

**RIFIZOSTREP – NEW DRUG AGAINST
TUBERCULOSIS****Mamadullaev G.Kh**

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Abstract: The article presents the results of bacteriological studies of the internal organs of guinea pigs and rabbits experimentally infected with *M. bovis* and *M. tuberculosis* with the subsequent use of a new anti-tuberculosis drug “Rifisostrep”.

Keywords: Rifizostrep, Drug, Antimicrobial, Mycobacterium, *M.Bovis*, *M.Tuberculosis*, Bacteriological, Against- Microbes' Strain, Resistance, Sensitivity.

This is an open-access article under the [CC-BY 4.0](https://creativecommons.org/licenses/by/4.0/) license**Introduction**

Tuberculosis is a chronic infectious disease found in farm animals, wild animals, ungulates and poultry. In diseased internal organs and tissues, special nodules are formed - bumps (tubercles). Tuberculosis is also common among people [1; 9].

Considering the extreme resistance of the pathogen to external environmental factors (sunlight, temperature, humidity, pressure, pH, disinfectants, etc.), it is necessary to strengthen the fight and prevention of animal tuberculosis in the republic, as well as diagnostic measures. [4;5].

According to the World Health Organization (WHO), in 2015, 10.4 million new cases of tuberculosis were registered worldwide, of which 5.9 million (56%) were men, 3.5 million (34%) were women, and 1.0 million (10%) children. That same year, 1.4 million people died from tuberculosis.

According to WHO websites, tuberculosis is currently the deadliest disease. In 2017 alone, 10 million people became infected with tuberculosis and 1.6 million people died (**Source: <https://mir24.tv>**).

In the modern period, many aspects of the disease have been studied and clarified by scientists around the world in the direction of studying tuberculosis infection using the science of molecular biology. The biology of the disease, the main epidemiological and epizootological patterns, pathogenesis, and diagnosis have been widely studied at the molecular genetic level. At the same time, many chemotherapy drugs are being discovered to combat this disease. In this regard, scientific research is widely conducted in leading scientific centers and higher educational institutions of the world [3; 6; 8].

In recent years, mono-, bi- and polyresistance of pathogenic strains to single-drug therapy for tuberculosis has begun to increase. To prevent this problem, scientists are conducting large-scale research to create a set of new combination anti-tuberculosis drugs. In medical physiology, there is an

international DOTS program (DOTS - (Directly Observed Treatment, Short-course under direct observation). The DOTS strategy to combat tuberculosis used a combination (mixture) of 2-3 or 4 different drugs. As a result of the implementation of the DOTS strategy, it was possible to reduce morbidity and mortality from the disease [2;7].

Our research has been carried out in this direction: a new combined anti-tuberculosis drug "Rifizostrep" has been created on the basis of the tuberculosis laboratory of the Veterinary Research Institute, and a large-scale test of its antibacterial activity against the pathogen is being carried out. The goal and objectives of our research are to determine the effectiveness of the drug "Rifizostrep" in the body of laboratory animals against strains of *M. tuberculosis* and *M. bovis* using the bacteriological method.

Methods

Scientific research was carried out in accordance with the methodological guidelines and instructions "Prevention and control of animal tuberculosis", approved by the Committee of Veterinary Medicine and Livestock Development of the Republic of Uzbekistan (M. 1982, 1988, Tashkent 1998, 2011).

Cultivation and storage of mycobacterial strains in the museum, examination of pathological samples taken from experimental animals in the laboratory, brought from livestock farms were carried out according to the instructions "Laboratory diagnosis of animal tuberculosis" (Omsk 1988), the manual "Laboratory diagnosis of tuberculosis" and the instructions "Diagnostics of animal tuberculosis" (Tashkent 2011), as well as on the basis of methodological manuals by T.N. Yashchenko, I.S. Mecheva "Guide to laboratory tests for tuberculosis. - M.: Medicine, 1973" [10;11].

Bacteriological tests of a new drug against strains of *M. bovis* and *M. tuberculosis* continued in order to test the combination of a new tuberculostatic against tuberculosis pathogens - the drug "Rifizostrep".

Lifetime trials of the drug "Rifizostrep" against tuberculosis pathogens were carried out on guinea pigs. 27 guinea pigs were infected with virulent strains of mycobacterium tuberculosis *M. bovis* 8-03, which causes disease in cattle, and *M. tuberculosis* 7880, which causes disease in humans, subcutaneously at a dose of 0.03 mg/kg.

