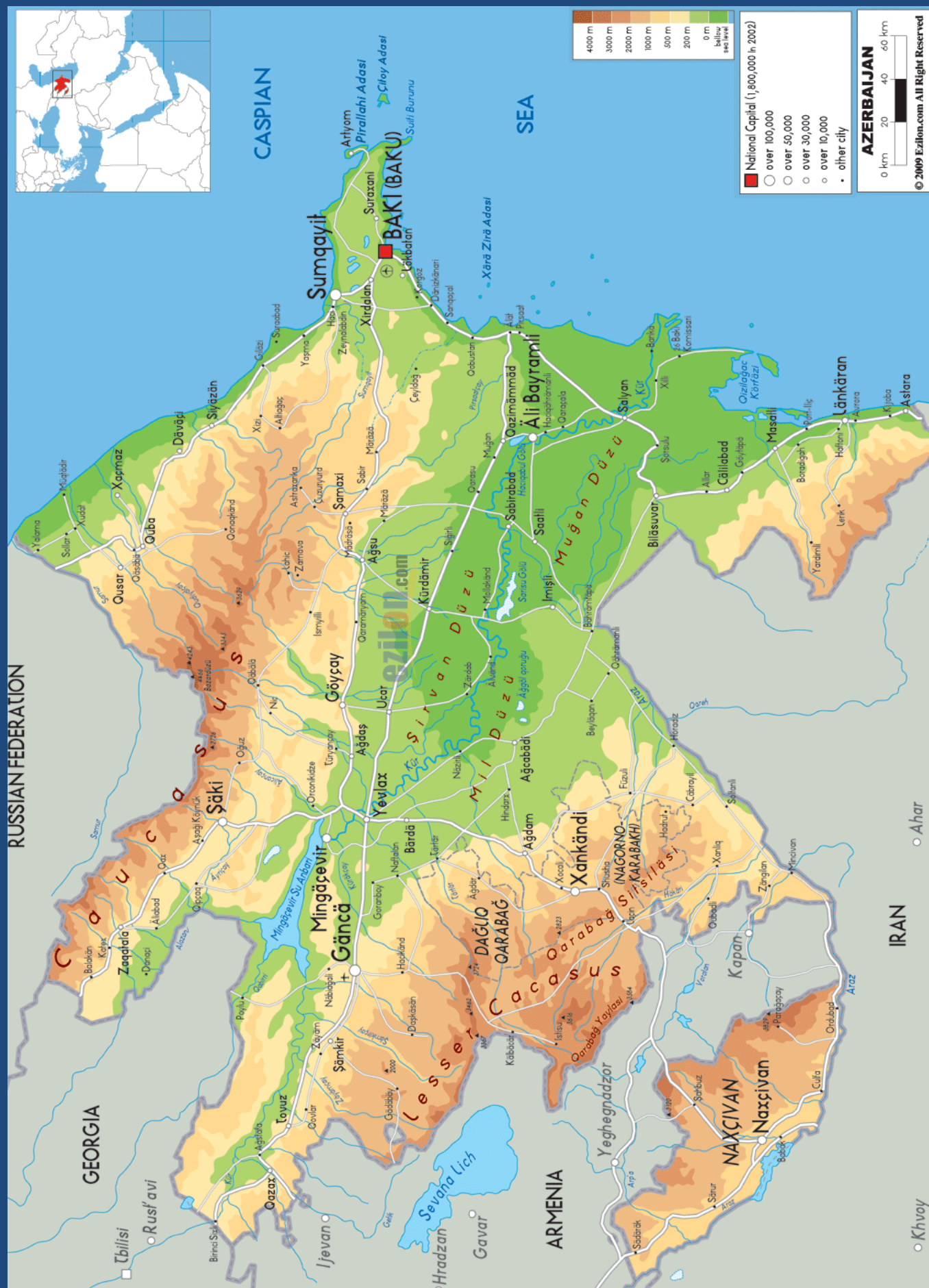


SOME FEATURES OF THE SPREAD OF TULAREMIA IN AZERBAIJAN

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Common information

Azerbaijan is located on the western shore of the Caspian Sea at the southeast extremity of the Caucasus.

