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TULAREMIA IN THE USSR

Selected translations from ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII
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CHANGES IN PROPERTIES OF TULAREMIA PATHOGEN UNDER THE INFLUENCE OF ERYTHROMYCIN

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 12, Dec 73 (manuscript received 12 Jul 73) pp 98-101

[Article by R. I. Kudelina, Tularemia Laboratory, Institute of Epidemiology and Microbiology imeni N. F. Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The highly virulent S form, which circulates in endemic tularemia sites, is the natural form of existence of *Francisella tularensis*. The R form, which has no virulent properties at all, like the intermediate SR form, appears when the S form is cultivated in the laboratory on artificial nutrient media [1, 3-6 and others].

We submit here data on changes in properties of the tularemia pathogen under the influence of erythromycin. This question had not been previously discussed in the literature.

Studies were conducted on models of the Schu strain (Nearctic race) isolated by Foshey in the United States from a patient with tularemia and strain No 543 (Central Asian race) isolated from a midday gerbil by Aykimbayev. Both strains had the typical morphological, cultural and biochemical properties (fermented glycerin; contained the enzyme, citrulline ureidase; caused death of white mice, guinea pigs, as well as rabbits in the case of the Schu strain, when isolated [a few?] microbial cells were given by hypodermic injection).

Unlike the Holarctic race strain, the above strains were sensitive to erythromycin in concentrations of 12-25 µg/ml [2].

Our study consisted of two main stages: production of erythromycin-resistant variants and comparative study of their morphological, cultural and biological properties. We used conventional methods in these studies: successive passages on solid agar medium consisting of fish hydrolysate with cystine, glucose and blood, with increasing concentrations of erythromycin. We performed up to 11 passages, and the maximum antibiotic concentration was 6400 µg/ml.

Morphological examination of the obtained variants under a light microscope failed to demonstrate appreciable differences. Electron microscopy by V. L. Popov revealed that the cells of erythromycin-resistant variants were larger than those of the original strains. For example, the dimensions of cells

of the original Schu strain were $650 \times 400 \mu\text{m}$, for its variant they were $700 \times 500 \mu\text{m}$, for strain No 543-- 500×250 and $700 \times 450 \mu\text{m}$, respectively. It should be noted that, unlike strain No 543, the Schu strain was larger and had more marked polymorphism, which is consistent with the data of Olsuf'yev et al. [3] and Olsuf'yev [4].

When erythromycin-resistant variants were grown on coagulated yolk medium, we failed to detect any distinctions related to start and nature of growth. Differences were demonstrated only when the cultures were plated in dishes with agar from fish hydrolysate with cystine, glucose and blood. After 4-day incubation at 37° and subsequent storage of dish contents at 4° for 1 day, the original Schu and No 543 strains formed in the dishes convex, shiny, smooth, light-blue--whitish, opaque and large (2-2.5 mm) colonies, while the erythromycin-resistant variants formed flat, grayish, transparent small (<1 mm) colonies. In both instances, the colonies were homogeneous, occasionally differing slightly in dimensions, which depended on density of plating and thickness of the agar layer.

We plated a suspension consisting of a mixture of cultures of the original strain and erythromycin variant (Figures 1 and 2 [photos not reproduced]) in order to better compare the morphology of colonies of original strains and their variants.

Cultures of the original strains and their variants were inoculated in test tubes with blood agar in order to determine the minimum inoculation dosage and demonstrate differences in growth rate. For inoculation, we used 2-day cultures grown in a coagulated yolk medium, standardized in accordance with the bacterial standard of the State Institute for Control and Standardization of Medical Biologicals imeni Tarasevich to 1 billion microbial cells per milliliter. The inoculation dosage constituted 0.1 ml of different 10-fold dilutions of cultures of 10^8 to 10 bacterial cells. We recorded colony growth daily for 9 days. Evaluation thereof was made on a 4-point scale. We failed to demonstrate differences in growth of cultures of original strains and variants when large doses of culture (10^8 - 10^7) were inoculated. Differences in intensity and time of appearance of growth were only noted with inoculation of doses of 10^5 or less. For example, when a culture of original Schu strain (10^5) was inoculated, growth in the form of isolated colonies was recorded from the 1st day, and by the 3d day was rated as 3+ (profuse), whereas with inoculation of the same dose of culture of the erythromycin variant growth was demonstrable only on the 6th day (2+). The minimum inoculation dosage, with which growth was observed, constituted 10 microbial cells for the original Schu strain and 10^3 for the variant.

We also observed less profuse and later growth in the variant of strain No 543, as compared to the original strain, although no differences were noted in inoculation dosage.

We tested virulence and immunogenicity of the variants in experiments on white mice weighing 12-15 g, which were infected subcutaneously by different 10-fold dilutions of the erythromycin variant cultures (1-100 million bacterial cells) and cultures of the original strains (0.1-1-10 microbial cells), using 3 mice

per dosage. In all, each strain was used to infect 36 mice (27 with the variant and 9 with the original strain). We assessed strain virulence on the basis of time of death, pathological anatomy, accumulation of pathogen in the spleen, liver and blood of the mice, as well as results of cultivating the spleen on a coagulated yolk medium.

Virulence of the new variants diminished drastically, as compared to the original strains: when infected with variants, the mice survived after being given 100 million microbial cells, whereas after infection with the original strains they died on the 5th-6th day after injection of 1-10 microbial cells, and we demonstrated considerable accumulation of the pathogen in the internal organs.

Concurrently with decrease in virulence, the erythromycin variants also showed attenuation of immunogenic properties, and this was more marked in the variant of strain No 543 than the variant of the Schu strain. Thus, all mice infected with different doses of a culture of the No 543 variant died of tularemia 1 month after giving them 100 microbial cells of the original (homologous) virulent strain. The average time of mouse death was 5-7 days, depending on the dosage of the initially given variant. All mice given 1-100,000 microbial cells of a culture of the Schu strain variant died on the 5th-7th day after being given 100 microbial cells of the homologous virulent strain. We observed only partial death among mice infected with 10 and 100 million microbial cells of the variant culture after administration of the virulent strain (2 out of 3 infected mice died). Evidently, the erythromycin variant did not lose its immunogenic properties entirely.

Control mice, which were infected concurrently with the experimental ones, given 1-10 microbial cells of Schu and No 543 died of tularemia on the 5th-7th day.

We studied the antigenic properties of the produced variants using the reaction of agglutination with rabbit sera obtained by immunizing animals with cultures of virulent strain No 503 (S form) and attenuated strain No 21/400 (R form). We ran the agglutination reaction with a live culture (1 billion microbial cells) of strain No 543 and Schu, and variants thereof with the above-mentioned sera in dilutions of 1:5-1:2560. We demonstrated some differences in titers and nature of the agglutinate in the original strains and their erythromycin variants. The original Schu strain was agglutinated by serum to strain No 503 to a titer of 1:640 (stable agglutinate) and not by serum to strain No 21/400; the variant reacted with both sera in a titer of 1:80, agglutination by serum to strain No 21/400 being unstable. Both the original and variant of strain No 543 reacted in a titer of 1:320 with serum to strain No 503. The original strain did not react with serum to strain No 21/400, whereas the variant yielded a positive agglutination reaction (unstable) in a titer of 1:10.

These data are indicative of certain changes in antigenic properties of the strains in the direction of transformation from the S to the R form, which is related, as we know, to loss of Vi antigen. These changes were more marked in the Schu strain variant than No 543, in which they were only incipient.

Thus, analysis of the submitted data warrants the conclusion that acquisition of resistance to erythromycin by the strains is associated with a change in

their morphological and cultural properties (change from S to R form), drastic attenuation of virulence and immunogenicity, and partial change in antigenic properties related to loss of surface Vi antigen. It should be noted that there was no parallelism of changes in antigenic and immunogenic properties. For example, strain No 543 lost its immunogenicity entirely but showed little change in antigenic properties, whereas the Schu variant showed more significant changes in antigenic properties than immunogenic ones. As indicated previously [2], the biochemical properties (attitude toward glycerin and citrulline) remained unchanged.

Conclusion

Avirulent R variants were obtained by using erythromycin on the Schu strain (Nearctic race) and No 543 strain (Central Asian race). They differ from the S forms of the pathogen of tularemia in morphology of colonies, cultural and biological properties.

PHOTO CAPTIONS

1. p 99. Mixture of culture of original and erythromycin variant of Schu strain (S and R forms); magnification 3×.
2. p 99. Mixture of cultures of original and erythromycin variant of strain No 543 (S and R forms); magnification 3×.

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IMMUNIZATION WITH BIVALENT AND TRIVALENT MIXED VACCINES AGAINST PLAGUE,
TULAREMIA AND ANTHRAX USING A JET INJECTOR. REPORT 1

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5,
May 1974 (manuscript received 5 Feb 73) pp 59-64

[Article by V. G. Pilipenko, M. A. Miroshnichenko and N. A. Loktev,
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Stavropol]

[Text] For the last 10-15 years, the attention of many researchers had been drawn to the jet (needle-less) method of administering vaccines which, it is believed, simplified and accelerated considerably vaccination and renders this procedure virtually painless [7, 1, 2, 5, 6].