90 days after the end of the experiment, all animals in the experimental and control groups were killed for pathological and bacteriological studies. A pathological sample taken from the internal organs of animals in the experimental and control groups was processed according to the Ghosn-Levenshtein-Sumiyoshi method and placed on a Levenshtein-Jensen nutrient medium; smears prepared from a suspension of pathological samples were stained using the Sil-Nielsen method. method and subjected to microscopy (magnification 12x100). Bacteriological examination of pathological samples lasted 3 months.

The specific bactericidal activity of the drug "Rifisostrep" against strains of mycobacterium tuberculosis *M. bovis* and *M. tuberculosis* was comparatively studied on 28 rabbits in comparison with the drug isoniazid [11].

After the end of the experiment, rabbits in all experimental, control and comparative groups were killed, the internal organs of the animals were examined for pathology, and samples were taken from the internal organs for bacteriological examination. The resulting pathological sample was processed according to the Ghosn-Levenshtein-Sumiyoshi method and sown on Levenshtein-Jensen nutrient medium; smears prepared from pathological samples were stained using the Seel-Neelsen method and subjected to microscopy. The period of bacteriological examination of pathological

samples was 3 months.

Based on the results of a bacteriological study of pathological samples, a conclusion was made about the level of antibacterial action of a new tuberculostatic combination of drugs - the drug Rifizostrep against tuberculosis pathogens.

Results and Discussion

According to the study design, the results of cultural and microscopic examination of pathological samples of guinea pigs receiving the drug Rifizostrep after tuberculosis infection are presented in Table 1. Table 1 shows that after infection with the *M. bovis* strain of group 1, during bacteriological examination of pathological samples of 9 guinea pigs that received Rifizostrep once every 5 days, internal *M. bovis* was not detected in any of the 9 guinea pigs. the pathogen has not been isolated. During bacterioscopic examination, no mycobacterial bacilli were found in any smear stained using the Seal-Neelsen method.

From the entrails of guinea pigs infected with the *M. bovis* strain in group 2 as a control and not receiving the drug, tuberculosis pathogens were found in the visceral samples cultures of all 3 guinea pigs on days 18-24 of Löwenstein-Jensen, and typical colonies were formed on the surface of the nutrient medium. As a control, in all test tubes planted in pure form, tuberculosis colonies grew and had the following characteristics: - growth rate - on average 18-24 days; Description of the colonies - uneven shape, lumpy surface, dry R-colonies, cloudy single number, small dewy volume, straight and curved shape, rough surface, dry and sticky consistency, ivory pigmented, medium dilution of the colonies. Consistency - crumbles.

Table 1

Results of a cultural study of pathological preparations of guinea pigs receiving the drug Rifizostrep after tuberculosis infection

№	Kind of animal	group	number of heads	strain name	Name of the drug and method of use	Bacteriological research
1	Guinea pig, experiment	I	9	<i>M. bovis</i> 8-03	Rifisostrep parenteral	-----
2	Guinea pig, control	II	3	<i>M. bovis</i> 8-03	Control, no drugs	+++
3	Guinea pig, experiment	III	9	<i>M. tuberculosis</i> 7880	Rifisostrep parenteral	-----
4	Guinea pig, control	IV	3	<i>M. tuberculosis</i> 7880	Control, no drugs	+++
5	Guinea pig, comparative group	V	3	<i>M. tuberculosis</i> 7880	Isoniazid orally	--+
	Jami		27			

Reminder: + tuberculosis detected;

- tuberculosis was not detected.

Microscopy of smears stained by the Ziel-Neelsen method of tuberculosis colonies obtained from the food environment using a bacterial bacillus revealed the following morphological and tinctorial features: the *M. bovis* strain absorbed the fuchsin dye in the smear and was colored red-violet. Polymorphism is clearly expressed in the morphology of *M. bovis* bacilli. Under the microscope, short, thick, thin-flat, pointed and somewhat thickened bacterial rods were detected.

Cocci-like forms were also detected in some visual fields, and granules were expressed in some senescent cells.

After infection with the *M. tuberculosis* 7880 strain in the 3rd group, during bacteriological examination of pathological samples from 9 guinea pigs receiving rifisostrep once every 5 days, the *M. tuberculosis* 7880 strain was not isolated. In smears stained using the Seal-Nielsen method during bacterioscopic examination, mycobacterial bacilli were not detected.

