AZERBAIJAN REPUBLIC

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

SALMONELLOSIS OF HONEY BEES IN AZERBAIJAN AND THE DEVELOPMENT OF NEW TREATMENT AND PREVENTIVE MEASURES AGAINST IT

Speciality: 3109.01 – "Veterinary microbiology, virology, epizootiology, mycology with mycotoxicology and immunology"

Field of science: Agrarian sciences

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INTRODUCTION

The Relevance of the Topic. As the development of the non-oil sector in Azerbaijan becoming priority, beekeeping in Azerbaijan is aimed at ensuring the necessary development of beekeeping, turning it into a competitive and profitable field while applying relevant advanced international practices and technologies in our country in order to improve the breeding work, as well as protecting and preserving the local aboriginal bee gene pool. Thus, it is quite demanding to carry out scientific research in the relevant direction regarding the development of new treatment and preventive measures grounded on scientific research against diseases of honey bees, including salmonellosis, which cause great economic damage to farms.

The development of beekeeping in the country is an important component of the State's agrarian policy in terms of ensuring food security and eliminating unemployment at the expense of honey bee products.

The "Food Safety Program of the Republic of Azerbaijan" approved by Decree No. 640 of the President of the Republic of Azerbaijan, Heydar Aliyev, dated March 2, 2001, is the first important normative-legal act adopted in the relevant field. Increasing the production and keep the prices of local agricultural food in affordable level is is one of the main goals of the Food Safety Program.

The Karabakh region and other regions around the region freed from occupation have a special importance in the development of beekeeping.

It is not excluded that there are a number of natural and anthropogenic factors that prevent the development of beekeeping to the required level and the harvesting of abundant and quality crops in our country. Climate change that often occurs in the world, excessively hot or rainy weather causes a decrease in productivity in beekeeping. However, other factors, diseases, mainly infectious diseases, also cause great damage to beekeeping.

One of the infectious diseases that cause serious economic damage to bee familiesis the Salmonellosis disease. Salmonellosis is an infectious disease of old bees, caused by various types of bacteria

belonging to the genus Salmonella (*S. typhimurium, S. gallinarium, S. pullorum*), which causes their mass loss and a sharp decrease in productivity. When salmonellosis occurs, mortality among older bees is 50-60%[1].

Salmonellosis of honey bees, its diagnosis, epizootiology, clinical symptoms, and prevention have been iesied in various countries, and the technology for the preparation of various drugs for its treatment has been developed, synthesized, and applied locally[2].

Despite the fact that the salmonellosis disease of honey bees has been sufficiently studied in many countries, there are very few scientific studies in this field in Azerbaijan, and the disease has not been studied extensively. The wide distribution of disease agents in the external environment, their resistance to external environmental factors, resistance to physical effects and many chemical substances require the development of more effective treatment and preventive measures for the prevention of the disease.

Therefore, in the current conditions, we set the goal of conducting scientific research in the relevant direction for the development of treatment-prophylactic measures against salmonellosis of honey bees in the country.

Although many chemical preparations have been proposed as a way of prevention of salmonellosis, such as Formaldehyde (2%), monochlorinated iodine, sodium hypochlorite solution, etc., many of these preperations have a number of missing properties. Some of them damage and corrode the equipment and things of apiaries, while others complicate and impede the principle of operation by adversely affecting the health of beekeepers during use, and some are not only expensive, but also very difficult to find. Researches in this field are continued and scientists and specialists are conducting researches in order to identify effective drugs. Therefore, in recent years, researchers have reported on the effectiveness of iodine preparations in the treatment and prevention

[1] Bal arısının xəstəlikləri və zərərvericiləri. Dərslik. / S. Məhərrəmov, E. Əsədov, H. Hüseynov [və b.] – Naxçıvan: "Əcəmi" nəşr. – Poliqrafiya Birliyi, – 2014. – 240 s.

[2] Гробов, О.Ф. Болезни и вредители пчел / О.Ф. Гробов, А.К. Лихотин. – Москва: ВО: Агропромиздат, – 1989. – 239 с.

of honey bee diseases[3,4].

Therefore, the identification of new disinfectants, the development of their disinfection regimen (especially aerosol disinfection), and conducting research are one of the most important issues facing veterinary medicine and science.

Taking into account the above, the aim was to study the spread of salmonellosis pathogens in honey bees in the Guba-Khachmaz, Lankaran-Astara, and Shirvan-Salyan economic regions of Azerbaijan depending on the season and vertical zones, obtaining a pure culture of the S. typhimurium pathogen, testing disinfectants in laboratory conditions, and applying treatment and prevention in the farm.

The object and subject of the study. The object of the study is honey bee colonies in private beekeeping farms in the plain, mid-mountainous and mountainous areas of the Guba-Khachmaz, Lankaran-Astara and Shirvan-Salyan economic regions, relevant laboratories of the institute, as well as laboratory animals involved in the study (white mice). The epizootiology and spread of salmonellosis of honey bees in the aforementioned regions, the study of its biological characteristics and the development of treatment and preventive measures against it, bee products and samples, the culture of the pathogen, and the applied veterinary drugs are the subjects of the study.

The purpose and function of the study. The aim of the project is to detect salmonellosis in honey bee colonies kept on farms or in backyards in the plains, mid-mountainous and mountainous areas of the Guba-Khachmaz, Lankaran-Astara and Shirvan-Salyan economic regions, to study its clinical features, epizootiology and to develop complex treatment and preventive measures against the disease. To achieve this goal, the following tasks are planned**:**

1. Study of the epizootiological situation of salmonellosis in beekeeping farms in the northeastern and southern regions of the country where the study was conducted, and to research on the dynamics of

[3]Рожков, К. А., Кузнецов. А. Ф. Опыт применения" Монклавит-1" в составе углеводной подкормки для медоносных пчел // Материалы II Международной научно-практической конференции, – Киров: – 3 – 4 марта, – 2015, – с.142-145.

[4] Салимов, Р.М. Подкормки с препаратами йода / Р.М. Салимов, М.Г. Гиниятуллин, Н.М. Ишмуратова // ж. Пчеловодство, – Москва: – 2009. № 7,– с. 16-19.

salmonella bacteria across the landscape according to seasonal and ecological levels of contamination and recovery.

2. Determining the prevalence of salmonellosis in the study areas by agroclimatic/territorial height zones.

3. Studying the dependence of salmonellosis infection on the resistance of honey bee families to the disease.

4. Bacteriological and microbiological examination of honey bees and beekeeping products obtaining a pure culture of the organism and the causative agent.

5. Study of biochemical properties of (*S. typhimurium)* causative agent.

6. Pathogenicity of laboratory animals *(S. typhimurium*) the lesson of

7. Study of the effect of salmonellosis on the yield (honey) of honey bees.

8. Study of bactericidal effect of sodium hypochlorite and Monklavit-1 preparation.

9. Development and implementation of treatment and preventive measures against salmonellosis in honey bees, calculation of economic benefits, and development of recommendations based on the results obtained.