Various killed (monovalent vaccines, combined and polyvalent vaccines) and live vaccines were tested with the jet injector, in particular, there have been published reports on using the jet injector for inoculation of live monovalent vaccines against tularemia, plague and anthrax [9, 8, 3, 4, 5]. It was noted that an intensive inflammatory reaction with production of necrosis in the middle developed after administration of tularemia vaccine in the usual dilution to animals (guinea pigs, rabbits) [9]. Increased reactogenicity was observed when a jet injector was used to administer STI anthrax vaccine in the usual dosage [12]. These circumstances prompted the recommendation to use for injections tularemia vaccine diluted 20-40 times and anthrax vaccine in 5-10-fold dilutions. In such cases, a rather satisfactory immunological response was obtained, with moderate local postinoculation reactions.

Thus, in addition to satisfactory immunological efficacy of tularemia and anthrax vaccines demonstrated in experiments with the jet injector, there were reports of increased reactogenicity of these vaccines with the above method of administration. This shows that it is necessary to continue with investigations of the feasibility of administering live bacterial vaccines using a jet injector.

In view of the good prospects for using jet injectors, our objective was to investigate the possibility of using this method for immunization using live associated divaccines and trivaccines [divalent and trivalent vaccines] for immunization against plague, tularemia and anthrax, information about testing of which we could not find in the literature. In experiments on guinea pigs, we made a comparative determination of reactogenicity, safety and immunological

efficacy of these live mixed vaccines and corresponding live monovaccines when administered with a jet injector.

In all, we conducted 7 experiments on 160 guinea pigs weighing 400-500 g. In the first experiment, we gave a mixture of plague and tularemia vaccines, in the second one a mixture of plague and anthrax vaccines, in the third a mixture of tularemia and anthrax, in the fourth a mixture of plague, tularemia and anthrax vaccines (there were 26 guinea pigs in each of the first 3 tests and 40 in the fourth). In the next experiments (5th, 6th, 7th), the animals were given monovalent plague, tularemia and anthrax vaccines, respectively (control).

The mixed vaccines were prepared from 2-day subcultures of vaccine strains of *P. pestis* EB and *F. tularensis* No 15 of Gayskiy (reconstituted). As the third component, we used standard dry STI anthrax vaccine (series No 123), produced at the Tbilisi Institute of Vaccines and Sera. The mixed vaccines, like the monovalent ones, were injected in a volume of 0.5 ml in the lateral shaved surface of the thigh using a Soviet BIP-4 jet injector at an effective pressure of 8 atm. In this volume of vaccine, there were 100 million plague, 10 million tularemia bacteria and 10 million live spores of STI vaccine, i.e., 20-30 times less than the usual doses of these vaccines when given separately by the epicutaneous method.

At the site of vaccination, all of the guinea pigs had noticeable infiltrates the size of a lentil, occasionally pale yellowish (lemon peel) in color with barely noticeable spot defect in the center (site of penetration of the jet). In some animals, blood seeped from this spot for the first min after injection.

Table 1. Local reaction of guinea pigs to mixed and monovalent vaccines

Vaccine	Size of site of inflammation (mm)		Necrosis	Duration of reaction (days)
	range of fluctuation	average		
Mixture of plague and tularemia	12×12-30×35	20×20	+	12-17
Mixture of plague & anthrax	15×15-30×40	26×30	+	15-17, less often 21
Mixture of tularemia & anthrax	12×12-35×37	22-24	-	14-19
Mixture of 3 vaccines	12×12-30×45	25×30	+	14-21
Plague	9×9-20×30	16×18	-	9-12
Tularemia	17×17-20×30	20×24	+	14-17
Anthrax	9×9-20×30	15-17	-	10-12

In guinea pigs immunized with a mixture of plague and tularemia vaccines, there were well-marked infiltrates and hyperemia of the skin at the injection site by the 2d-3d day (Table 1). By the 5th day, necrosis of the skin developed in the center of the infiltrated region. In most guinea pigs, the local reaction lasted 12-17 days, and in some animals it disappeared by the end of the 3d week. An equally strong local reaction, with formation of a necrotic area in the center of the focus of inflammation, developed in guinea pigs given a

mixture of plague and anthrax vaccines (see Table 1). In the vast majority of animals the reaction disappeared by the 15th-17th day, less often the 21st. A moderate local reaction without marked necrosis of the skin was observed in animals inoculated with a mixture of tularemia and anthrax vaccines (see Table 1). The reaction usually regressed by the 14th, 17th and 19th days.

The most intensive reaction was observed in guinea pigs inoculated with a mixture of all three vaccines (see Table 1). In eight animals, the reaction was associated with formation of abscesses and extensive necrosis of the skin, which resembled the scabs of anthrax carbuncles in density, unevenness and dark color (see Figure, A [photo not reproduced]). The reactions regressed by the 12th-21st day, whereas complete recovery of tissue was observed at the end of the 4th week in some animals.

Thus, unlike inoculations by the cutaneous method with mixed bivalent and trivalent vaccines [10, 11], intracutaneous-subcutaneous administration using the BIP-4 jet injector for the same vaccines but 20-30 times less concentrated led to summation of reactogenic properties of their constituents and development of intensive local postvaccinal reactions.

In control guinea pigs inoculated with the jet injector using live monovalent vaccines, the local reactions were moderate (see Table 1 and Figure, B, B, Γ)

There was an 0.5-1° elevation of temperature in virtually all animals for 5-8 days after immunization. The animals were less active up to the 10th day, they did not take enough feed, lost an average of 24-25 g weight each and, in some cases, particularly in the second and fourth experiments, they lost 115-150 g. On subsequent days, the animals gained weight evenly, and by the end of the 40th day the weight of most guinea pigs returned to the initial level or increased somewhat.

We tested the serological response and allergic status on 12 guinea pigs from each experiment 40 days after immunization.

We found positive allergic reactions to percutaneous injection of tularin and anthraxin in 100% of the immunized animals (Table 2).

Blood serum of all animals immunized with bivalent and trivalent vaccines showed positive interaction with tularemia diagnosticum in the agglutination reaction, with mean titers of 1:148, 1:216 and 1:220, respectively; in animals given monovalent tularemia vaccine the titer was 1:406.

Serum from 75-100% of the animals showed a positive agglutination reaction with plague (erythrocyte) diagnosticum (PHAR [passive hemagglutination reaction]). The highest mean serum titer (1:218) was obtained in guinea pigs inoculated with monovalent plague vaccine and the lowest (1:72) with serum from animals given a mixture of plague and tularemia vaccines. The serum of animals inoculated with a mixture of plague and anthrax vaccine interacted positively with plague diagnosticum in higher dilutions--1:143 (Table 3).

Thus, all of the guinea pigs inoculated with mixed vaccines using a jet injector presented allergic alteration for tularemia and anthrax antigens to almost the same

Table 2.

Allergic change in guinea pigs 40 days after inoculation with mixed and monovalent vaccines using jet injector

Vaccine	Experiment No	Allergen	Number of animals	Guinea pigs with positive reaction	Average reaction (in mm)
EV + LTV	1	Tularin	12	12	12×12
EV + STI	2	Anthraxin	12	12	12×12
LTV + STI	3	Tularin	12	12	13×14
EV+LTV+STI	4	Anthraxin	12	12	12×12
LTV	6	Tularin	12	12	9×9
STI	7	Anthraxin	12	12	14×14

Key for this and Tables 3 and 4:

- EV) plague vaccine
- LTV) live tularemia vaccine
- STI) anthrax

reactions were distinct, being somewhat more marked to the tularemia vaccine used in mixture with other vaccines and separately. Serum titers referable to tularemia and plague antigens were higher in animals inoculated with monovalent vaccines than in those inoculated with mixed vaccines.

We assessed immunity to plague, tularemia and anthrax according to results of infecting the guinea pigs with virulent cultures of the corresponding pathogens 40-45 days after immunization. The animals immunized with mixed bivalent and trivalent vaccines were divided into two and three equal groups, respectively, then submitted to hypodermic infection with one of the pathogens. Guinea pigs immunized with monovalent vaccines and nonimmunized animals infected with the same pathogens served as controls.

We infected 12 animals from each experiment with 200 Dcl [?] of virulent strain No 461 of *Y. pestis*, 1000 Dcl of virulent culture of *F. tularensis* strain No 144/713 and 10 Dcl of anthrax vaccine No 2 of Tsenkovskiy. Among guinea pigs immunized with mixed and monovalent vaccines, 91-100% were found to be highly resistant to plague, 83-100% resistant to tularemia and 91-100% to anthrax (Table 4). Only in the third experiment did 1 out of 12 guinea pigs survive after immunization with anthrax vaccine. As shown by our subsequent tests with the Pchelka [Bee] jet injector, the results of which will be published in our next report, such a low yield of animals immune to anthrax after administration of a mixture of tularemia and anthrax vaccine is not a chance phenomenon. Having recurred in other analogous experiments, it warrants reference with every justification to competition between these two antigens--

Figure 3.