- Characterized by a diverse landscape**
- There are two major forms of landscape-plains and mountains**
- Full name: Republic of Azerbaijan**
- Area: 86,600 sq km.**
- Population: about 9.6 million**
- Capital: Baku**
- Language: Azeri**

Common information (continued)

- The climate in Azerbaijan is majorly influenced by geographical position, landscape and the Caspian Sea.**
- The semi desert and dry, subtropical, temperate and frigid types of climate dominate in the country.**
- It has been determined that Azerbaijan accounts for 8 out of 11 climate zones, existing in the world.**
- The animal kingdom of Azerbaijan is rich:**
- There are 107 species of mammals, 357 sp. of birds, 67 sp. of reptiles and amphibians, 97 sp. of fish and over 15 000 sp. of invertebrates.**

Introduction

- In Azerbaijan *F. tularemia* first time was isolated in 1941 at the RAPS
- Then it was identified in 1948, 1949, 1955, and 1958.
- The natural foci were registered in 1958. Two types of tularemia foci exist: mountain-stream zone, where main carrier is the *Microtus arvalis* and lake-bog zone, where main carrier is *Arvicola terrestris*.
- Subspecies of *Francisella tularensis* named *F. tularensis holarctica* circulates in the territory of Azerbaijan.
- Starting in 1958 until 1989, 515 tularemia strains were isolated from samples obtained in both natural foci.

Historical information

- After 1958 tularemia epizooties have been reported almost every year in various parts of the country, and the most important ones are 1964, 1967, 1983 and 1986.
- Human infection was first reported in 1943.
- Outbreaks were recorded among the population in 1964 (75 people), 1967 (129 people), 1995 (42 people) and 2000 (23 people).
- The way of human infection is mainly alimentary (water or food), there are cases of contact, transmissible and aspiration infections.
- The main carriers of tularemia are a common and a water voles, transporters - ticks, and other bloodsucking dipterans - horseflies, flies - stable fly, mosquitoes, etc.

Purpose of investigations

- Since 1987 to 2011 years investigations on the identification of the causative agent of tularemia have not been conducted.
- The aim of this work was identification of a real borders of the spread of this infection in the country and clarification some of the features of tularemia spread in recent years.

Materials and methods

- During the investigations were used epidemiological, bacteriological and molecular biological (PCR) methods.

Materials and methods (continued)

- Individual study of rodents was carried out by method of imprints of parenchymal organ pieces (spleen, liver, lungs) on an agar plate with the following inoculation by loop.**
- Biological tests were put on laboratory animals (white mice) when examining the rodents, which were caught in a natural foci.**
- 10 gerbils were pooled in one group for infecting the white mice.**
- Suspensions, prepared from the spleen, liver and lungs were inoculated into the white mice in a volume of 0.5 ml.**

RESULTS

Tularemia cultures were isolated from the following animals during the period of 1958-2012:

- Vinogradov Jird – *Meriones vinogradovi*
- Persian Jird – *Meriones persicus*
- Libyan Jird – *Meriones erythraeus*
- Small Asian Jird – *Meriones tristrami*
- Social vole – *Microtus socialis*
- Common vole – *Microtus arvalis*
- Water vole – *Arvicola terrestris*
- Wood rat – *Apodemus sylvaticus*
- House mouse – *Mus musculus*
- Dwarf hamster – *Cricetulus migratorius*
- Transcaucasian hamster – *Mesocricetus brandti*

Tularemia cultures were isolated from the following animals (continued)

- **Wather shrew – *Neomys sehelkovnikovi***
- **Forest dormouse – *Dyromus nitedula***
- **Caucasian white-toothed shrew – *Crocidura suaveolcus***
- **Shrew-Soricidae**
- **Weasel – *Mustela nivalis***
- **Cat – carcass**
- **Common whetear – bird**
- **Bugs**
- **Fox – carcass**
- **Frog**
- **Typical tortoise**

List of the administrative regions where detected tularemia epizootia/epidemia among rodents *Table 1*

R E G I O N S	Y E A R S
SHAHBUZ	1958 - 1971, 1973, 1977, 1983 - 1986
SHARUR	1958
BABEK	1958, 1964
BILASUVAR	1958, 1959, 1966, 1969
SAATLI	1961
GAKH	1963, 1964, 1969
ZAQATALA	1964, 1965, 1972
KHOCAVEND	1964, 1966
QAZAX	1964, 1983, 1984
TOVUZ	1964, 2012
GOYGOL, GULFA, GORANBOY	1964
OQUZ, AQDAM, KURDAMIR	1964
SHAMKIR, GANJA	1964

List of the administrative regions where detected tularemia epizootia among rodents (Continued)