Research methods. In the course of research, microbiological and bacteriological examinations were carried out to determine infection with salmonellosis in live, sick and dead honey bees collected from bee farms, as well as in samples of honeycomp, honey, and rose[5,6,7].

Biochemical properties of Salmonella culture, pathogenicity and sensitivity to drugs were studied.

The dependence of the spread of salmonellosis in honey bees on vertical zones was investigated, disinfectants against the

[5] Sultanlı, Q.H. Başlıca ari xəstəliklərinin diaqnostikasına dair metodik vəsait. Bakı: "Müəllim" nəşriyyatı. - 2011. - 69 s.

[6]Гробов, О.Ф. Диагностика болезней пчел / О.Ф.Гробов, Л.Н. Гусеева // ж. Пчеловодство, – Москва: – 1991. № 5, – с. 23-31.

[7]. Микитюк, П.В. Диагностика болезней пчел. (Методическое пособие) / П.В. Микитюк, С.Т. Рягин – Белая Церковь: Белоцерковский сельскохозяйстве нный институт, – 1977. – 65 с.

causative agent of salmonellosis were tested in laboratory and farm conditions, the economic efficiency was calculated with the appropriate formulas, and a recommendation was drawn up based on the results.

The main provisions presented to the defense:

1. The epizootological situation and seasonal dynamics of salmonellosis in honey bees in the beekeeping farms of the northeastern and southern regions of the republic were studied, and it was determined that the prevalence of the disease is higher in the northern region than in the southern region.
2. Infection with salmonellosis and tolerance to the disease depend directly on the strength of honey bee colonies.
3. The biochemical properties of salmonellosis and its effect on bee honey productivity, its quantitative and qualitative indicators were identified.
4. It is possible to completely neutralize salmonellosis-infected honey bee colonies and hives by spraying (with aerosol method) the Monklavit-1 preparation.
5. In addition to having a disinfectant effect, the Monklavit-1 preparation is also more effective in the treatment of salmonellosis according to the scheme we propose.

Scientific novelty of the study:

- For the first time in Azerbaijan, the rates of salmonellosis infection of honey bee families in the Guba-Khacmaz, Shirvan-Salyan and Lankaran-Astara economic regions of the country, the dynamics of the seasonal spread of the disease and the causative agent in both regions were studied;

- For the first time in the Republic, the influence dynamics of honeybees against salmonellosis and the effect of the disease on bees' honey productivity were studied;

- For the first time in the republic, a pure culture of *S. typhimurium*, the causative agent of the disease, was obtained, its biochemical properties, degree of sensitivity to antibiotics and pathogenicity in laboratory animals were studied;

- For the first time, by studying the bactericidal properties

(effects) of sodium hypochlorite and monclavit-1 against the causative agent of the disease, rules for the use of monclavit-1 in bee and bee products, as well as equipment used in beekeeping, were developed and tested in a wide range of farm conditions. It was determined that monclavit-1 drug is more effective, the economic benefit of one bee family during its application is 442.86 AZN. However, the methodological instruction (recommendation) "New disinfection scheme against the causative agents of salmonellosis in honey bees" was developed and approved for use.

Theoretical and practical significance of the research. Since the salmonellosis disease of honey bees has not ever been studied under the conditions of Azerbaijan, the causes of the origin and spread of the disease in research facilities in Guba-Khacmaz, Shirvan-Salyan and Lankaran-Astara economic regions of the country were determined on scientific grounds.

It is not excluded that the successful results of the conducted research will be applied in beekeeping in other regions of the republic, as well as in the liberated Karabakh region and its surrounding regions.

For the first time in Azerbaijan, we determined the optimal disinfectant and treatment dose of Monclavit-1 drug for the treatment and prevention of salmonellosis in the beekeeping farms of its Guba-Khacmaz, Shirvan-Salyan and Lankaran-Astara economic regions, studying the biological characteristics of the causative agent of S. typhimurium, in the plains, foothills and mountainous zones of the Adyghe regions. The study of the spread of salmonellosis (the causative agent) by season is of great theoretical and practical importance.

Therefore, thanks to the successful solution of the issues, the monclavit-1 preparation, prepared based on the results, is intended to be used in the treatment and prophylaxis of honey bees with salmonellosis in bee farms, and in the neutralization of the causative agent of the disease. It is not excluded that the scientific results obtained by us can be used in the teaching of relevant subjects in higher and secondary schools, and can be referred to when writing works on the microbiological study of honey bees.

Approbation and application of the work. The main provisions of the research work were reported and widely discussed in the annual reports of the the Fish and Bee Diseases Department of the Veterinary Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan, in the in the Scientific Council, and in the official meetings of this Institute, as well as in the national and international (local and foreign) scientific conferences mentioned below.

The results and proposals arising from the research were presented at the following conferences:

1. III-Nakhchivan International beekeeping conference, Nakhchivan (May 26-27, 2017).
2. IV-Nakhchivan International beekeeping conference, Nakhchivan (May 18-19, 2018).
3. V-Nakhchivan International beekeeping conference, Nakhchivan (24-25 May, 2019).
4. Materials of the international scientific-practical conference on the application of innovations in the direction of the development of Veterinary Science, - Baku (November 25-26, 2019).
5. IX International Conference "Current problems of society, economics and law in the context of global challenges", Moscow (April 4, 2022).

Based on the research materials, 5 articles (2 abroad), 5 conference materials (1 abroad), 1 methodical tool and 1 recommendation were published, containing the main content of the dissertation work.

The name of the institution where the dissertation work was performed. The research work was carried out in the laboratories of the Fish and bee Diseases Department of the Veterinary Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan.

The structure and scope of the dissertation work. The dissertation work is presented on 145 pages, 190,788 characters. The dissertation work consists of an introduction (18,702 characters), 6 chapters (Chapter I 57,611 characters, Chapter II 25,256 characters, Chapter III 21,469 characters, Chapter IV 12,741 characters, Chapter V 11,942 characters, Chapter VI 21,496 characters), discussion of the

research (17,813 characters), results (2,511 characters), practical suggestions (1,247 characters), a list of used literature, 16 figures, 22 tables and 2 graphs and 1 scheme. The list of used literature includes 157 sources cited in the dissertation. 48 of them are Azerbaijani, 93 are Russian, and 16 are other foreign literature.

CHAPTER I
LITERATURE SUMMARY

This chapter of the dissertation describes the history of studying salmonellosis of honey bees in Azerbaijan and abroad, the species composition of the pathogens, the degree of study of their spread, treatment and prevention, the physical geographical characteristics of the researched areas of the regions, as well as the vegetation cover, landscapes by altitudinal zones, climatic indicators and other features[8].