Serological change in guinea pigs 40 days after immunization with mixed and monovalent vaccines

Vaccine	Exper. No	Diagnosti-cum	Animals		Mean titer
			total	positive reaction	
EV + LTV	1	Plague	12	9	1:72
		Tularemia	12	12	1:148
EV + STI	2	Plague	12	12	1:143
		Tularemia	12	12	1:216
EV+LTV+STI	4	Plague	12	11	1:98
		Tularemia	12	12	1:220
EV LTV	5 6	Plague	12	12	1:218
		Tularemia	12	12	1:406

the adverse effect of tularemia vaccine on immunizing properties of anthrax vaccine, when administered together with a jet injector. However, addition of a third constituent, EV plague vaccine, to the mixture of the two above-mentioned vaccines eliminated entirely the inhibitory effect of tularemia vaccine on immunological efficacy of STI vaccine.

Table 4.
Immunity of guinea pigs immunized with mixed and monovalent vaccines against plague, tularemia and anthrax

Vaccine	Number of animals immunized against					
	plague		tularemia		anthrax	
	infected	survived	infected	survived	infected	survived
EV + LTV	12	11	11	11	—	—
EV + STI	12	12	—	—	12	12
LTV + STI	—	—	12	12	12	11
EV	12	12	12	12	12	11
EV	12	12	—	—	—	—
LTV	—	—	12	10	—	—
STI	—	—	—	—	12	9
Unimmunized guinea pigs	10	0	10	0	10	0

Thus, animals immunized with mixed and, particularly trivalent vaccines using a jet injector showed as good an intensity of immunity as guinea pigs given monovalent vaccines. In all instances, development of resistance in the guinea pigs to the pathogens of plague, tularemia and anthrax was associated with distinct serological and allergic changes.

The pathomorphological and histological changes in the viscera of guinea pigs 60 days after administration of mixed vaccines by the jet injector method were essentially similar to the changes following use of the corresponding monovalent vaccines. They consisted of signs of moderate lymphoid and reticular hyperplasia, plasma cell reaction in lymph nodes, some hemosiderosis of the spleen, small pro-

liferates of Kupffer's cells in the liver, as well as insignificant perivascular lymphohistiocytic infiltration in the lungs. In only one case did the lungs show signs of interstitial pneumonia after immunization with a mixture of tularemia and anthrax vaccines. At the same time, it should be stressed that residual manifestations of nonsuppurative lymphadenitis and periadenitis were observed more often with use of a jet injector to give EV monovalent plague vaccine than to give other monovalent and polyvalent vaccines.

Conclusions

1. When given with a BIP-4 jet injector, mixed bivalent and trivalent vaccines containing one-twentieth and one-thirtieth, respectively, of the usual doses for cutaneous inoculation of plague, tularemia and anthrax vaccines resulted in development of highly persistent resistance to plague, tularemia and anthrax in 90-100% of the guinea pigs; however, a significant part of the animals developed severe postvaccinal reactions at the site of injection of the mixed vaccines, with signs of necrosis, whereas guinea pigs immunized with the corresponding monovalent vaccines presented moderate local postimmunization reactions.

2. The severe reactions at the administration site were indicative of summation of reactogenic properties of the constituents of live mixed vaccine when given with a jet injector, which makes it imperative to continue studies for determination of optimum doses of constituents of mixed vaccine for administration with a jet injector.

PHOTO CAPTION

- p 61. Dynamics of local reaction of guinea pigs inoculated with mixed vaccine (A) and polyvalent--plague (B), tularemia (B) and anthrax (T)--vaccines. The numbers refer to postimmunization day of reaction

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P ANTIGEN OF FRANCISELLA TULARENSIS

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[Article by V. V. Sukhar', Rostov-on-Don Plague Control Institute]

[Text] El'bert and Gayskiy established the existence of antigenic differences between virulent and avirulent strains of *F. tularensis* [9]. Olsuf'yev and Yemel'yanova [3] found that attenuation to an avirulent, nonimmunogenic state deprives the pathogen of tularemia of the so-called Vi antigen complex. However, it remained unclear whether the Vi complex is an individual antigen or corresponds to several antigens. We [4] discovered an individual antigen A, using the gel precipitation method, and demonstrated that it was wanting in avirulent cells [6], and it has been isolated in pure form [7]. In this report, we shall demonstrate that avirulent cells are wanting in yet another individual antigen, which was first discovered in an immunochemical study of the sline [mucus] fraction of *F. tularensis* and named P antigen.

In our study, we used two strains of *F. tularensis*--virulent No 128 (1 Dcl for white mice is 10 bacteria) and completely avirulent, nonimmunogenic No 21/400, as well as *F. novicida* (1 Dcl for white mice equals 10 bacteria). We recovered bacterial mass by desiccating with acetone 3-day cultures grown at 37° in glucose-cystine fish agar.

The fraction of mucous substance was obtained in the following manner: 122 g bacterial mass of strain No 128 was suspended in a 10-fold (weight/volume) amount of 0.85% NaCl solution (pH 7.0), heated, stirring occasionally, in a water bath to 57° and kept at this temperature for 1 h. The suspension was then placed in a beaker heated to 57° and centrifuged for 15 min at 30,000 G. The cellular sediment was discarded and, after cooling, we added to the centrifugate an aliquot of cold (-20°) acetone; the precipitate was separated from the liquid phase by centrifugation, dissolved at 57° in 250 ml 0.85% NaCl and placed in the refrigerator (1-2°) overnight, then centrifuged for 1 h at 30,000 G. The precipitated light bluish, semiclear mucous gel was dialyzed against distilled water and lyophilized.

We identified the antigen composition of the fraction using the reaction of double diffusion precipitation in agar (RDP) according to Ouchterlony [20] and a micromodification of this method--microprecipitation reaction according to

Kanchukh [1], using a multiwell plate [5] where the distance between wells is 3-5 mm.

Immunoelectrophoretic analysis of the fraction was made under the following conditions. The wells (4 mm in diameter) in a plate (120x90x2 mm) of 1% agar or agarose on Michaelis veronal buffer (pH 8.6, $\mu = 0.06$) were filled with a solution of the mucoid substance treated according to Sevag [23] to remove protein; we then added Sephadex G-25 to the well to create a continuous gel structure. After electrophoresis ($I = 25$ mA, potential gradient 8 V/cm, 1.5-3 h) the troughs 3 mm away from the wells were filled with immune serum.

We tested the antigenic properties of the fraction on chinchilla rabbits weighing 2.5-3 kg. The animals were given intravenous injections of 0.1--0.2--0.5--1--2--4--8 mg fraction at 6-day intervals. Before each injection, we took 1 ml blood from the marginal vein of the ear to determine the titer of serum agglutinins.

Agglutinating antisera to the fraction were obtained from rabbits given intravenous injections of 0.25--0.5--1--2 mg of the preparation at 6-day intervals. Antisera to live strain No 128 bacteria inactivated with 0.25% formalin were obtained by intravenous injection of 0.25--2--3--3 mg lyophilized cells at 1-week intervals.

A water-salt extract of dry bacteria was recovered by a method that included maceration with sand and extraction with 2.5% NaCl solution [4].

The yield of mucoid fraction constituted 2.54% of the weight of bacterial mass. In this fraction, we demonstrated 10.41% nitrogen by the method of Fels and Veatch [13], 3.4% phosphorus by the method of Fiske and Subbarow [14], 4.59% protein by the method of Lowry et al. [17], 16.8% carbohydrates according to Dubois et al. [12], 0.13% nucleic acids according to Spirin [8], but no DNA using the method of Dische. Maximum amount of reducing substances (10.43%) when scaled to glucose was assayed according to Hagedorn and Jensen [16] after 6 h hydrolysis with 2 N HCl at 100°, amino sugars (10.4%) according to Cessi and Piliego [10] after 7 h hydrolysis with 0.2 N HCl at 100°, N-acetylated amino sugars (3.86%) according to Reissig et al. [21] after 1 h hydrolysis with 0.2 N HCl at 100°. After hydrolysis with 6 N HCl, there was precipitation of a significant sediment of substance soluble in chloroform and mixture of chloroform with methanol (2:1), which was indicative of presence of a lipid component in the preparation.

Using the reaction of diffusion precipitation with tularemia equine and rabbit antisera to live strain No 128 bacteria, within the fraction we demonstrated A antigen and 2 antigens in common in tularemia strains No 128 and 21/400, as well as F. novicida. Antiserum to formalin-treated strain No 128 bacteria and antifraction serum demonstrated only common antigens, but not A antigen.

Figure 1 illustrates the results of testing the antigenic properties of the mucoid fraction; it had good agglutinogenic properties: 0.8-3.8 mg of the substance injected during the first 3-5 injections elicited a significant elevation of agglutinin titers. However, upon subsequent injections of large doses (4-8 mg) they declined.

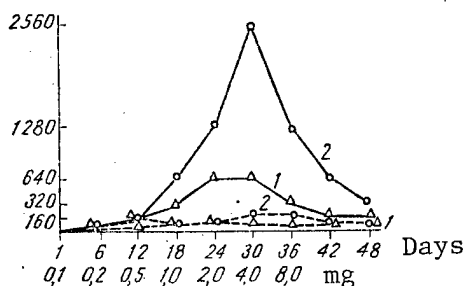


Figure 1.

Dynamics of agglutinin titers in blood serum of rabbits No 1 (1) and 2 (2) in response to intravenous injection of mucoid fraction of strain No 128 bacteria

Solid lines--agglutinins to virulent strain No 128; dash lines--to virulent strain No 21/400.

