstitial between *M. bovis* and *M. tuberculosis* (Kubica et al., 2006). The pathogens of tuberculosis are often isolated from caseous lesions (68%), as well as milk of infected animals (18%), which is a risk to consumers of livestock products (Leite et al., 2003). Mycobacteria of *M. bovis* are straight, or slightly curved, short, thin, with rounded ends of a stick 0.3–1.6 microns wide and 1.5–2 microns long. Inside the cells there are sometimes fills ("Grains Fly"), which are located at the ends of mycobacteria. The size of mycobacteria and the number of inclusions depend on the duration and conditions of cultivation, and the ability to polymorphism is observed both in the cultivated in nutrient media, and in the allocation of them from pathological material, where there are coccoid forms and rods (Brennan & Nikaido, 1995).

Mycobacterium *M. avium* – thin, oblong, curved, immobile, acid resistant rods with rounded edges, stained by the Ziehl-Neelsen method in a bright red color. *M. avium* is characterized by a strong polymorphism, as a result of which colored smears reveal forms of coccoid, long-grained and non-granular rods, while coccoid forms or branched forms predominate in old cultures (Runyon, 1965). Mycobacterium *M. avium* is a major pathogen of domestic, synanthropic, zoo and wild bird tuberculosis. An infected bird dies from a generalized form of tuberculosis in 30–60 days (Mutalib & Riddell, 1988).

Mycobacterioses caused by atypical mycobacteria of *M. fortuitum*, the clinical manifestations of which are characterized by pneumonia and damage to the digestive tract (Okamori et al., 2018), are quite common among people. This type of mycobacterium is widespread in livestock farms in Ukraine, but it does not represent an epizootological risk for animal husbandry, but only predetermines the state of increased sensitivity to tuberculin and allergen from atypical mycobacteria (Kotlyar, 2016).

The aim of our work was to study the effect of pathogenic and atypical mycobacteria on the immune condition of laboratory animals.

Materials and methods

Experimental researches were carried out in the laboratory for the study of tuberculosis and the laboratory of clinical biochemistry of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine". The effect of pathogenic and atypical mycobacteria on the immune status of guinea pigs was studied by infecting them with a suspension from the bacterial mass of each individual mycobacterial culture *M. bovis* (strain Vallee), *M. avium* (strain IEKVM UAAN) and *M. fortuitum* (strain № 122). The suspension was prepared at the rate of 1.0 mg/ml of sterile NaCl and injected subcutaneously into animals at a dose of 1.0 ml. To research the most informative indicators of the immunological state of animals on the 7th, 14th, 30th and 60th days after the injection of mycobacterial cultures blood samples were taken. In the blood of experimental animals the amount of neutrophils and phagocytic activity were determined according to the methodological recommendations "Methods of researches markers of the functional state of the cells of peripheral blood and bone marrow of animals". In the serum of guinea pigs the total protein level was determined spectrophotometrically, the level of circulating immune complexes (CIC) of average molecular weight – by the method of Grinevich and Alferov (1985), seromukoida – by Weimer and Moshin (1952).

Results

The results of conducted scientific experiments to determine the immunobiological parameters of experimental guinea pigs for infection with various types of mycobacteria are shown in Table 1.

For the analysis of data in the Table 1, two-way analysis of Fisher variance was used (the first one was the type of culture, the second one – time or age) with the help of "Two-way analysis of variance without repetition" due to "Data mining" ASP MS

Table 1. Immunological indicator of experimental guinea pigs

Cultures	Biomarkers of nonspecific immunity	Blood analysis through the days			
		7	14	30	60
M. bovis	Total protein, gr/l	78.23 ± 1.25	74.31 ± 2.03	76.13 ± 1.97	67.35 ± 2.31
	CIC , mg/ml	0.161 ± 0.070	0.172 ± 0.060	0.190 ± 0.040	0.138 ± 0.050
	Seromucoids, mg/ml	0.099 ± 0.012	0.097 ± 0.016	0.094 ± 0.024	0.091 ± 0.013
M. avium	Total protein, gr/l	75.24 ± 1.24	73.43 ± 2.15	72.52 ± 2.32	65.61 ± 1.85
	CIC, mg/ml	0.144 ± 0.040	0.149 ± 0.070	0.167 ± 0.050	0.128 ± 0.040
	Seromucoids, mg/ml	0.096 ± 0.018	0.094 ± 0.021	0.095 ± 0.019	0.088 ± 0.022
M. fortuitum	Total protein, gr/l	73.54 ± 1.31	71.95 ± 1.97	69.11 ± 2.02	62.34 ± 1.85
	CIC, mg/ml	0.132 ± 0.030	0.138 ± 0.050	0.161 ± 0.070	0.127 ± 0.050
	Seromucoids, mg/ml	0.095 ± 0.013	0.094 ± 0.015	0.096 ± 0.011	0.086 ± 0.016
Control	Total protein, gr/l	66.25 ± 1.34	67.71 ± 2.23	66.52 ± 2.57	66.31 ± 2.18
	CIC, mg/ml)	0.121 ± 0.060	0.122 ± 0.070	0.121 ± 0.030	0.122 ± 0.050
	Seromucoids, mg/ml	0.092 ± 0.013	0.091 ± 0.018	0.092 ± 0.015	0.092 ± 0.011