CHAPTER II
RESEARCH MATERIALS AND METHODS

The studies were conducted in 2013-2021 in the laboratory of Fish and bee Diseases of the Veterinary Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan and on the basis of materials collected from individual beekeeping farms located in the plain, mid-mountainous and mountainous areas of the Guba-Khachmaz, Lankaran-Astara and Shirvan-Salyan economic regions. (Figure 1).

In order to determine the infection with salmonellosis in honey bees, the collected samples were inoculated with bacteriological EPB (meat peptone broth), EPA (meat peptone agar), Bismuth sulfite agar, Endo agar, MacConkey agar, XLD agar, nutrient media using generally accepted methods, and thin smears were prepared using the Gram method and microbiological examinations were performed.

[8] Azərbaycan Respublikasının coğrafiyası. Regional cografiya / AMEA, akad. H.Ə. Əliyev adına Coğrafiya İnstitutu - Bakı: "Avropa" Nəşriyyatı, - 2015. -c.3, -400 s.

Literature was used in conducting biological examinations[9].

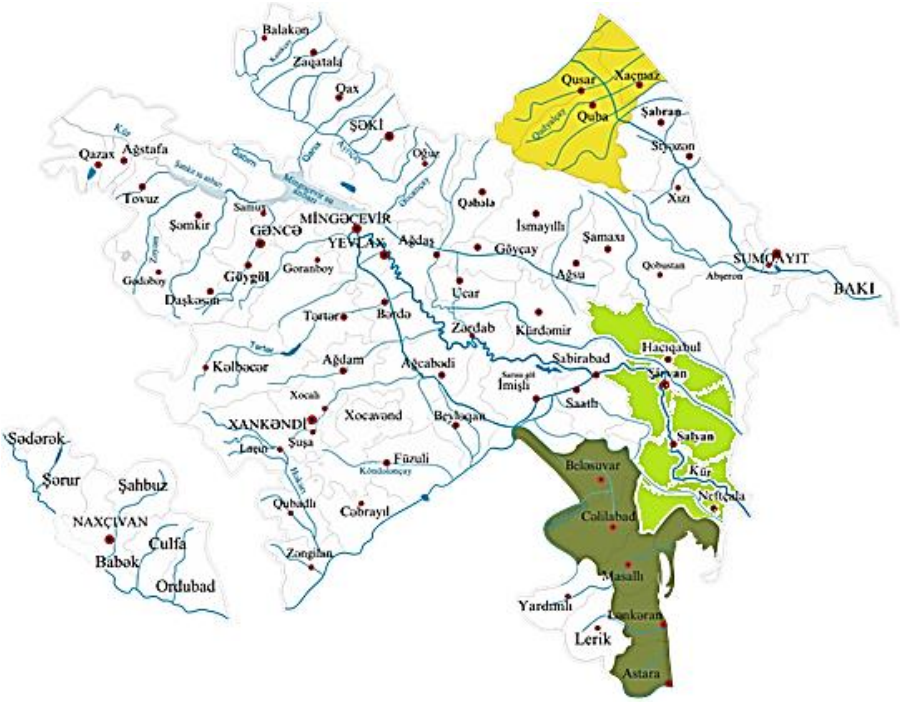


Figure 1. Geographical map of the researched areas

To determine the infection with salmonellosis pathogens in honey bees, a Motic №6105283 and Solaris BEL binocular microscope were used.

The biochemical properties of some carbohydrates of the causative agent of *S.typhimurium* were studied, and the *S.typhimurium* ATCC 14028 LOT 363-701-2 strain culture was used for control purposes.

The biochemical properties of some carbohydrates, pathogenicity properties of the causative agent of salmonellosis, as well as the degree of sensitivity to drugs were studied on white mice and bees.

The pathogenic properties of the causative agent of *S.typhimurium*

[9] Аликин, Ю.С. Диагностика и профилактика вирусных болезней пчел // – Москва: ж. Пчеловодство, – 2011. №5, – с. 28-30.

were studied on white mice and bees, as well as the degree of sensitivity to drugs and tested under farm conditions.

During the study, the dimensions of honey bee species were measured using an MBC-10 stereoscopic microscope with an ocular micrometer.

To determine the amount of nitrogen in the bee organism, generally accepted methods were used. For this purpose, the bee's digestive apparatus was removed together with the honey comb, and the amount of nitrogen was determined using the Kjeldahl apparatus.

Comparative bactericidal properties of monclavit-1 and sodium hypochlorite preparations were studied for the purpose of preventing salmonellosis.

In order to study the effect of disinfectants on the causative agent of *S.typhimurium,* monclavit-1 and sodium hypochlorite preparations were used, and ordinary water was used as a control. In order to prepare solutions of sodium alkali with different percentages, a RADWAG Elektronik, Model: AS220.R2 scale was used. In order to continue the experiments, the disinfectant effect of these solutions was studied in a laboratory experiment when a swab was infected with a *S.typhimurium* microbial culture in a box per 1m³ area.

An effective disinfectant (5% Monclavit-1 preparation) was tested in farm conditions and the economic efficiency of the applied preparations was calculated based on the positive results.

The statistical significance of the difference based on the tests was checked using the Student's t-test[10].

CHAPTER III
EPIZOOTIOLOGICAL SITUATION OF SALMONELLOSIS IN HONEY BEE SPECİES IN BEEKEEPING FARMS IN THE QUBA-KHACMAZ ECONOMIC REGION

In this chapter, the degree of spread of salmonellosis in beekeeping farms in the north-eastern and southern regions of the republic, the

[10] Hesablama texnikasının və eksperimentin riyazi nəzəriyyəsinin elmi tədqiqatlarda tətbiqi. T.Ə.Babayev, A.R.Bünyatov, Q.C. Əfəndiyev [və b] / – Bakı: Elm, – 1999. – 102 s.

spread of the disease in vertical zones, the seasonal dynamics of infection and the strength of honey bee families during the wintering period on bee loss and the effect of salmonellosis infection are discussed.

The epizootiological situation of the relevant farms in the studied regions was determined based on the detection of salmonella pathogens in samples taken from a total of 720 bee colonies (Table 1).

Table 1

Indicators of salmonellosis infection in samples taken from bee families in Guba-Khacmaz Economic Region

№	City	Number of samples taken from bee colonies	Number of bee colonies infected with salmonellosis	Number of bee colonies infected with salmonellosis (in %)
1	Guba	240	180	75,0
2	Gusar	240	150	62,5
3	Khacmaz	240	90	37,5
	Total	720	420	58,3

During the research, the epizootiological status of the relevant farms of the researched regions was determined according to the indicators of salmonella causative agent detection in the samples taken from a total of 720 bee colonies in Guba-Khacmaz, Shirvan-Salyan and Lankaran-Astara economic regions of the republic.