X-axis, dosage given; y-axis, reciprocals of agglutinin titers

Proceeding from the data obtained in the last experiment, we prepared and tested the following system for recovering agglutinating antifracton serum: rabbits were given intravenous injections of 0.25, 0.5 1 and 2 mg fraction at 6-day intervals. The sera thus obtained presented titers of 1:640-1:2560 in the agglutination reaction with virulent *F. tularensis* and 1/32-1/64 these levels with avirulent tularemia bacteria. In the RDP with water-salt extracts (1-15 mg) of both tularemia strains and *F. novicida*, the antifracton sera caused formation of only 1-2 lines of precipitation common to the tested strains, but did not precipitate A antigen, which was indicative of absence of A antibodies.

Agglutination by antifracton sera of tularemia bacteria wanting in A antibodies should mean that the sera con-

tain agglutinins to an unknown surface antigen (agglutininogen) differing from antigen A. We named the new agglutininogen P antigen. The low agglutination titers of antifracton sera to strain No 21/400 warranted the belief that P antigen is absent in avirulent tularemia cells. We obtained direct proof of this in the experiment where 2 antifracton sera with agglutination titers of 1:640 to strain No 128 and 1:80 to strain No 21/400 lost the agglutinins to the avirulent strain, but retained them completely for the virulent strain after absorption of 100 mg bacterial mass of strain No 21/400 (2 h at 37° and 24 h at 4°).

We were unable to demonstrate P antigen in the RDP according to Ouchterlony: 4-16 mg water-salt extract or 20 mg bacterial mass of strain No 128 did not cause appearance of visible precipitation lines with the antifracton serum (P serum) absorbed by the avirulent strain, whereas only 1-2 lines in common with the virulent strain were formed with unsorbed serum, and they could not belong to P antigen. Conversely, the microprecipitation reaction with P serum diluted in such a way that its titer in the agglutination reaction was 1:160-1:640 and the mucoid fraction (1:100-1:1000) or water-salt extract (1:10-1:100) of bacteria of virulent strain No 128 consistently demonstrated P antigen in the form of a line, which was usually narrower than the A antigen line (Figure 2 [photo not reproduced]).

Rabbit antisera to live strain No 128 bacteria absorbed by the bacterial mass of strain No 21/400, or equine tularemia sera contained A and P antibodies (AP sera). We were able to demonstrate A and P antigens immediately with use thereof in the microprecipitation reaction.

Immunoelectrophoresis of the mucoid fraction (1:250-1:2500) using rabbit AP serum (titer after dilution 1:200-1:400) also demonstrated P antigen: on agar

electrophoregrams there was a line of A antigen closer to the cathode; between it and the well was a line of P antigen (Figure 3 [photo not reproduced]). Immunoelectrophoresis in agarose gel showed that A and P antigens remained where they had been applied, which was indicative of their electrophoretic neutrality.

All of the tested virulent (Nos 128, 1657, 1658) and vaccine (Nos 9, 10, 11, No 15 of Gayskiy--reconstituted and American No 38) strains revealed P antigen by the method of bacterial agglutination. They were agglutinated to a titer or one-half by P serum. The minimally immunogenic vaccine strain No 15 Gayskiy (nonreconstituted) was an exception, and it agglutinated to one-quarter titer. Evidently the extremely low P antigen content of this strain (about 1/500th of the amount in strain No 15 Gayskiy--reconstituted--and 1/1000th of that in virulent strains) is the cause of its poor agglutinability.

Brucella (Br. abortus 554, Br. melitensis 16M, Br. suis 1330, the single *brucella* diagnosticum of series No 19, Odessa Institute of Virology and Epidemiology) and *F. novicida* were not agglutinated by P serum, which was indicative of species-specificity of P antigen.

In addition to the extracts already mentioned, P antigen was demonstrated in trichloroacetic (according to Boivin), water-ether [19] and water-acetone (1:1) extracts. A method of isolating it in pure form has not yet been developed, but distribution is already known according to some methods of separating the extract [7]. According to these data, P antigen is eluted in the external part of the column when filtered through Sephadex G-100, migrates to the cathode upon electrophoresis in a starch block (pH 8.75), is not sorbed on a column with the acetate form of DEAE-Sephadex, but is sorbed and partially separates from A antigen on a column with the borate form of this anion-exchange resin.

The properties of this antigen have been studied. It was found to be resistant at 20° to trichloroacetic acid, formamide, 0.25% formalin and detergents (1-5% sodium desoxycholate and sodium dodecylsulfate), boiling for 2 h and autoclaving (110°, 40 min). When boiled for 30 min in 0.1 M NaOH or 0.2 M Na₂CO₃, antigens A and P were destroyed, whereas only P antigen was destroyed when boiled for 1 h in 0.1 M acetic acid, and this could be used as a differential test for quantitative assays of A antigen.

In the fraction obtained by chromatography on DEAE [diethylaminoethyl] Sephadex, which contained P antigen, carbohydrates were demonstrated without admixture of proteins and nucleic acids [7]. These data, as well as resistance to denaturing factors, warrant the assumption that P antigen is a carbohydrate.

In order to produce a P line in the microprecipitation reaction, 300 times more water-salt or water-ether extract is required than for formation of the A line. This difficulty in demonstration of P antigen is apparently the reason why, in the experiments of Saslaw et al. [22], some sera with high agglutination titers from people immunized with Foshey killed tularemia vaccine did not cause formation of precipitation lines, whereas in the experiments of Nutter [18] agglutinating rabbit antisera to extracts of tularemia bacteria caused only a precipitation band reduced to the form of a mist near the antigen well.

It was previously reported [7] that only live, rather than killed bacteria and extracts of both, elicited A antibody production in rabbits. In contrast, considerable titers of P antibodies were recovered with administration of formalin-treated bacteria or the mucoid fraction. It can be assumed that expressly P antibodies determine the agglutination titers of antisera to killed bacteria and extracts, whereas in antisera to live cells apparently A antibodies are also agglutinins.

The antigenic receptors that are lost with attenuation of *F. tularensis* have been designated heretofore as Vi antigenic substances, Vi antigen complex or Vi antigen [2, 3]. It was assumed that this complex is a membrane that invests the cell [3]. In view of the specificity for immunogenic (virulent and vaccinal) strains, antigens A and P could be viewed as the first individual antigens of the Vi complex to be identified. Thus, it can be concluded that the Vi antigen complex is heterogeneous, and the hypothesis can be expounded that the surface of the pathogen of tularemia constitutes a complex mosaic pattern made of up A and P antigens, as well as two agglutinogens in common with *Brucella*, the existence of which was mentioned already by Francis and Evans [15].

We have yet to determine the significance of A and P antigens in the mechanism of virulence and immunogenicity. For this purpose, it will be necessary to recover them in pure form and select A^+P^- and A^-P^+ variants of *F. tularensis*.

Conclusions

1. An antigen, named P antigen, specific to virulent and vaccine strains of *F. tularensis*, has been discovered.
2. P antigen is a thermostable, species-specific agglutinin.
3. Monoreceptor P serum has been recovered for quantitative assay of P antigen using serological methods.

PHOTO CAPTIONS

2. p 54. Demonstration of P antigen by microprecipitation reaction
- a: 1) rabbit P serum (1:320*); 2) rabbit AP serum (1:640*);
3) equine AP serum series 38-2 (1:320*); 4) equine AP serum series 38-2 (1:1280); 5) equine AP serum series No 68-2 (1:1280*); 6) mucoid fraction** (1:500)
- б: 1) 0.85% NaCl; 2) equine AP serum series No 38-2 (1:1280);
3) rabbit P serum (1:320*); 4) rabbit AP serum (1:320*);
5) rabbit AP serum (1:1280); 6) mucoid fraction** (1:500)
- в: 1) water-salt extract of strain No 128 (1:40); 2) mucoid fraction** (1:500); 3) mucoid fraction** (1:250); 4) 0.85% NaCl; 5) water-salt extract of strain No 21/400 (1:20);
6) rabbit AP serum (1:640)

*Agglutination titers after diluting sera.

**Here and in Figure 3, the fraction recovered from strain No 128 and treated according to Sevag.

3. p 55 Demonstration of P antigen by immunoelectrophoresis
 α , ϵ) rabbit AP serum (1:320); δ) normal rabbit serum; 1) mucoid
fraction** (1:250); 2) mucoid fraction** (1:2500); 3) mucoid
fraction** (1:20,000)

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EVALUATION OF VACCINE STRAINS OF *F. TULARENSIS* IN EXPERIMENTS ON RABBITS

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[Article by L. V. Sirotuk, State Institute for Standardization and Control of Medical Biologicals imeni Tarasevich, Moscow]

[Text] Immunological quality of vaccine strains is of paramount importance to high efficacy of live tularemia vaccine. Immunogenicity of vaccine strains of *F. tularensis* is determined on guinea pigs, which are highly sensitive and highly susceptible to tularemia (infective and lethal dosage for them consists of a few bacteria). Man is also highly susceptible to tularemia and can be easily infected, but because of low sensitivity the disease usually ends with recovery. The rabbit is just as susceptible to tularemia, and a few bacteria are sufficient to infect it, but even 10 billion bacterial cells does not constitute an unconditionally lethal dose for it [3]. For this reason, we were interested in the rabbit as, perhaps, a closer model to man in order to evaluate vaccine strains of *F. tularensis*.