R E G I O N S	Y E A R S
AGJABADI	1964, 1967, 1968, 1969
SHAMAXI	1964, 1967, 1968
KELBAJAR	1965, 1967, 1969
IMISHLI	1966, 1979
LAÇIN	1966, 1981
SHEKI, BEYLAGAN	1967
FUZULİ, JEBRAİL, GUBADLI	1967, 1968
ZENGILAN, ABŞERON	1968
ORDUBAD	1970, 1974, 1982, 1983, 1985, 1986
DAŞKESAN	1980, 1984
GADABAY	1981, 1984, 1985, 1986

List of water reservoirs, where tularemia strains were isolated:

Tularemia cultures were also recovered from fleas - *C. mokrzevski*, *Ct. secundus*, *C. consimilis*; from ixoid (*Rh. turanicus*, *Rh. sanguineus*), argasid (*Alactorobus alveonatus*), and gamasid ticks.

- Sarisu Lake – Saatli Region**
- Ajinohur Lake – Gakh Region**
- Alazan River – Zagatala Region**
- Shpartu Spring, Charakanchay Channel - Fuzuli Region**
- Shpartu-Garasu – Aghjabadi Region**
- A spring – Imishli Region**

List of water reservoirs, where tularemia strains were isolated: (continued)

- **Jeyrankechmaz River – Shamakhi Region**
- **Saribulag – Kalbajar Region**
- **Goy - Gol Lake – Khanlar Region**
- **Irrigation channel in Zirnali Village – Zangilan Region**
- **Akerachay, Bazarchay – Gubadli-Zangilan Region**
- **Kendelanchay – Fuzuli Region**
- **Bolgarchay – Mughan**
- **Mahmudchala Lake – Imishli Region**
- **Aghchala Lake – Bilasuvar Region**

List of territories for tularemia examination:

- Kur-Araz lowland
- Small Caucasian foothill
- Big Caucasian foothill
- Mil plain
- Mughan plain
- Ajinohur steppe
- Northern steppes of Small Caucasus
- Shirvan steppe
- Western shore of Mingachevir Water Reservoir
- Sardachol
- Shaki plateau
- Mil-Garabagh plain
- Ganja-Gazakh Regions (Shamkir, Goranboy, Gazakh, Samukh, Tovuz, Ganja)
- Region located within lower stream of Kur River – Bilasuvar, Imishli
- Big Caucasus (Zagatala, Shaki, Oghuz)
- Small Caucasus (Yevlakh, Goranboy)

Epizootiological surveys

Epizootiological studies have been carried out by the zoological group (6 person) in spring and fall, in 2012-2013 (240 business trip days) in field conditions at the areas of Eastern and Western Jeyranchol, Ganja-Gazakh plain and Small Caucasus, which is located at the Highland zone, with 494 thousands hectare of total area.

Epizootiological surveys (continued)

- Field samples were taken from 554 epidemiological points at 168 sectors.
- 2525 rodents of 7 types (mostly *M.erythrurus*), 13674 ectoparasites (11914 fleas of 11 types and 1760 ticks of 10 types) were caught for laboratory tests.
- Ectoparasites were collected when examining the entrances of burrow ways (13698) and burrows (56) of rodents.
- The results of Epizootiological tests are shown by seasons in the table 2.

Epizootiological study results

Table 2

MESOFOCI	Jeyranchol, Ganja – Gazakh plain, Highland zone		
OBJECTS	YEARS: 2012	2013	TOTAL
FIELD (thousand ha.)	241,5	252,5	494
SECTOR	91	77	168
EPID. POINT	297	257	554
RODENTS:	1419	1106	2525
M. Erythrourus	1131	949	2280
M. tristrami	26	11	37
M. musculus	37	37	74
A. elater	1	1	2
M. sociales	7	2	9
M. arvales	6	96	102
A. sylvaticus	-	5	5
Cr. guldenstaedti	11	5	16
V. vulpes	2	-	2
Chiroptera	2	-	2
Small cattle	40	-	40
Cattle	10	263	273

Epizootiological study results Table 2 (continued)

OBJECTS	YEARS: 2012	2013	TOTAL
FLEAS:	4787	7127	11914
X. conformis	4318	6348	10666
C. laeviceps	400	751	1151
St. tripectinata	25	4	29
P. irritaus	20	2	22
Copt. caucasika	20	-	20
Rh. ucrainica	4	3	7
A. rossica	-	6	6
St. secundus	-	3	3
L. taschenbergi	-	4	4
C. consimilis	-	4	4
Ct. bogatschevi	-	2	2