Salmonellosis infection was recorded in 75,0% of Guba, 62,5% in Gusar, 37,5% in Khachmaz and 58,3% of bee families in beekeeping farms in the north-eastern region of the Republic (Table 1). Proportion of infection in beekeeping farms located in Quba and Qusar districts the number of bees, the location of bee families closer to each other and the profile. It is believed that the main reason is the timely implementation of construction measures.

The epizootiological situation of the spread of the disease in the beehives populated along the vertical belts in the north-eastern regions of Azerbaijan, as well as in the Guba region, was determined. During the conducted microbiological and bacteriological examinations, the causative agent of *S. typhimurium* was found in various food environments and its culture was separated (Figure 2;3).

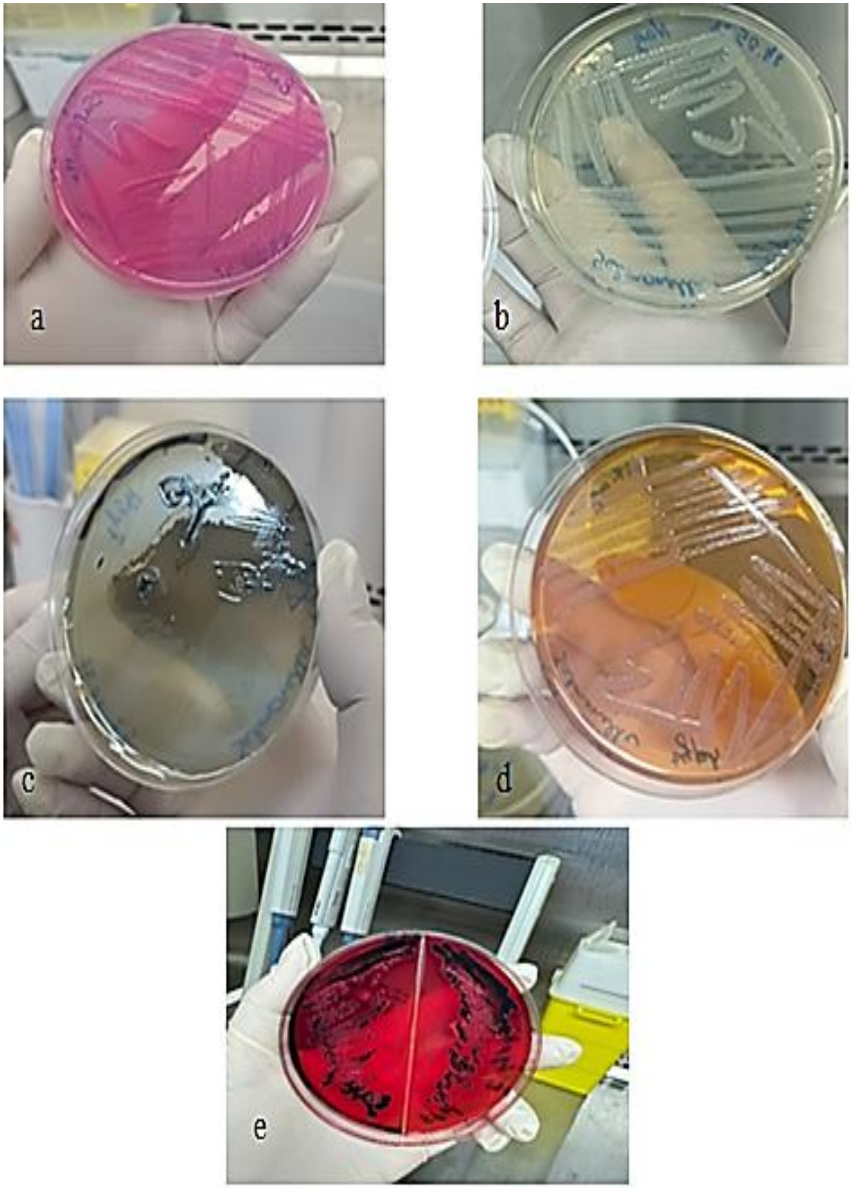


Figure 2. (a,b,c,d) *S. typhimurium* causative agent investigated in different food environments

a) Endo agar; b) Meat peptone agar (EPA); c) Bismuth sulfite agar;d) MacConkey agar e) XID agar

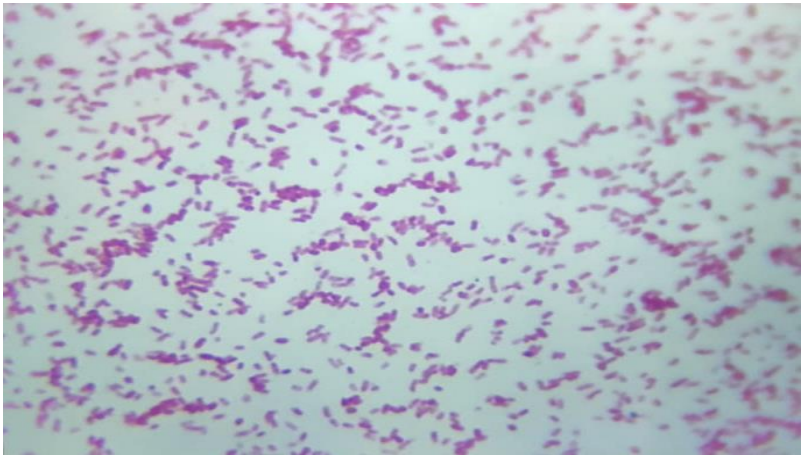
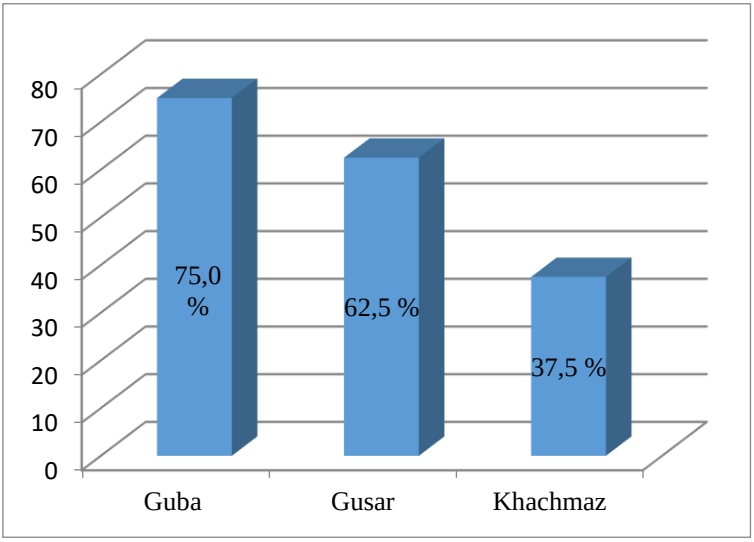


Figure 3. Microscopic appearance of *S. typhimurium* causative agent

Salmonellosis infection in beekeeping farms in the Guba-Khachmaz economic region was 75,0% in Guba, 62,5% in Gusar, and 37,5% in Khachmaz (Graph 1).