Since rabbits do not die when infected with highly virulent strains of the Palearctic variant of *F. tularensis*, we decided not to study immunity by the infection method, and we conducted a comparative study of antigenicity and "taking" of several vaccine strains of *F. tularensis* in the collection of the Institute imeni Tarasevich. We used genetically related variants of tularemia vaccine strain Gayskiy No 15, which differed in immunogenicity for guinea pigs: highly immunogenic variant No 15 NIEG, the ED₅₀ of which for guinea pigs numbers a few bacteria, moderately immunogenic variant No 15 v/IIIs with ED₅₀ constituting thousands of bacteria and the mildly immunogenic variant No 15/38, the ED₅₀ of which for guinea pigs is up to millions of bacteria.

In our tests, we used 48-h second-generation cultures grown on solid yolk medium at 37°. We inoculated chinchilla rabbits weighing 2-2.5 kg with cultures suspended in isotonic solution of sodium chloride to a density of 2 billion bacteria per milliliter, according to the optical standard of cloudiness for *F. tularensis*. The inoculation was administered by the scarification method: we applied 2 drops of suspension (one inoculation dose for man) to a depilated skin area on the animals side and, using a smallpox vaccination quill, made parallel scratches 0.8-1.0 cm long through the drops, after which we rubbed the suspension in with the flat side of the quill for 30 s, then allowed it to dry in the air for 5-10 min. The

reaction was considered positive when hyperemia and an infiltrate at least 0.5 cm in cross section appeared at the site of scarification on the 5th postvaccinal day.

We took blood from the marginal vein of the ear from all rabbits, before inoculation and every 7 days for 1 month. We ran the hemagglutination reaction and passive hemagglutination reaction (PHA), being governed by the techniques described in the technical documentation for tularemia diagnosticum and erythrocytic tularemia diagnosticum. As antigen, we used a suspension of second-generation culture of vaccine strain No 15 v/IIs, which was grown on McCoy medium at 37°. The suspension contained 5 billion bacterial cells per milliliter isotonic solution of sodium chloride, pH 7.0. Rabbit sera were inactivated prior to the PHA by heating at 56° for 30 min. Erythrocytes sensitized with tularemia antigen were obtained from the Institute of Epidemiology and Microbiology imeni Gamaleya.

The highest elevation of agglutinin titer was observed in most rabbits on the 14th day, then the quantity of agglutinins started to diminish and held at an average level (Table 1). More intensive antibody production was observed when strains No 15 NIEG [Scientific Research Institute of Epidemiology and Hygiene] and 15 v/IIs were used for vaccination. When the rabbits were immunized with strain No 15/38, most of them failed to produce any agglutinins at all. Maximum accumulation of hemagglutinins was also observed on the 14th day in most rabbits, after which they began to diminish and held at an average level (see Table 1) for the duration of the observation period (28 days). The three strains differed drastically in capacity to elicit hemagglutinin production: NIEG No 15 presented the greatest antigenicity (hemagglutinin titer exceeded 1:8000 on the 14th day in most rabbits), strain No 15 v/IIs elicited moderate hemagglutinin production (titers did not exceed 1:2000) and strain No 15/38 had a mild effect (in most rabbits hemagglutinin titers did not exceed 1:160).

Table 1. Dynamics of agglutinins and hemagglutinins in blood of rabbits immunized with variants of Gayskiy No 15 strain

Antibodies	Strain No	Mean titers (log)					Number of rabbits
		be- fore imm.	after immunization (day)				
			7th	14th	21st	28th	
Agglutinins	15v/IIs	0	2,21	2,31	1,98	1,79	9
	15 NIEG	0	1,95	2,31	2,19	2,19	9
	15/38	0	0	1,34	1,32	1,0	9
Hemagglutinins	15v/IIs	0	2,28	3,26	3,04	2,94	9
	15 NIEG	0	3,53	3,81	3,61	3,47	9
	15/38	0	1,83	3,02	2,80	2,30	9

Thus, only immunogenically active variants of Gayskiy vaccine strain No 15 v/IIs and 15 NIEG elicited agglutinin production. Mashkov and Belkina [2] made analogous findings: when rabbits were given Gayskiy strain No 15 vaccine,

agglutinins appeared in their blood in a titer of up to 1:640, whereas with administration of an avirulent tularemia strain no agglutinin production was observed. Production of hemagglutinins in the blood of immunized rabbits occurred concurrently, and the maximum levels of both were observed at the same time, on the 14th day. The capacity to elicit hemagglutinin production was related to immunogenicity of the vaccine strains: the more immunogenic the strain, the higher the hemagglutinin titer in blood of rabbits immunized with it. Hemagglutinin titers exceeded significantly the agglutinin titers in the same rabbits, and they were often present in blood in the complete absence of agglutinins. On the basis of the foregoing, it can be considered that use of PHA as a test for antigenicity with sera of rabbits immunized cutaneously could be useful in the study of vaccine strains.

In addition to antibody production, we examined acceptance of cultures of different vaccine strains of *F. tularensis* in immunized rabbits, since this is one of the criteria for evaluating strains on guinea pigs [1]. In humans, a close link was also noted between acceptance and quality of the strain [5].

In the tests of acceptance ["take"], in addition to the strains used to test antigenicity, we used the mildly immunogenic variant of Gayskiy strain No 15/Kb, the ED₅₀ of which for guinea pigs constituted millions of bacteria, mildly immune strain No 33 NIEG with ED₅₀ of hundreds of thousands of bacteria and highly immunogenic strains of NIEG Nos 10 and 53, the ED₅₀ of which constitute less than 10 bacterial cells [7].

Table 2.
Take in rabbits of vaccine strains
of *F. tularensis*

Strain No	Rabbits, out of 12, with positive reaction to dose of			
	50 000	500 000	5 000 000	50 000 000
15v/IIIs	7	11	12	12
15 NIEG	9	11	12	12
15/38	0	2	4	9
15/Kb	1	3	5	8
10 NIEG	11	12	12	12
33 NIEG	8	10	12	12
53 NIEG	10	12	12	12

Experiments were conducted in three variants. In the first variant, we tested 4 doses at a time on each rabbit, from 50,000 to 50 million bacteria of the same strain (4 rabbits per strain). We use dose to refer to the quantity of bacteria in two drops of suspension applied to the rabbit's skin for immunization. We conducted three tests, in which all of the strains except the mildly immunogenic ones, No 15/38 and 15/Kb elicited similar results (Table 2). Thus, with use of even mildly immunogenic strain No 15 Kb one rabbit presented a distinct take up to a dosage of 50,000

bacteria, whereas with administration of highly immunogenic strain No 15 NIEG one rabbit presented a positive reaction only up to a dosage of 500,000 bacteria. Apparently, such a system of titration for acceptance by one animal is unsuitable for determining the quality of a strain.

In the second variant of experiments, we immunized one rabbit simultaneously with all strains, but in one dilution which, if the strain had immunogenic properties, elicited positive immunization reactions in most rabbits. Five rabbits were immunized cutaneously with all strains in a dosage of 50,000 bacteria. The vaccinal reactions were the same in all animals: positive with strains No 15v/IIIs and NIEG No 10, 15 and 53; negative with strains No 15/38,

15/Kb and 33 NIEG. Thus, this method is apparently more promising for comparative studies of acceptance of vaccine strains.

In the third variant, we tested three No 15 strains differing in immunogenicity--No 15v/IIs, 15 NIEG and 15/38. Each strain was tested on the same rabbit in the same inoculation dosage, which constituted 200 million bacteria according to the opacity standard. We conducted three experiments, with three rabbits per strain in each test. On the 5th day, all rabbits immunized with No 15v/IIs and 15 NIEG presented positive immunization reactions in all three experiments; however, with use of strain No 15/38, a take was observed only in the first experiment in one out of the three rabbits, whereas no immunization reaction was present in the rest of the cases.

Consequently, the set-up of the first and third variants of experiments made it possible to differentiate the acceptance only of strains that had changes which were advanced in the direction of saprophytization of the type in strains 15/38 and 15/Kb. When all strains were applied in the same dilution simultaneously to one rabbit (second variant), we were already able to single out, according to immunogenicity, even the mildly immunogenic strains such as No 33 NIEG. We were unable to demonstrate the initial stages of decline of immunogenicity in the 15v/IIs type strain according to immunization reactions of the rabbit. It should be noted that we obtained a response in rabbits that is similar to the type of response of man, since tests of humans also failed to demonstrate, on the basis of take, any appreciable differences between highly immunogenic strain No 15 NIEG and moderately immunogenic strain No 15v/IIs [4]. Thus, although the results of rabbit studies referable to the take test are not suitable for evaluation of immunogenicity of commercial vaccine strains, they can still be used in experimental work. Studies of rabbits are also quite promising for evaluation of commercial strains with regard to testing antigenicity of vaccine strains, since hemagglutinin production was consistent with degree of immunogenicity.