Excel-2010 (Baranovsky et al., 2017; Blanco et al., 2012). Summary tables consist of:

Total protein (Variance analysis)						
Source of variation	SS	df	MS	F factual	P-datum	F critical
Type of cultures	119.006	3	39.668	7.02676	0.00985	3.8625
Age, days	144.093	3	48.031	8.50807	0.00541	3.8625
Arbitrary factors	50.8082	9	5.6453			
Total:	313.907	15				
Power of influence	%		Significance of influence			
Type of cultures	37.91		p < 0.0099			
Age, days	45.90		p < 0.0054			
Arbitrary factors	16.19		0.05 < p			

CIC (Variance analysis)						
Source of variation	SS	df	MS	F factual	P-datum	F critical
Type of cultures	0.00394	3	0.0013	7,02676	0,00985	3,8625
Age, days	0.00200	3	0.0007	8,50807	0,00541	3,8625
Arbitrary factors	0.00087	9	9,65E-05			
Total:	0.00681	15				
Power of influence	%		Significance of influence			
Type of cultures	57.85		p < 0.001			
Age, days	29.39		p < 0.01			
Arbitrary factors	12.75		0.05 < p			

Seromucoids (Variance analysis)						
Source of variation	SS	df	MS	F factual	P-datum	F critical
Type of cultures	2.6E-05	3	8.67E-06	1.6082	0.2541	3,8625
Age, days	9.05E-05	3	3.02E-05	5.5979	0.019	3,8625
Arbitrary factors	4.85E-05	9	5.39E-06			
Total:	0.00016	15				
Power of influence	%		Significance of influence			
Type of cultures	15.76		p < 0.25			
Age, days	54.85		p < 0.02			
Arbitrary factors	29.39		0.05 < p			

Analyzing the data of the Table 1, an increased level of total protein in the blood serum of the experimental guinea pigs was established during the entire study, but the greatest difference by 18.1% and 14.4% was observed on 7th and 30th days after infection of animals with *M. bovis*.

In the blood serum of animals that were infected with the culture of *M. fortuitum*, the slightest increase in the level of this indicator by 11.0% on 7th day and its greater decrease to 6.0% for 60th day was registered. Specifically, a decrease level of total protein in animals after the action of all the investigational mycobacterial cultures on 60th day compared with the control.

The CIC level, which is the activator of *β*-lymphocytes and the complement system, was higher than the control and agreed on the nature of changes in the total protein content during the research. In the 1st table is shown that a high level of circulating immune complexes by 57.0% was observed in the serum of guinea pigs under the action of *M. bovis* 30 days after the injection of the investigational mycobacterial culture. However, animals that were infected with *M. avium* and *M. fortuitum*, the slightest differences of this index were recorded to 4.9% on 60th day regarding to the control.

The level of seromucoids, which belong to suppressor proteins, within 30 days of the research, was increased in all experimental groups of animals with the greatest distinction of 7.6% on the 7th day in the serum of guinea pigs infected with *M. bovis* against control. On the 60th day, an index decrease in the serum of animals of all groups was recorded with a maximum difference up to 6.5% in the group of guinea pigs infected with *M. fortuitum*.

One of the types of leukocytes that are involved in the protective reactions of the organism, in particular phagocytosis, are neutrophils. Consequently, the dynamic of changing in the number of neutrophils in the blood of laboratory animals infected with various types of mycobacteria was determined (Fig. 1).

The data submitted in the 1st Figure shows that under the influence of all three species of mycobacteria, a percentage increase of neutrophils in the blood of guinea pigs was recorded during a 30-day study with a maximum difference by 28.6% animals infected with *M. bovis* as compared with control. It is also important to note that on the 60th day the decrease of these cells by 10.7% in the blood of animals infected with *M. fortuitum* was observed, it might be connected to the increased destruction of neutrophils affected by the infection development.

Each neutrophil cell is capable for only one phagocytic event; therefore, their number reflects and agrees upon the data on phagocytic activity (Fig. 2).

The Figure 2 shows the percentage increase of phagocytic neutrophils activity in the blood of animals infected with all three types of mycobacteria studied in comparison with tested animals, but the greatest deviation by 15.8% on the 30th day was noted in the blood of animals affected by *M. bovis*. The increase number of active phagocytes in the blood, which retain a high phagocytic activity, indicates an increased immune response. During the latter stages of research, a 3.2% decrease of FA in the blood of animals that were infected with *M. fortuitum* was recorded. Reduction of FA might be connected to excessive formation of immature neutrophils in the bone marrow.