Epizootiological study results Table 2 (continued)

OBJECTS	YEARS: 2012	2013	TOTAL
TICKS:	867	893	1760
Orn. alactagales	640	439	1079
H. plumbeum	21	140	161
H. aegyptium	1	-	1
H. asiaticum	27	184	211
I. ricinus	79	38	117
R. bursa	99	10	109
B. carcaratus	-	5	5
Dermosentor N	-	2	2
Haemophisalis N	-	11	11
Gamasoidea	-	64	64
Burrow way	6147	7551	13698
Burrow	30	26	56
Ectoparasites	5654	8020	13674

Bacteriological investigations

- **In the laboratory, during the reporting period were obtained and analyzed for the presence of pathogens of tularemia all:**
- **2525 specimens from rodents and other mammals**
- **13674 specimens from ectoparasites (11914 fleas, 1760 ixode and argasid ticks)**
- **From the isolated field material 10726 inoculations were done on a nutrient medium and 418 biological tests were put on white mice.**

Bacteriological investigations (continued)

- **During the bacteriological examination of the field material, the plating of the samples of internal organs of rodents and ectoparasites were done on elective nutrient mediums - Hottinger agar and meat-peptone agar.**
- **Individual study of rodents was carried out by method of imprints of parenchymal organ pieces (spleen, liver, lungs) on an agar plate with the following inoculation by loop. Biological tests were put on white mice when examining the rodents, which were caught in a natural foci.**

Bacteriological investigations (continued)

10 gerbils were pooled in one group for infecting the biotest animals.

Suspensions, prepared from the spleen, liver and lungs of rodents were inoculated into the white mice in a volume of 0.5 ml.

- Media with cystine (tularemia agar, enriched blood agar, Mac-Koe) were used for researching the causative agent of tularemia. During the reporting period, 2231 rodents and 1760 ticks were bacteriologically and biologically examined for tularemia.

Bacteriological investigations (continued)

- As a result, 3 strains of tularemia microbe were isolated at the following addresses:
- East Djeyranchol Guyrugenchi village, 30 ° -2 km:
- 1. Libyan jird - 3 copies = Strain N 61 F. Tularensis
- West Djeyranchol, Sazhdag mountain, 290 ° - 2 km:
- Ticks collected from 20 small cattle:
- 2. Ixodes ricinus (39 copies) = Strain N 105 F. tularensis
- 3. Rhipicephalus bursa (60 copies) = Strain N =111 F. tularensis