Conclusions

1. There was a correlation between antigenicity of tularemia strains for rabbits when cultures were applied on the skin and their immunogenicity in tests with guinea pigs.
2. A study of hemagglutinin titers in epicutaneously immunized rabbits made it possible to demonstrate an initial decline of immunogenicity of vaccine strains of *F. tularensis*, whereas agglutinin titers in similarly immunized rabbits reflected only advanced changes in the strain, in the direction of saprophytization.
3. Vaccinal skin reactions in the rabbits made it possible to demonstrate only a significant decline of immunogenicity of vaccine strains of *F. tularensis*.

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STUDY OF ENDEMIC INFECTIONS IN LITHUANIAN SSR. REPORT 3: TULAREMIA INFECTION AMONG RURAL POPULATION ACCORDING TO RESULTS OF SEROLOGICAL AND ALLERGOLOGICAL TESTS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 8, Aug 74 (manuscript received 14 Aug 73) pp 33-37

[Article* by L. I. Moteyunas, Republic Sanitary and Epidemiological Station, Vilnius]

[Text] In the period of keeping records in the USSR, tularemia has been found in all administrative regions and republics of the European part of our country, with the exception of Lithuanian SSR [7]. In order to determine whether there are also endemic sites for tularemia in the last mentioned republic, initially a study was made of such environmental elements as landform, natural biocenoses and parasitocenoses. On the basis of landform features, it was established [3] that there are conditions in Lithuanian SSR for existence of sites of the meadow-field, forest and backwater-swamp types. The fauna referable to small wild mammals is represented in Lithuania by 17 species of highly susceptible and highly sensitive animals [6] with respect to tularemia (group I according to Olsuf'yev and Dunayeva), which are capable of maintaining a continuous epizootic process in nature. In this republic, there are 18 species of bloodsucking arthropods (6 species of ticks, 4 of gadflies [or horseflies] and 8 mosquito species), which are potential vectors of *F. tularensis*, and ticks are also capable of maintaining persistence of endemic sites. On the basis of evaluation of the gathered data, from the standpoint of medical geography and landform epidemiology, the hypothesis was expounded that there are endemic tularemia sites in Lithuania [3]. It was checked by means of allergological and serological testing of the rural population in 1969-1972, including amateur hunters who are exposed to a higher risk of tularemia infection.

In our survey, we took into consideration the age, time of residence in the screened region, occupation, how long the subject had been engaged in hunting and how the hare carcasses were handled. Allergic tests were performed by percutaneous injection of 0.1 ml tularin. The reaction was checked after 24-48 h. It was considered positive if there was an infiltration (induration) of the skin at the site of tularin injection at least 5 mm in diameter. In

*ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII, 1973, No 5, p 83 and No 11, p 130.

view of the strict specificity of the reaction to tularin and the fact that preventive inoculations against tularemia had never been given to the inhabitants in LiSSR, all cases of positive allergic reactions to tularin were evaluated as retrospectively detected cases of prior infection. On the basis of the results of the epidemiological survey, all individuals who reacted positively to tularin were divided into three groups: the first consisted of individuals infected by *F. tularensis* within the territory of Lithuanian SSR, the second those who very probably were infected in this republic, but there was no absolute certainty of this; the third group consisted of individuals who had been infected (reliably and probably) or immunized outside Lithuania.

We took blood for the agglutination reaction (AR) just prior to the allergic skin test. We used the volumetric AR with tularemia diagnosticum, which is considered highly sensitive and strictly specific [1, 5, 8]. We recorded reactions with a titer of at least 1:20. Considering the antigenic similarity of pathogens of tularemia and brucellosis, in all positive AR cases we also ran a test with brucella diagnosticum. We recorded the AR and allergic skin test separately, without taking into consideration the results of the other test.

We performed the allergy test on 73,534 people ranging in age from 18 to 69 years; 5372 of them were hunters (all men) living in 25 administrative regions of the republic and the other 68,162 lived in rural areas in 18 (out of 44) regions. Of those screened, 84.8% had lived in the area of the screening for over 10 years, 79.9% of the hunters had hunted for over 5 years and 68.4% always hunted in the same administrative district.

Since Lithuanian SSR is a territory that consists of several separate ecological and faunal regions (EFR), the obtained data were analyzed not only all together, but for the different regions [9].

According to the allergy test, the highest percentage of positive reactions was found among hunters in the southeastern EFR (Table 1), which is in first place among other regions with regard to lakes and forests.

Table 1. Results of allergy test with tularin made on hunters

EFR	Number of hunters		Incidence of positive reactions			
	under Obser- vat.*	tested	epidemiological groups			total
Northwestern	2576	1868 (72,5)	50	12	13	75 (4,0±0,4)
Central Lithuanian	2827	2003 (70,9)	30	17	8	55 (2,8±0,4)
Southwestern	892	725 (81,3)	23	4	5	32 (4,4±0,8)
Southeastern	1368	776 (56,7)	27	11	10	48 (6,2±0,9)
Totals	7663	5372 (70,1)	130 (2,4±0,2)	44 (0,8±0,1)	36 (0,7±0,1)	210 (3,9±0,3)

*In the tested administrative regions of the indicated EFR.

Note: Percentages are given in parentheses.

Hunters with positive reactions (including those referable to 1st and 2d epidemiological groups) were found in all 25 regions surveyed; however, the percentage thereof fluctuated over a wide range (from 0.8 ± 0.5 to 7.8 ± 2.4). The largest number of individuals with positive tularin reactions ($8.2 \pm 1.2\%$) was found among hunters who are agricultural machine operators, who are exposed to the additional risk of tularemia infection during their work (dismantling stacks, loading, transporting and unloading fodder, sugar and feed beets, etc.). They repeatedly mentioned seeing epizootics in the stacks (according to epidemiological screening charts). In second place according to incidence of positive reactions were foresters ($4.6 \pm 1.0\%$).

The highest percentage of positive reactions among hunters was noted in the 20-29 and 30-39 year age groups (4.6 ± 1.0 and 5.6 ± 0.5 , respectively). After 40 years, there was gradual decline in number of individuals with positive tests with each passing decade (4.1 ± 0.4 and $1.7 \pm 0.4\%$, respectively), and it was the lowest ($1.3 \pm 0.4\%$) in the age group of 60 or more years old. An analogous tendency was found when we analyzed data with consideration of duration of hunting activities: the highest incidence of positive tularin reactions was found among hunters who had been hunting for up to 10 years (4.5 ± 0.6 and $5.2 \pm 0.6\%$ for the first and second 5-year periods, respectively). This percentage gradually dropped in subsequent periods.

However, not only and not so much the actual hunting process as the process of primary handling of hare carcasses influence infection of hunters (Table 2).

Table 2. Allergic reaction of hunters to intracutaneous injection of tularemia as related to frequency of stripping hare carcasses

Indicator	Total	Did strip carcasses		
		always	sometimes	did not
Number tested: absolute	5180	4840	152	188
%	100	93,4	3,0	3,6
Positive reactions: abs.	199	193	4	2
%	$3,8 \pm 0,3$	$4,0 \pm 0,3$	$2,6 \pm 1,3$	$1,0 \pm 0,7$

Among the rest of the inhabitants, positive reactions to tularin constituted $0.68 \pm 0.04\%$ among women (out of 36,398) and $2.09 \pm 0.08\%$ (out of 31,764 men), i.e., 3.1 times more. Of the 909 people with positive tularin reaction, 369 (40.6%) were referred to the first epidemiological group, 211 (23.2%) to the second and 329 (36.2%) to the third. The lowest incidence of positive reactions ($0.43 \pm 0.07\%$) was found among individuals up to 19 years of age. In other age groups, it was higher: 1.4 ± 0.2 at 20-29 years, 1.9 ± 0.3 at 30-39 years, 1.4 ± 0.3 at 40-49 years, 1.0 ± 0.1 at 50-59 years and $0.6 \pm 0.1\%$ at over 60 years of age.

The distribution of individuals with positive tularin reactions in different occupational groups was as follows: $4.2 \pm 0.4\%$ among foresters, $3.3 \pm 0.3\%$ among

land reclamation workers, $2.5 \pm 0.2\%$ among agricultural machine operators, $1.6 \pm 0.1\%$ among blue- and white-collar workers, $0.8 \pm 0.05\%$ among agricultural workers and $0.55 \pm 0.07\%$ among "others" (students, housewives, retirees and others).

In a scheduled serological screening of 3953 foresters and land reclamation workers, a positive AR was found in $4.5 \pm 0.3\%$ (3.9 ± 0.4 and $5.0 \pm 0.5\%$, respectively). In an analogous scheduled screening of 5629 foresters and land reclamation workers of the same regions using the allergy test, positive results were found in $3.7 \pm 0.2\%$ of the cases. Consequently, one can pick up a somewhat larger number of immune individuals with the AR test than with the allergy test alone ($t = 1.9$). The results of the allergy and AR test did not coincide in a considerable number of individuals. For this reason, if we consider all those in whom either of these tests was positive, the actual immune element in the relevant groups with regard to tularemia would be larger than established solely by the allergy test.

A comparative study of infection of hunters and other population groups was made on the basis of data for 13 regions (in the rest of the regions, such a study was made only for hunters, whereas other strata of the population were not studied, or vice versa). The group of hunters consisted of 2792 people and the group of other rural population, identical in age and sex to the hunters, consisted of 20,476 people. As shown by a comparison for only the first epidemiological group, which is the most correct, there were 3.6 times more hunters with positive tularin reactions than among the rest of the inhabitants (2.9 ± 0.3 and $0.8 \pm 0.06\%$, respectively).