Graph 1. Prevalence of salmonellosis in beekeeping farms of some northeastern regions of Azerbaijan (in %)

Considering the high incidence of salmonellosis infection in bees in the farms of the Guba region, it was considered important to determine the prevalence of the disease in some farms located at high altitudes in the region (Table 2). As can be seen from the table, farms located in mountainous areas were more susceptible to infection than other areas.

Table 2

Epizootiological situation of salmonellosis infection of bee families in the plain, mid-mountainous and mountainous zones of Guba region

№	Name of the Area	Number of Apiaries	The number of bee families	The number of infected bee families	The number of infected bee familiesin %
Plain					
1	Qonaqkand	11	1107	290	26,2
2	Jimi	9	991	218	22,0
3	Khashi	7	721	174	24,1
	Total	27	2819	682	24,1
Middle Highlands					
4	Khinalig	7	653	230	35,3
5	Griz	9	1014	426	42,1
6	Galykhudat	5	452	165	36,6
	Total	21	2119	821	38,7
Mountainous					
7	Jek	8	570	243	42,7
8	I Nughadi	10	998	436	43,7
9	II Nughadi	11	1082	477	44,1
	Total	29	2650	1156	43,5

As can be seen from Table 2, the impacts located in mountainous areas are different from other areas. Those areas are relatively more exposed to infection. We come to conclusion that the high infection rate in those areas is due to the continuous presence of bee colonies in the same location. Because a disease in one bee colony

is more likely to spread to other nearby bee colonies, and all bees soon become infected with the disease.

CHAPTER IV
EPIZOOTIOLOGICAL SITUATION OF SALMONELLOSIS IN HONEY BEE SPECİES IN BEEKEEPING FARMS IN THE LANKARAN-ASTARA AND SHIRVAN-SALYAN ECONOMIC REGIONS

In 2013-2021, studies were conducted to determine the prevalence of salmonellosis in honey bees in Lankaran, Astara, Masalli, Jalilabad and Bilasuvar regions (Table 3).

Table 3

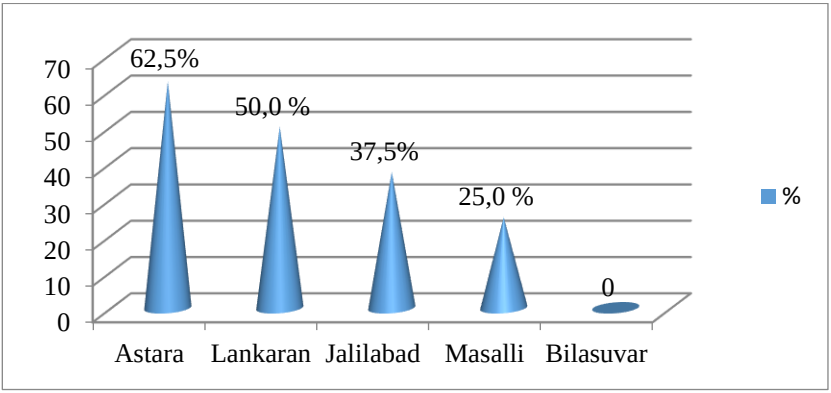
Indicators of salmonellosis infection in samples taken from bee colonies by Lankaran-Astara and Shirvan-Salyan Economic Region

№	City	Number of samples taken from bee colonies	Number of bee colonies infected with salmonellosis	Number of bee colonies infected with salmonellosis (in %)
4	Astara	240	150	62,5
5	Lankaran	240	141	59,0
6	Masalli	240	60	25,0
7	Jalilabad	240	90	37,5
8	Bilasuvar	240	0	0
	Total	Total 1200	441	36,8

In beekeeping farms in Shirvan-Salyan and Lankaran-Astara economic regions, 62,5% salmonellosis infection was recorded in Astara, 50,0% in Lankaran, 25,0% in Masalli, 37,5% in Jalilabad. İn Bilasuvar though the disease has not been discovered.

As can be seen from the Graphs 2, salmonellosis infection of bees in the Shirvan-Salyan and Lankaran-Astara economic regions of the

republic varies depending on the regions position varied in accordance with geographical location.



Graph 2. Epizootiological situation of salmonellosis infection of bee families in Lankaran-Astara and Shirvan-Salyan economic region

Depending on the geographical location of the beehives in the areas of Masalli region, which differ from each other due to different natural and climatic conditions, the course of salmonellosis in bee families has different forms. The duration of salmonellosis disease and the degree of infection of bees depends on the condition of bee colonies, as well as on the geographical location and conditions of the apiaries. For this reason, it is important to study the dynamics of the spread of salmonellosis in lowland, mid-mountainous and mountainous areas and to determine the epizootiological condition of the area or the apiary according to the clinical signs of the disease.

As a result of the research, the number of bee colonies and their degree of infection with salmonella were determined in the apiaries of villages located in the plain, mid-mountain and mountainous areas of Masalli district. The research was conducted on pathological materials collected from beekeeping farms. The infection was on average 24,1% in the plain areas, 45,8% in the mountainous areas and 38,0% in the mid-mountain areas.

Based on the result of the observations, it was found that only 30-35% of the existing apiaries in Masalli district are moved to another place only once or twice a year due to the season. Due to the non-compliance

with the existing rules on moving apiaries at least 4-5 times depending on the flowering period of plants due to the season, the development of bee colonies weakens (loses strength) and weak bee colonies are placed for wintering, which ultimately increases the infection rate due to a decrease in the immunity of bees.

The rate of salmonellosis infection in beekeeping farms in Masalli district, depending on the strength of bee colonies, was determined in the fall of 2016 and spring of 2017, and the corresponding epizootiological situation of the area was assessed. For this purpose, 3 experimental groups consisting of 15 similar bee colonies from each of the plain, mid-mountainous and mountainous areas were organized (Table 4).