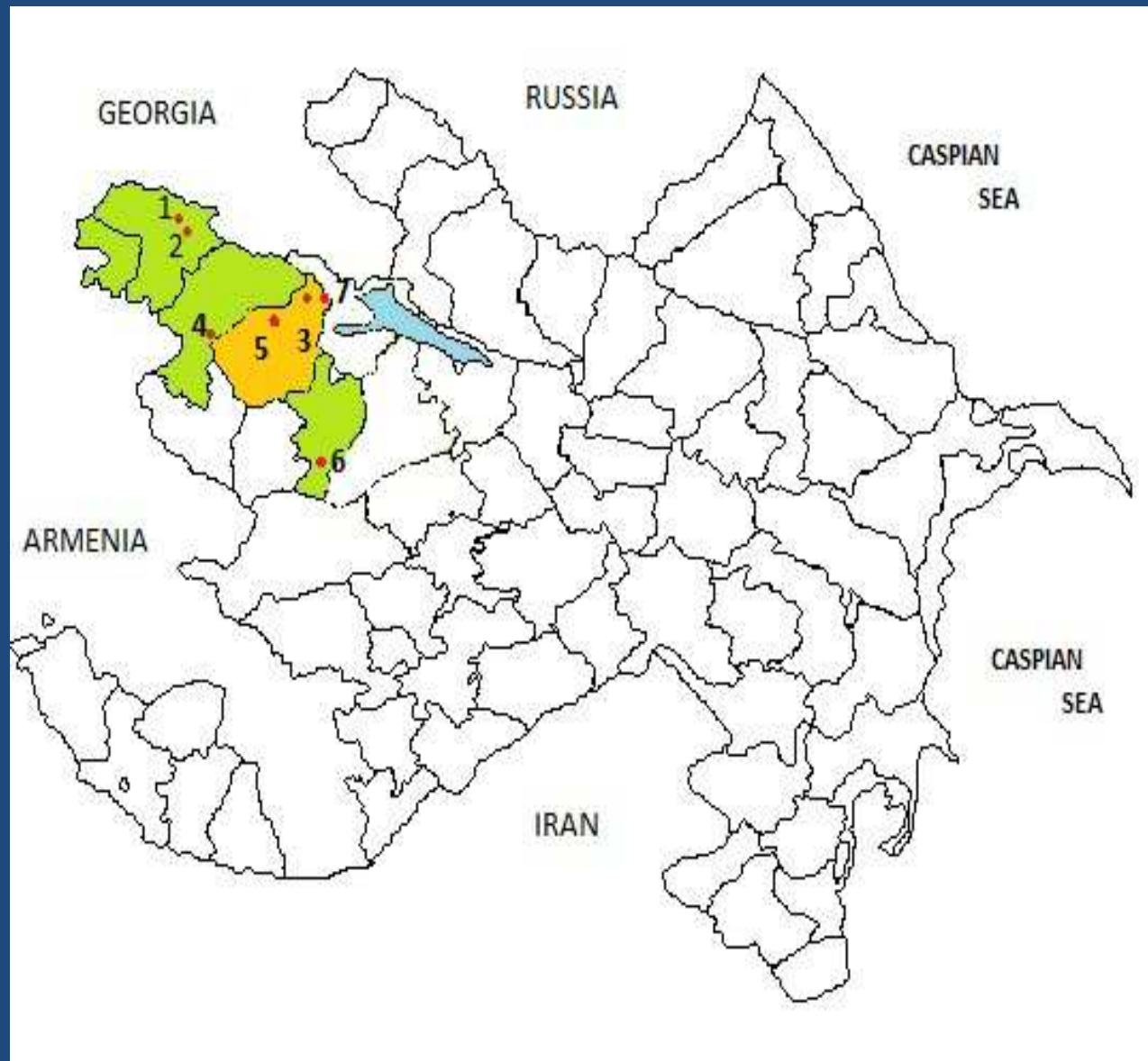
Bacteriological investigations (continued)

- All 3 strains were isolated through a biological sample (white mice) at the second passage. These strains were identified as tularemia microbes in the Central Laboratory of the RAPS
- - Color and morphology of bacteria were identical to *F. tularensis* in smears;
- - a specific growth observed on nutrient media (tularemia agar, Mac-Koe),
- - microbe growth does not observed on simple nutrient media (beef-extract agar)
- - were giving agglutination with a specific tularemia serum;
- - Were pathogenic for white mice and guinea pigs.

Results obtained by checking with biochemical methods were inherent to *F.tularensis*:

- - Oxidase & Urease - negative;
- - Catalase & Beta-lactamase - positive

Areas, where *Fr.tularensis holarktica* was detected in 2012-2013.



Legend:

- 1) Sazhdag mountain
- 2) Sazhdag mountain
- 3) Bayramly village
- 4) Guyrugenchi village
- 5) Djeyranchol village
- 6) Mursaltepe village
- 7) Gasymalilar village

● - places of
Fr. tularensis

PCR studies

- During the reporting period, tularemia molecular tests were done using the apparatus of Real Time PCR. Extracted, from pieces of internal organs, DNAs of 394 rodents were examined. Got 7 positive results:

West Djeyranchol:

- 1) Sazhdag mountain, 290 ° - 2 km, the tick *Ixodes ricinus* N 47
- 2) Sazhdag mountain, 290 ° - 2 km, the tick *Rhipicephalus bursa* N 48
- 3) Bayramly village, 135 ° - 4 km, from Libyan jird N 69

Eastern Djeyranchol:

- 4) Guyrugenchi village, 30 ° - 2 km, from libyan jird N 7
- 5) Djeyranchol village 85 ° - 5 km, from libyan jird N 24
- 6) Mursaltepe village, 150 ° - 9 km, from libyan jird N 49
- 7) Gasymalilar village, 260 ° - 3 km, from social vole N 10
- 1423 rodents were studied in 2012 - 2013 for the presence of intestinal yersiniosis. The results are negative.

Conclusions

- As a result of the two-year monitoring revealed new areas, where earlier epizootic of tularemia have not been noted.
- For the first time the culture of the causative agent of tularemia was isolated from red-gerbils (*M.erythrurus*) and ticks (*Ixodes ricinus* and *Rhipicephalus bursa*), collected from small ruminants in this area.

Thank you for your attention!