We evaluated the sensitivity of both methods and suitability of retrospective detection of individuals who had suffered from tularemia infection by means of simultaneous screening of 1573 hunters living in 8 rural regions, using the allergological and serological (AR) tests. The results revealed that when the selected population group (1573 people) was screened by either of the above tests the percentage of positive findings constituted $4.1-4.3 \pm 0.4$, whereas with concurrent use of both tests it reached 6.3 ± 0.6 .

Since agglutinins and allergic sensitivity, which are highly sensitive and specific in individuals with a history of tularemia, persist for decades [2, 5, 8], it was necessary to determine whether the endemic sites had retained activity up to recent years, or whether the demonstrated immune stratum constituted merely a trace of prior (long ago) activity. For this purpose, we screened 3159 school children 7 to 16 years of age from 23 schools in 2 regions of the republic. Our choice of schools was governed by data from the allergy screening of the adult population (in areas of maximum accumulation of positive reactions). We found 20 children ($0.6 \pm 0.1\%$) from 7 schools who reacted positively to intracutaneous injection of tularin, including 8 children with positive reactions out of 198 tested in one school. Of the 20 children with positive reactions, 9 were up to 10 years of age. According to the epidemiological survey, all 20 children could have been infected with tularemia only within the borders of the republic, and 18 of them only within the borders of their administrative regions. Serological testing of 14 school children with positive tularin tests revealed that AR was positive in titers of 1:20 and 1:40 in only 2 of them. Other authors

have also observed that the results of allergy and serological tests on children did not coincide [2]. The submitted data indicate that, in addition to endemic sites for arboviral infections [4, 9], there are also tularemia sites in Lithuanian SSR.

Conclusions

1. In Lithuanian SSR, there are endemic tularemia sites, in which hares are the reservoirs of infection in nature and source of human infection.
2. For the population of Lithuania there are other routes of tularemia infection, in addition to a route such as hunting.
3. The most complete retrospective detection of individuals infected with tularemia is achieved by concurrent use of allergological and serological tests.

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TULAREMIA IN THE ARCTIC

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[Article by L. I. Nekrasova, Arkhangelsk Medical Institute]

[Text] The region least explored with regard to tularemia is the tundra zone, which occupies vast expanses of the Soviet Union. There are sparse data in the literature concerning tularemia in the Arctic, and they deal with detection of this infection primarily in the Extreme North of European USSR. Thus, in 1953-55, 17 cases of tularemia in man, confirmed by serological and allergic skin tests were diagnosed in the tundra zone of Kola Peninsula. The outbreaks were preceded by an unidentified epizootic among lemmings and other rodents; these animals were not examined bacteriologically at that time [5]. Endemic tularemia sites were also discovered beyond the North Pole circle and in the Nenetsk National Okrug of Arkhangelsk Oblast [4]. As for the extreme northern Asian part of the USSR, a tularemia site was found on the lakes of the forest-tundra in the suburbs of Norilsk [1, 3]; an extensive zoological and parasitological survey of the fauna and epidemiological screening of the public proved the existence of endemic tularemia sites on the banks of the Ob River inlet, at the latitude of the Arctic circle [2]. There is no information concerning tularemia in the rest of the territory of the Arctic region in the Asian part of the USSR.

For this reason, we tried to determine the distribution of tularemia and intensity of epidemic manifestation of endemic sites of infection in the extreme northern part of Siberia and the Far East, by means of serological screening of inhabitants, who are permanent residents of this region and who were not immunized against tularemia. We excluded from this survey individuals who were serving in the Soviet Army. We determined the natural immune element among aborigenes (Nenets, Dolgan, Nganasan, Yakut, Chukchi, Eskimo and others) in the summertime, in different areas: Chukotsk National Okrug in 1970, Taymyr National Okrug in 1971, Yamalo-Nenetsk National Okrug in 1972, Bulunskiy, Allaykhovskiy and Nizhnekolymskiy Rayons of Yakutsk ASSR. All of the populated centers surveyed were situated in the tundra zone and only two villages (Kolymskoye and Andryushkino) in the forest-tundra zone.

For the epidemiological survey, we used the agglutination test with highly sensitive diagnosticum from a strain of *F. tularensis* of the Neoarctic subspecies (the diagnosticum was obtained from the Institute of Epidemiology

and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences). We ran the agglutination reaction with 1:20 serum dilution, and a very distinct (+++) reaction was considered positive.

Results of serological testing for tularemia of indigenous population in the extreme northern part of Siberia and the Far East

Testing site	Number of people tested	Number of people tested who showed positive agglutination test result ($\geq 1:20$)	
		absolute	% $\pm$ m
Yamalo-Nenetsk National Okrug:			
Yar-Sale	103	21	20.3 $\pm$ 4
Taymyr National Okrug:			
Novorybnoye	138	27	18.9 $\pm$ 3
Novaya	44	4	9 $\pm$ 4
Yakutsk ASSR:			
Nayba	160	32	20 $\pm$ 3
Chokurdakh	102	16	15.6 $\pm$ 3
Kotenko	90	13	14.4 $\pm$ 4
Oyotung	87	13	14.9 $\pm$ 4
Kolymskoye	53	6	11.3 $\pm$ 4
Andryushkino	104	16	15.4 $\pm$ 3
Chukotsk National Okrug:			
Uelen	81	13	16 $\pm$ 4
Nunyamo	83	7	8.4 $\pm$ 3
Yanranay	22	2	9 $\pm$ 6
Ryrkaypiy	48	5	10.4 $\pm$ 4

We tested 1115 people 10 to 70 years of age in 13 populated centers. We found positive agglutination reactions in a serum dilution of 1:20-1:160 (mainly in dilution of 1:20-1:40) in inhabitants of all regions screened (see Table). In the youngest age group (10-14 years), positive reactions were obtained in 4% (out of 113 tested). The percentage of people immune to tularemia was highest among those over 20 years of age, and it ranged from 9 to 23 in the different localities. There was a history of protracted sore throat, lymphadenitis, fever, i.e., diseases that were suspicious for tularemia, in only 24 subjects, whose blood showed specific agglutinins.

We also used the Wright test in all cases of positive serum reactions to tularemia. In two cases, we obtained a positive result in titers that did not exceed the ones with tularemia antigen (1:20-1:40).

Aside from the existence of a naturally immune stratum among the indigenous inhabitants, the cases of tularemia among individuals who came to these regions from other places for temporary work also served as confirmation of the presence of active endemic sites for tularemia. Thus, there were two cases of tularemia in scientists who had worked in an expedition to Taymyr Peninsula, the region of Pyasina River, near Tareya (73° north latitude) in July and August 1967. The infection occurred against a background of numerous lemmings, which is the background species of tundra rodents.

Here is a brief excerpt from a case history:

Patient P., 38 years old, participated in expedition to Taymyr starting on 10 July. She had been sick since 15 August. For 5 days there was general malaise, increased fatigability. On 22 August, her temperature rose to 39.2°, she suffered from severe chills, headache and pain in the right inguinal region. On 23 August, the patient noticed enlargement of lymph nodes in the right inguinal region and swelling with hyperemia of the skin, 3 cm in diameter, at the site of a bite, in the middle third of the right lower leg. Her temperature was 38° for 1 week. She was treated with tetracycline. Her well-being improved, so that she was able to continue working in the field. On 2 September, her condition worsened again: temperature rose again to 38°, pain reappeared and lymph nodes were enlarged in the right inguinal region. After returning to Leningrad on 6 September she was hospitalized in the surgical department of the hospital of the Academy of Sciences, from which she was transferred to the Infectious Hospital imeni Botkin with suspicion of tularemia. The diagnosis of ulcerative-bubonic form of tularemia was made on the basis of her history, clinical findings and results of immunological test (on 20 October positive agglutination reaction in a dilution of 1:320; a 1x1.5 cm papule appeared at the site of tularin injection 48 h later, which was surrounded by a hyperemic zone 4 cm in diameter). She responded well to streptomycin.

Another participant of this expedition became sick on 30 July. After a few days he noticed a bubo in the inguinal region. He took tetracycline on his own. Infection by the bubonic form of tularemia with localization of the bubo in the neck region had already been observed in 1965 in one scientist during expeditionary work in this same region of Taymyr Peninsula. In that case, the diagnosis of tularemia was confirmed serologically and positive result of skin test with tularin upon retrospective examination in 1967.

Conclusions

1. A serological screening of a sample of 1115 indigenous residents, who had not been immunized against tularemia, made it possible to retrospectively ascertain prior tularemia infection, which is indicative of the existence of endemic tularemia sites in the Arctic region of Asian USSR.
2. The northernmost endemic tularemia site was found on Taymyr Peninsula (73° north latitude), where three cases of tularemia were recorded among members of an expedition.
3. The obtained data validate the desirability of immunizing individuals for whom there is an occupational risk of tularemia infection in the Extreme North.