Table 4

Strength and salmonellosis infection of bee colonies during and after hibernation in the southern region (n=15)

S/N	Areas	Experience regions	The strength of bee colonies when put into hibernation	The strength of bee colonies after hibernation	Bee loss	Salmonella infection rate of bees %
1	Plain	Weak	1.19±0.12	0.75±0.06	36,9	33,5
		Medium	1.60±0.18	1.21±0.12	24,4	27,1
		Strong	2.19±0.20	1.85±0.17	15,5	17,5
		On average	1.66±0.17	1.24±0.14	25,3	26,0
2	Middle highlands	Weak	1.31±0.15	0.85±0.09	35,1	41,2
		Medium	1.81±0.19	1.38±0.16	23,8	32,4
		Strong	2.28±0.24	1.91±0.19	83,8	20,4
		On average	1.80±0.19	1.16±0.15	35,5	31,3
3	Mountainous	Weak	1.37±0.14	0.70±0.09	51,1	53,1
		Medium	1.72±0.17	1.15±0.14	66,9	39,6
		Strong	2.15±0.21	1.66±0.21	77,2	22,1
		On average	1.75±0.18	1.56±0.15	10,9	38,2

As can be seen from Table 4, each of the 3 experimental groups with 15 bee families each consisted of 1/3 of low power, 1/3 of medium power and 1/3 of strong bee families. (with 50 bees per colony) two weeks after coming out of hibernation, the degree of infection of bees was determined by collecting the older bees after they had performed a cleaning flight.

It can be seen from the table that the rate of salmonellosis infection of bees in weak bee families living in plain, mid-mountainous and mountainous regions was 26,0%, 31,3% and 38,2%, respectively.

In apiaries consisting of weak families located in the plain area, the rate of salmonellosis infection of bees in bee families was 7,7% and 19,6% lower, respectively, compared to bees in the foothills and mountainous regions.

These indicators are similarly observed in bee families with medium strength. Thus, compared to families located in the plains, salmonellosis infection was 5,3% and 12,5% higher in the families located in the mountainous and mountainous areas, respectively.

It can be seen that weak colonies are more susceptible to the disease than medium-strength and strong colonies, regardless of the region in which they are located.

The study found that the rate of infection with the disease varies depending on the area where honey bees live and the strength of the colony in the Masalli region. Since strong colonies are less susceptible to the disease during the winter and early spring, they have an easier winter and develop rapidly in early spring, collecting more honey.

Since bees from weak colonies are more susceptible to the disease during the winter, they have a worse winter, and their early spring development is delayed and their productivity decreases due to their weakening.

Considering that bees from strong colonies are 50-55% less susceptible than those from weak colonies, it is appropriate to put mainly strong colonies in apiaries for wintering.

CHAPTER V
BIOCHEMICAL, PATHOLOGICAL AND CLINICAL SYMPTOMS IN THE HONEY BEE ORGANISM INFECTED WITH SALMONELLOSIS

In this chapter, the salmonellosis disease of honey bees, the sensitivity of the salmonellosis agent to antibiotics, the effect of the disease on the amount of nitrogen in the honey bee body, and other characteristics are explained.

Salmonella typhumurium, isolated during salmonellosis disease of honey bees, was planted in food media and tested with some carbohydrates on one-day culture. During the experiments, maltose, glucose, mannitol and fructose and TCA (triple sugar agar) were broken down, gas and acid formation were observed. It was determined that the causative agent had no effect on lactose, dulcite, sucrose, inulin and raffinose carbohydrates, that is, it was not subjected to decomposition. During the experiments, *S. typhimurium* did not spoil milk or dissolve gelatin. The results obtained are consistent with *S. typhimurium* strain culture and literature data.

In the research process, the pathogenicity of *S. typhimurium* culture obtained from honey bees was studied on laboratory animals, the first studies were conducted on white mice.

In order to study pathogenicity, a suspension was prepared from a 24-hour agar culture of one billion *S. typhimurium* culture in physiological solution, 10 mice were injected into the thigh muscles of white mice in an amount of 0,3 ml, and 3 were kept under control. All infected mice died within 4-5 days. Bone marrow of dead white mice was inoculated into Meat Peptone Broth (MPB) medium and placed in a $3,7C^0$ thermostat. After one day, agar medium was inoculated into Meat Peptone Agar (MPA), Endo Agar, MacConkey Agar, Bismuth Sulfite Agar medium and placed in a $3,7C^0$ thermostat again, and after 72 hours, a suspension was prepared from the *S. typhimurium* culture in physiological solution in a ratio of 1:10.

The experiment was continued on honey bees. In order to study the pathogenicity of the cultures on live bees, 50 bees in the cage were fed with a salmonella culture prepared with syrup. 50 bees in another

control cage were fed only with the prepared syrup. As a result, all bees fed with salmonella culture died within 4-5 days, while the bees in the control did not die.

Bees from both groups were recorded and it was found that the mortality rate during infection was 80%.

In order to comparatively determine the effectiveness of the drugs against salmonellosis in beekeeping farms of the Lankaran-Astara economic regions, the sensitivity of Zinaprim and Intravit drugs was first studied in laboratory conditions, the infection rates of this disease were determined before and after using the drugs, and then the effectiveness of the drugs was tested in farm conditions.

According to the results of the studies, the effectiveness of zinaprim and intravit preparations was tested and it was determined that zinaprim gave 78,2% efficiency when used alone, and 86,9% efficiency when zinaprim and intravit preparations were used together.

Salmonellosis, as an infectious disease of bees, not only causes their mass mortality, but also has a serious negative impact on the development of bee colonies, biochemical and physiological processes in the bees' bodies, leading to a decrease in their productivity.

The amount of nitrogen in the body of one bee in 5; 15; 25-day-old bees of bee colonies infected with salmonellosis was 1,51; 2,09 and 1,93 mg, respectively, which was 13,0% (t_2=13,9); 9,0% (t_2=2,19) and 15,4% (t_2=2,81) less than in bees of healthy bee colonies, respectively.

In both healthy and infected bees, the amount of nitrogen in the body of a bee increases as it ages compared to 5-day-old bees. 25-day-old bees do not participate in the nutrition of bee larvae. However, the amount of nitrogen in the body of infected bees increases first in 15-day-old bees, and then decreases in 25-day-old bees compared to healthy bees.

Since bees in infected colonies produce more bee brood, more royal jelly is secreted from their glands in their bodies, which results in the colonies losing more energy because they consume more food, which causes nitrogen to decrease. Therefore, the amount of nitrogen

in the body of healthy bees increases slightly at first, and the rate of nitrogen increase decreases due to the excess energy consumed.

CHAPTER VI
THE EFFECTS OF DRUGS AGAINST SALMONELLOSIS IN HONEY BEES AND THE TREATMENT AND PREVENTIVE MEASURES

Taking into account the economic damage that salmonellosis can cause to beekeeping, which is a profitable industry whose role in ensuring the food security of the population in the country cannot be ruled out, we considered it appropriate to implement the recommended treatment and prevention measures in beekeeping farms based on the results of our research. In this regard, the research work carried out is relevant.