As a control, in group 4, internal organs of guinea pigs infected with strain *M. tuberculosis* 7880 and not receiving the drug were grown from the internal organs of all 3 guinea pigs grown on the surface of the medium, forming typical colonies. As a control, colonies of tubercles grew in all tubes grown alone and had the following characteristics:

- growth rate – on average 26-30 days; Description of colonies - smooth, bumpy surface, dry
- R-colonies, single and numerous, small volume, moist color, regular shape, rough surface, dry and sticky consistency, ivory pigmented, medium dilution of colonies. Consistency - crumbles.

Microscopy of tuberculosis colonies obtained from a food environment using a bacterial bacillus revealed the following morphological and tinctorial characteristics: in the smear, the *M. tuberculosis* 7880 strain was stained red-violet, and polymorphism was expressed in the morphology of the bacterial bacillus. Under the microscope, long and short, thick, thin, flat-ended and some thickened bacterial rods were found. In some fields of view, coccoid forms are also found; granules are formed in some old cells; granules are not expressed in young cells.

After infection with the *M. tuberculosis* strain in the 5th group, during a bacteriological study of pathological samples of guinea pigs from the comparative control group receiving the drug Isoniazid, colonies of tuberculosis grew from the internal organs of one of the 3 guinea pigs; in 2, no tuberculosis colonies were produced.

Thus, we can conclude that based on the results of a comparative study of the antibacterial activity of the drug Rifizostrep against strains of tuberculosis in guinea pigs with the drug isoniazid, the drug Rifizostrep has a more active antibacterial effect on tuberculosis pathogens than the drug isoniazid. During bacterioscopy of smears stained using the Ziel-Neelsen method, tuberculosis bacilli with typical morphological and tinctorial properties were identified from the bacterial mass formed during cultural examination.

The specific antibacterial activity of the drug "Rifisostrep" against the *M.bovis* and *M.tuberculosis* strains of *Mycobacterium tuberculosis* was studied in 28 rabbits. The activity of the new drug against pathogens has been comparatively studied in comparison with the medical drug isoniazid.

Table 2.

Results of a cultural study of pathological samples of rabbits treated with the drug Rifizostrep after tuberculosis infection

№	Kind of animal	group	number of heads	Name of the infected strain	Infectious dose	Dosage of the drug	Name of the drug and method of administration	Result
1	Rabbits, experience	I	4 4	<i>M.bovis</i> 8-03	0,03 mg/kg	0,5 ml 1,0 ml	Rifizostrep parenteral	70 % 100 %
2	Rabbits, control	II	3	<i>M.bovis</i> 8-03	0,03 mg/kg		Control, no drugs	+

3	Rabbits, experience	III	4 4	M.tuber culosis 7880	0,03 mg/kg	0,5 ml 1,0 ml	Rifizostrep parenteral	70% 90 %
4	Rabbits, control	IV	3	M.tuber culosis 7880	0,03 mg/kg		Control, no drugs	+
5	rabbits, comparison group	V	3	M.tuber culosis 7880	0,03 mg/kg	10 mg/kg	Isoniazid, orally	50 %
6	rabbits, comparison group	VI	3	M.bovis 8-03	0,03 mg/kg	10 mg/kg	Isoniazid, orally	60 %
	Total		28					

Reminder: + tuberculosis detected;

- tuberculosis was not detected.

As can be seen from the results of Table 4, after infection with the M. bovis strain of group 1, during a bacteriological examination of pathological samples of rabbits that received Rifizostrep once every 5 days, none of the 8 rabbits were found to have M. bovis isolated from their internal organs. During bacterioscopic examination, no mycobacterial bacilli were found in any smear stained using the Seal-Neelsen method.

As a control in the 2nd group, tuberculosis pathogens grew by forming typical colonies on the surface of the Lowenstein-Jensen nutrient medium in culture samples from internal organ samples of 3 rabbits infected with the M. bovis strain and not receiving the drug. As a control, colonies of tubercles grew in all tubes grown in pure form, and they had the following characteristics:

- growth rate - 26-30 days on average; colony description - uneven shape, bumpy surface, dry R-colonies, single, small dewy volume, regular shape, rough surface, dry and sticky consistency, ivory pigmented, colony thinning level is average. Consistency - crumbles.

Microscopy of smears of mycobacterial species stained by the Siel-Neelsen method from tuberculosis colonies obtained from the food environment using a bacterial bacillus revealed the following morphological and tinctorial features: the M. bovis strain was stained red-violet. Polymorphism is expressed in the morphology of M. bovis bacilli. Under the microscope, short, thick, thin-flat-pointed and somewhat thickened bacterial rods were discovered. In some fields of view, cocci-like forms were also revealed; granules were expressed in the composition of old cells.