Discussion

Other studies have found that infection of cattle apart with such cultures as *M. bovis, M. tuberculosis* and *M. kansasii* also shows a different immune response of animals. The delayed-type hypersensitivity was revealed by inoculation of each culture of mycobacteria; however, the response to the injection of *M. bovis* and *M. tuberculosis* exceeded the response to infection with *M. kansasii.*

Specific responses were given by all animals due to injection of *M. tuberculosis* and *M. bovis* in 3 weeks after inoculation. After 6 weeks animals infected with *M. tuberculosis* had a decreased immune response, whereas animals with *M. bovis* remained the same. When inoculating *M. kansasii*, early initial antibody responses decreased 10 weeks after the start of the experiment. These results show that the immune response of animals is due to antigenic load, and not the development of the pathological process (Waters et al., 2010).

Various natural mechanisms of different macro organisms for inactivation of the pathogen of *M. tuberculosis* reported, which in turn causes the induction of adaptive immunity (Crevel et al., 2002).

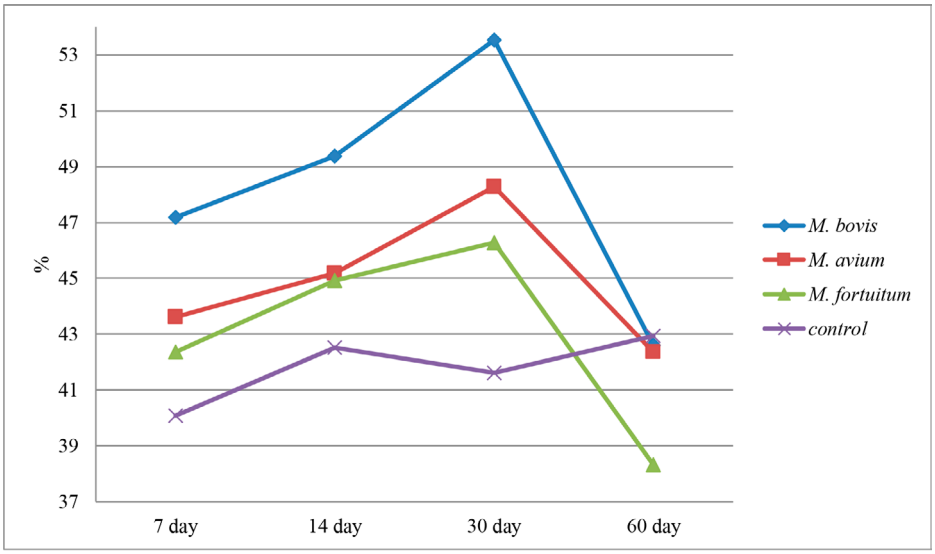

Figure 1. The percentage of neutrophils in guinea pigs blood

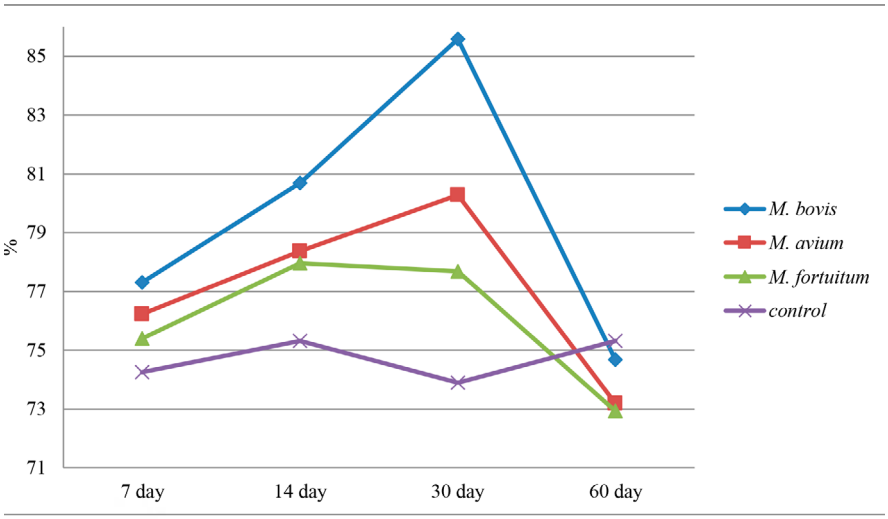

Figure 2. The level of neutrophils phagocytic activity in the blood of guinea pigs

A detailed study of the immune response of the macro organism to its infection with various types of mycobacteria, including those with natural and artificial mutations, opens the way to the creation of effective vaccines (Blanco et al., 2012).

Conclusions

Comparative studies about the effect of the cultures *M. bovis*, *M. avium* and *M. fortuitum* on the immune status of guinea pigs indicate that *M. bovis*, the pathogen of tuberculosis, on the 30th day after infection, caused the animals the greatest increase in some indicators of cellular and humoral immunity (neutrophils by 28.6% and their phagocytic activity by 15.8%, the level of total protein was 76.13 g/l, circulating immune complexes 0.190 mg/ml and seromukoids per day 7 (0.099 mg/ml) in comparison with animals of the control group.

Animals infected with the culture of atypical mycobacteria *M. fortuitum* showed less noticeable changes in immunity, compared to animals infected with the pathogen of tuberculosis.

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