6.1. Bactericidal effects of monclavit-1 and sodium-hypochlorite preparations

Taking into account the fact that salmonellosis, the causative agent of salmonellosis, is highly resistant to physical and chemical effects and is widespread in the external environment (soil, water, etc.), taking into account the comprehensive measures to combat the disease, disinfection of the appropriate equipment and supplies used in beekeeping (tools, bee hives, etc.) implementation is important.

In recent years, aerosol disinfection methods for equipment and supplies have been developed and are being implemented against many infectious diseases of bees. At the same time, by applying this method, which also saves human labor, it is possible to save 4-5 times on medicines, as well as completely neutralize pathogens in all parts of the relevant tools, including bee boxes.

The disinfectant properties of sodium hypochlorite and monclavite-1 preparations, disinfectants against salmonellosis of honey bees, were comparatively studied in laboratory conditions in private beekeeping farms of the studied regions.

To study the bactericidal indicators of both preparations, a mixture of the aforementioned preparations in water at different concentrations was used in a 24-hour culture of the *S. typhimurium* pathogen.

As a result, it was found that a 5% solution of the Monclavit-1 preparation destroyed the culture in 30 minutes, a 3% solution of the sodium hypochloride preparation in 30 minutes, and a 5% solution in 20 minutes(Table 5).

Table 5

Bactericidal indicators of Monclavit-1 and sodium hypochlorite preparation

The concentration of the drug	**Growth of microorganisms due to exposure to disinfectants**														
	Effects of Monclavit-1 over different periods					Effects of sodium hypochlorite over different periods					Control group (distilled water)				
	10 min	20 min	30 min	40 min	60 min	10 min	20 min	30 min	40 min	60 min	10 min	20 min	30 min	40 min	60 min
1%	+	+	+	+	+	+	+	+	+	_	+	+	+	+	+
2%	+	+	+	+	+	+	+	+	_	_	+	+	+	+	+
3%	+	+	+	+	+	+	+	+	_	_	+	+	+	+	+
4%	+	+	+	+	+	±	+	_	_	_	+	+	+	+	+
5%	+	+	_	_	_	+	_	_	_	_	+	+	+	+	+
100%	_	_	_	_	_	_	_	_	_	_	+	+	+	+	+

Note: + the height of the microbe ± short stature

- did not give birth

In the next stage, taking into account the above-mentioned advantages of aerosol disinfection, the comparative effectiveness of

these two preparations was studied to study the bactericidal properties of monclavit-1 and sodium hypochlorite preparations.

In order to study the bactericidal properties of both preparations used in aerosol form, an aqueous solution of 2 billion 24-hour culture of the *S. typhimurium* pathogen was used. For this, after swab test samples (2x2cm) were infected with microbial culture, the effect of both preparations in aerosol form on the corresponding culture was studied separately. The experiment was set up in separate boxes, 7 test objects were placed in each box, and 12 test objects were treated with distilled water as a control.

During the experiments, it became clear that after the 3% sodium hypochlorite solution was used in aerosol form three times, and the monclavit-1 preparation was used a second time, the corresponding objects were neutralized by the pathogen. None of the test objects in the control group were neutralized.

During the experiments, it became clear that after three repeated applications of 3% sodium hypochlorite solution in the form of an aerosol, and a second application of 5% Monclavit-1, the relevant test objects were neutralized by the pathogen. None of the test objects in the control group were neutralized.

Both preparations have a high bactericidal effect and are suitable for use in beekeeping. As a result of the experiments, it was determined that the sodium hypochlorite and Monclavit-1 preparations proposed for disinfection of bee hives in case of salmonellosis of bees can be used in the form of an aerosol.

6.2. On-farm testing of the treatment and prevention measures of the Monclavit-1 preparation against salmonellosis of honey bees

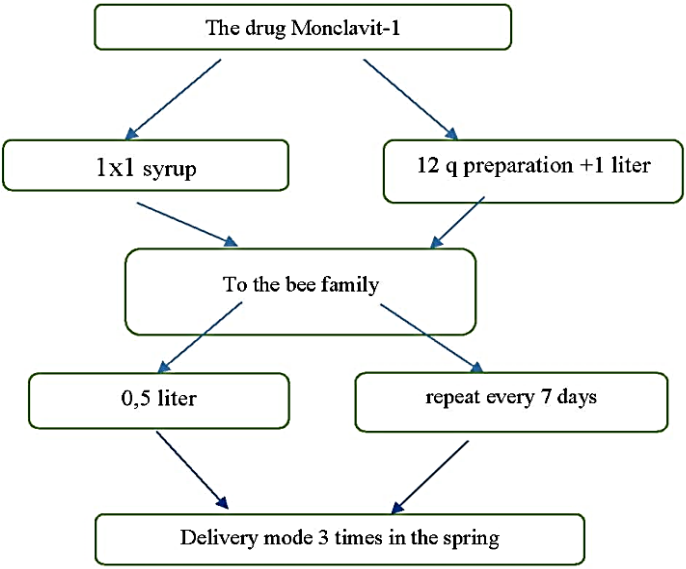
Based on the results of our research, the final stage of treatment and disinfection work was tested in farm conditions and applied on the farm.

It was determined by the conducted experiments that despite the fact that it is possible to use the sodium hypochlorite preparation in the form of an aerosol for the disinfection of beehives and other equipment during salmonellosis of bees, there is a need for additional experiments

for this, and considering the fact that there are some difficulties in

The study of the effectiveness of the Monklavit-1 preparation as a new treatment and prevention for salmonellosis of honey bees was tested on 60 bee colonies in the beekeeping farm of entrepreneur Y. Maharramov in the Sarchuvar village of Masalli district. For this purpose, 4 experimental groups were created according to the degree of infection and the indicated Monklavit-1 preparation was applied as a commission in production conditions in the following manner.

We have proposed a scheme for the use of Monklavit-1 preparation as a treatment for salmonellosis of bees. Thus, a solution containing 12 g of Monklavit-1 preparation added to 1 liter of syrup solution prepared in a 1:1 ratio of water and sugar was applied for treatment purposes. The appropriate solution form for each bee colony was applied to 25 bee colonies at the rate of half a liter. The above solution was administered for treatment purposes, repeated every 7 days, 3 times in spring and 3 times in autumn (Scheme 1).



Scheme 1. Treatment scheme of Monclavit-1 drug in salmonellosis of bees

The disinfectant effect of 3% sodium hypochlorite solution on 25 bee colonies and 5% solution of the Monklavit-1 preparation on 25 bee colonies against the causative agent of salmonellosis was tested in the same farm. 10 bee colonies were kept as a control group.

The effectiveness of the Monklavit-1 preparation was studied by disinfecting hives, combs, inventory, places where combs are stored, wax (wax raw materials), equipment, other items (special clothing, etc.), the wintering building and the workshop in the apiary area in the form of an aerosol.