After infection with strain M. tuberculosis 7880 in the 3rd group, pathological samples from 8 rabbits that received rifizostrep once every 5 days were bacteriologically examined. No mycobacteria bacilli were found in smears stained using the Seal-Nielsen method during bacterioscopic examination.

As a control, in the 4th group, the M. tuberculosis strain 7880 was infected and did not receive the drug in culture studies from samples of internal organs of rabbits; tuberculosis pathogens grew on the surface of the Lowenstein-Jensen nutrient medium, forming typical colonies. After 26-28 days, typical tuberculosis colonies grew rapidly in the control tubes in the form of small growths. Colonies of pathogens formed R-colonies on the surface of the nutrient medium, pigmented ivory-colored, in the form of a dewy form, in fragments or connected to each other. The consistency of the colonies is dry and crumbly, diffusely scattered, some have a slightly sticky consistency.

Microscopy of tuberculosis colonies obtained from a nutrient medium using bacterial bacilli revealed the following morphological and tinctorial features: in the smear, the M. tuberculosis 7880

strain was stained red-violet, and polymorphism was expressed in the morphology of the bacterial bacilli. Under the microscope, long and short, thick, thin, flat-ended and some thickened bacterial rods were found. In some fields of view, coccus-like forms are also found; granules are formed in some old cells; granules are not expressed in young cells.

- During a bacteriological study of pathological samples of rabbits from a comparative control group that received the drug Isoniazid after infection with strains of *M. tuberculosis* and *M. bovis* in groups 5 and 6, colonies of tuberculosis were found in the internal organs of two of them. 6 rabbits grew up in the 5th and 6th groups, 4 of them did not form colonies. Smears were prepared from the bacterial mass formed during cultural examination and stained using the Seel-Neelsen method. Bacterioscopy revealed tuberculosis bacilli with typical morphological and tinctorial features in smears.

- Thus, we can conclude that based on the results of a comparative study of the antibacterial activity of the drug Rifizostrep against strains of tuberculosis in rabbits with the drug isoniazid, the drug Rifizostrep has an active antibacterial effect on tuberculosis pathogens compared to the drug isoniazid.

Conclusion

1. Bacteriological studies have established that the drug Rifizostrep has a more active antibacterial effect on tuberculosis pathogens compared to the drug Isoniazid;

2. During cultural examination and bacterioscopy of smears in samples from guinea pigs of the experimental group that received the drug, no tuberculosis was detected, and in pathological samples from animals in the control group that did not receive the drug, tubercle bacilli with typical morphological and tinctorial characteristics were revealed;

3. According to the results of a comparative study of the antibacterial activity of the drug Rifizostrep against strains of tuberculosis in rabbits with the drug isoniazid, the drug Rifizostrep showed an active antibacterial effect on tuberculosis pathogens;

4. The drug "Rifisostrep" - exhibits synergism (increasing the effect of one drug by another) and prolongation (increasing the duration of action of the drug);

5. The pharmacokinetics of the drug "Rifizostrep" against pathogenic and atypical mycobacteria, leprosy, gram-negative (*Escherichia coli*, salmonella, *Klebsiella*, tularemia, etc.) and some gram-positive (staphylococci, pneumococci, streptococci, anthrax, rickettsia) has a bactericidal and bacteriostatic effect on microorganisms, also has a virucidal effect.

References

- [1] Antibiotics, sulfonamides and nitrofurans in veterinary medicine. Kovalev V.F., Volkov I.B. etc./Moscow All-Union Association "AGROPROMIZDAT" 1988.– 222 p.
- [2] Donchenko N.A. Improvement of means and methods for diagnosing and preventing tuberculosis in cattle // Abstract of the dissertation of the Doctor of Veterinary Sciences. - Novosibirsk 2008. p. 36
- [3] Lysenko A.P. Development and implementation of new methods for diagnosing and preventing tuberculosis in the Republic of Belarus / A.P. Lysenko, A.E. Vysotsky, T.N. Ageeva // Veterinary Pathology-2004-No. 1-2.-P.41-43.
- [4] Mamadullaev G.Kh. et al. Rifizostrep is a new combined drug against *Mycobacterium tuberculosis* / National Academy of Sciences of Belarus Republican Unitary Enterprise "Institute of Experimental Veterinary Medicine named after. S.N. Vyshel'sky" 2/2022 //International