In order to determine the comparative effectiveness of the preparations in salmonellosis of bees, samples were taken and examined in the appropriate manner before and after their application. First, cleaning work was carried out on the hives, frames, and partition boards contaminated with secretions. The preparation was sprayed in aerosol form at a rate of 5 ml per 1 m^3 of area. The air temperature during disinfection was 18-20^{0}C. After disinfection, the hives were kept outdoors for some time.

The results of the studies showed that by spraying (spraying) 5 ml per 1 m^3 of area with boxes containing honey bees infected with salmonellosis in aerosol form 3 times every 30 minutes, it is possible to completely neutralize bee colonies and boxes after 3 hours.

Thus, a total of 20 samples were taken from beehives, hives, and beekeeping inventory after the appropriate procedure within 3 hours. These samples were cultured in various nutrient media (EPB, EPA, Endo agar, MacConkey agar, Bismuth-sulfite agar) in the laboratory and incubated for 48 hours at 37C^0 in a thermostat. No growth of the pathogen was observed in the nutrient media and no salmonella pathogens were detected during microscopic examinations. As a result, the monclavit-1 preparation, when used in the indicated mode, did not grow in the cultured nutrient media, in which salmonella pathogens were completely neutralized in beekeeping farms.

Thus, as a result of the conducted test, it was confirmed that both preparations have a disinfectant effect. When comparing the

effectiveness of the Monklavit-1 preparation with the sodium hypochloride preparation, it was found that the Monklavit-1 preparation has high bactericidal properties, is easy to transport and use, and does not require special clothing during disinfection. Unlike the sodium hypochloride preparation, the Monklavit-1 preparation was found to be effective and efficient in the treatment and prevention of salmonellosis in bees.

The results of our experiments were applied in laboratory and farm conditions with the participation of the relevant commission of the Veterinary Science and Research Institute and were documented (the acts are attached).

6.3. Economic efficiency of the preparation Monclavit-1 used against salmonellosis of honey bees

Salmonellosis is widespread in honey bees in all regions of Azerbaijan and causes great economic damage to beekeeping farms. Thus, the occurrence of losses in bee colonies with salmonellosis causes significant damage to beekeeping farms as a result of a decrease in the quality of bees, honey, other products and income from beekeeping.

Based on the results of the studies conducted in farm conditions and the implementation of disinfection measures against the causative agent of salmonellosis of honey bees, the economic damage and the economic benefit were calculated.

As a result of the studies, when the weight of honey taken from 25 bee colonies infected with salmonellosis was compared with the weight of honey taken from a healthy bee colony, 7 kg less honey was obtained per bee colony per year.

As a result of our scientific research and experiments, it was confirmed that the economic efficiency of the disinfection method proposed by us using the monclavit-1 preparation is 442.86 manat per bee colony. The research and results obtained showed that the application of the Monclavit-1 preparation is more economically efficient as a disinfectant.

RESULTS

1. As a result of the conducted research, the epizootiological condition, distribution and seasonal dynamics of salmonellosis of honey bees in the beekeeping farms of the south and northeast regions of the republic were studied, and it was determined that the prevalence rate of the disease was 36,8% in the south region and 58,3% in the northeast region[3,8,9].

2. In the territory of the north-eastern region of the republic, it was determined for the first time that the rate of salmonellosis infection of bee families in the Guba region was 24,1% in the plain area, 38,0% in the mid-mountainous area and 43,5% in the mountainous area[8].

3. In the Shirvan-Salyan and Lankaran-Astara economic regions of the republic, it was determined for the first time that the rate of infection of honey bees with salmonellosis was 24,1% in the plain area, 38,0% in the middle mountainous area and 45,8% in the mountainous area [9].

4. During bacteriological and biological examinations, the culture of the causative agent of *S.typhmurium* was separated from the sick bees and its biochemical indicator was studied on carbohydrates, it was determined that the causative agent breaks down maltose, glucose, mannitol and fructose, and has no effect on lactose, dulcite, sucrose, inulin and raffinose carbohydrates[11].

5. In order to determine the sensitivity of salmonellosis agents to antibiotics and other drugs, in a one-day culture of *S. typhimurium* isolated from individual bee farms, antibiotics such as enrolin, gentamicin, colistin, tylasin, neomycin and zinoprim and intravitin, which belong to moderately dangerous substances according to the degree of their effect on the organisms of different types of animals, were tested (a mixture of two drugs) first studied the degree of sensitivity in laboratory conditions[2,6].

6. Zinaprim was 78,2% effective when used alone, and 86,9% when Zinaprim and Intravit drugs were used together[6].

7. As a result of the conducted research, it was found that after honey bees are infected with salmonellosis, it is possible to

completely disinfect the bee colonies and hives after 3 hours by spraying the preparation at the rate of 5 ml per m^3 area 3 times every 30 minutes (aerosol method) [10].

8. It was found that in the studied regions of the republic, beekeepers did not follow the current recommendations regarding the transplantation of plants at least 3-4 times, depending on the flowering period, which resulted in the weakening of the development of bee colonies and, as a result, the increase in disease transmission [9].

9. Based on the results of the research, monclavit-1 drug completely neutralizes salmonellosis agents in bee farms and salmonellas affected by the drug do not grow in cultivated food environments, monclavit-1 drugs have disinfectant properties, and according to our proposed scheme, monclavit-1 drug can prevent salmonellosis of bees. It has been determined that it is a more effective means of treatment and prevention, an average of 442.86 manats of economic profit was obtained from 1 bee colony [10,12].

PRACTICAL SUGGESTIONS

1. In order to prevent the infection of healthy honey bee colonies with salmonellosis, frames with larvae or queens and beekeeping tools and equipment should not be allowed to be brought into the apiary;

2. Unlike sodium hypochlorite, the use of Monclavit-1 in the treatment and prevention of salmonellosis in bees, as well as for feeding purposes, should be considered;

3. In order to strengthen bee colonies in the prevention of salmonellosis, it is more expedient to provide them with abundant and high-quality feed (honey, sugar syrup, honey and protein feed), create optimal temperature conditions during wintering, implement treatment and prevention measures (diagnosis, treatment and disinfection of the disease) in a timely manner, and to prevent the disease from causing more serious losses and spreading widely, it is strongly adviseble to carry out preventive measures mainly in spring and autumn;

4. It should be ensured that bee colonies collect pollen and nectar for their development by flying early, and be transferred to lowland areas at the end of February to ensure normal spring development;

5. Since strong honey bee colonies are 50-55% less infected than weak bee colonies, mainly strong bee colonies should be placed in apiaries for wintering;

6. The results obtained should be applied to beekeeping farms in other regions of the country, including in the liberated territories;

7. The positive results achieved by the Monklavit-1 preparation in preventive measures against salmonellosis in bees and in feeding have been confirmed and the application of this method is considered as appropriate by the Veterinary Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan.

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