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SEROLOGICAL EXAMINATION OF PREDATORY BIRD PELLETS IN THE VICINITY OF THE
IMAM-BABA STATION IN TURKMEN SSR

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Dec 74 (manuscript received 2 Jul 73) pp 38-40

[Article by V. M. Neronov, B. P. Dobrokhotoy and I. S. Meshcheryakova,
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[Text] In 1962, a base was organized in the vicinity of the Imam-Baba Station, which is situated in the central course of Murgaba River, by the Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, in order to investigate the many-year changes in main elements of a site of zoonotic cutaneous leishmaniasis, and for the last 2 years tularemia and plague.

In 1951, Khodukin [11], who examined the history of tularemia in Asia, arrived at the conclusion that the minor epidemic outbreak described by Voskresenskiy, which was recorded in 1884 in the garrison at Merv, resembled tularemia in all of its features. Subsequently, Strelyayeva et al. [8], who screened the local inhabitants (using the tularin skin test) and serum from cattle, goats and sheep from different parts of Turkmenia, also concluded that there was a possibility of tularemia sites in the Murgaba valley.

An outbreak of pneumonic plague was observed in late 1912 in the vicinity of Merv. In the fall of the same year, many deaths were observed among rodents, but it was not possible to confirm bacteriologically that a plague epizootic had occurred [10]. In 1950 and 1957, a plague epizootic was discovered among gerbils far to the south of the base (in the vicinity of Kushka, village of Chemen-i-Bit and Pobeda Sovkhoz), but the isolated strains presented attenuated virulence [3]. A similar type of *Y. pestis* strain had also been isolated in the vicinity of Iolotan, north of the base [2]. On the basis of analysis of environmental conditions and distinctions of the isolated strains, Akiyev [1] assumed that it is unlikely for the plague enzootic to persist in Southeastern Kara-Kumy, whereas the elevations of Badkhyz and Karabil to the south could merely be a zone to which *Y. pestis* is carried from other endemic plague sites.

As had already been repeatedly demonstrated [4-6, 12], serological testing of predatory bird pellets is a rather sensitive method for detection of epizootics

of both tularemia and plague. On the basis of these studies, we collected pellets in the valley and desert-sand regions in the vicinity of our base [7]. The first batch of pellets (112) was collected from 7 to 15 February and the second (172 units) from 28 April to 11 May 1973. It should be noted that, in the winter of 1973, there was a considerable increase in number of great gerbils in most parts of the base after several years of a low population thereof (the preceding peak was reported in 1964-1965, and in 1966-1967 there was a depression). In 1973, there was also an increase in number of red-tailed Libyan jirds and house mice. In turn, the abundance of feed and the features of the winter of 1972-1973 caused an increased concentration of predatory birds (kestrels [Falco species], long-legged buzzards, kites, steppe eagles, vultures and others) in the vicinity of Imam-Baba: we had occasion to observe up to 9-10 birds per field of vision in February over the third terrace [bench] of Murgaba. But, by the end of April, most of them had migrated or flown to the north, and the usual pattern became established in these sites with prevalence of the common and lesser kestrels [9] and rare sightings of other, larger species (long-legged buzzard in the sand and kite near the village). In February 1973, we collected pellets under the power-line poles in the hilly-ridged sands about 1-1.5 km from the left main bank of Murgaba. We failed to collect fresh pellets there 2.5 months later, although this was expressly the location of the highest density of great gerbil burrows (see Table) and the poles were very convenient as resting places for birds of prey--on the hills, next to the road and cattle-driving areas. In addition to these poles, some pellets were collected under telegraph poles on the second terrace on the left bank of Murgaba, under a few turanga trees in the tugays [vegetation-covered bottomland] on the right bank of the Murgaba, on the hills, under bushes and under the two triangulation marks on the third terrace and flattened-wavy sands on the right bank of the Murgaba. But, in general there were few pellets, 1-2 at some points and up to 10 in rare cases. All were relatively fresh; old and decomposed pellets that could be attributed to prior years were virtually absent.

The gathered pellets were distributed as follows with regard to weight: first batch (February 1973, total of 112)--under 2 g for 13 pellets, 2-5 g for 73, over 5 g for 26; second batch (April and May 1973, total of 172)--under 2 g 49 pellets, 2-5 g 59 pellets and over 5 g 64. The smaller ones were apparently referable to the kestrels, and were gathered chiefly in the tugays; average-sized ones were referable to kites and buzzards, large ones to spotted and steppe eagles, and others.

All of the pellets were tested by the antibody neutralization reaction, using standard methods [4, 5], for detection of tularemia and plague antigens. No tularemia antigen was demonstrable in the pellets. In four cases, plague antigen was found in the first batch of pellets: No 13 (from the hilly and ridged sands, residues of the great gerbil, pellet weight 7 g, titer 1:24), No 67 (third terrace of the Murgaba, residue of the great gerbil, pellet weight 7.4 g, titer 1:96), No 77 (third terrace, residues of great gerbil and insects, pellet weight 15.3 g, titer 1:48) and No 92 (second terrace on the left bank of the Murgaba, fur and bone residue, species unknown, pellet weight 6.3 g, titer 1:12). The last dilution of tested material causing complete arrest of passive hemagglutination scaled to dry pellet weight was

taken as the reaction titer. We used plague erythrocytic diagnosticum (dry) prepared by the Alma-Ata Plague-Control Institute as antigen to run these reactions. No plague antigen was found in the second batch of pellets. It should be noted that they were gathered beyond the limits of the area where pellets had been gathered in February, and there were virtually no fresh pellets at the old collection sites. Typically enough, plague antigen was demonstrated in the largest pellets of the first batch, and three of them contained residue of the great gerbil, which is the principal carrier in endemic plague sites of Central Asia.

Results of recording density of great gerbil burrows, number of predatory birds and distribution of their pellets under power line poles (hilly-ridged sands on the left bank of the Murgaba River)

Place and time of recording and gathering pellets	Distance traveled (km)	Number of active great gerbil burrows ¹	Number of predatory birds encount- ered	Number of power line poles examined	Number of poles where pellets were gathered (quantity of pellets in paren.)
Between Bolshevik village and Imam-Baba station, 28 Apr 73 (1100-1315 hours)	5	13	1 kestrel	20	12(32)
Between Beriket and Imam-Baba 29 Apr 73 (1000-1245 hours)	3.25	44	0	13	4(12) ³
North of road, Egri well Imam-Baba station, 11 May 73 (1000-1200 hours)	2.75	13	3 kestrels, 2 black vultures ²	11	4(18)
Total during 7-h excursion	11	70 or 1.6/ha	4 kestrels, 2 vultures	44	20(62)

¹Three recorders walked along the power lines, width of record tape 40 m.

²The carcass of a long-legged buzzard was found under one of the poles.

³On this segment of the route, pellets were collected on 12 Feb 73.

Conclusions

1. Examination of 284 pellets of predatory birds, collected in the vicinity of the Imam-Baba station in February and April-May 1973 using the antibody neutralization reaction failed to demonstrate tularemia antigen; plague antigen was demonstrated in titers of 1:12, 1:24, 1:48 and 1:96 in 4 pellets, in 3 of which there was residue from great gerbils.

2. In order to give a definitive answer to the question of whether there are endemic plague sites in this territory, as well as describe them, further investigations are needed.

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POSSIBILITY OF PREDICTING PLASMOCYTE REACTION OF MICE TO SOLUBLE ANTIGEN

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[Article by M. I. Levi, N. N. Sakayan, M. M. Livshits, I. S. Meshcheryakova and I. V. Rodionova, Moscow Municipal Disinfection Station]

[Text] Zdrodovskiy [3] proposed the plasmocyte reaction as one of the indicators of cellular immunity. It was subsequently shown that accumulation of cells of the plasmocyte class in a regional lymphoid organ is a function of the antigenic stimulus, which determines the quantitative characteristics of the plasmocyte reaction [2, 7, 20]. The increment in plasma cells was proportionate to the square of the logarithm of administered dose of antigen, as well as modulus of transition from antigenic function to accumulation of plasma cells (a), and inversely proportionate to the specific rate of antigen elimination from the injection site (k). Since there was no appreciable increase in incidence of mitosis in the lymphoid system after administration of soluble antigen, in accordance with data in the literature [14, 15, 17, 22] it was concluded that the plasma cell reaction to soluble antigen was largely attributable to an influx of plasma cell precursors in the regional lymphoid organ from other lymphoid structures [9]. Moreover, it was found useful to record the intensity of the plasma cell reaction when calculating precursors of antibody-producing cells, since it was necessary to add to the precursors in the regional lymphoid organ before administration of antigen the precursor cells that were found there as a result of influx of mobile cells from other lymphoid formations.

We undertook this study in order to determine the concrete value of the modulus of change from antigen function to plasma cells (a). In previous studies [7, 9, 20], modulus a was determined on the basis of overall increment of cells of the plasmocyte class in a regional lymphoid organ (spleen) and dynamics of antigen concentration at the site of administration (abdominal cavity). However, since the spleen cannot, strictly speaking, be considered a regional lymphoid organ for the abdominal cavity [19], we considered here the concentration of soluble antigen in blood, for which the spleen is, of course, a regional lymphoid organ.

We used two soluble antigens in our experiments: capsular antigen (fraction I) of *P. pestis* [18] and Boivin's complete antigen of *F. tularensis* [12, 13]. The

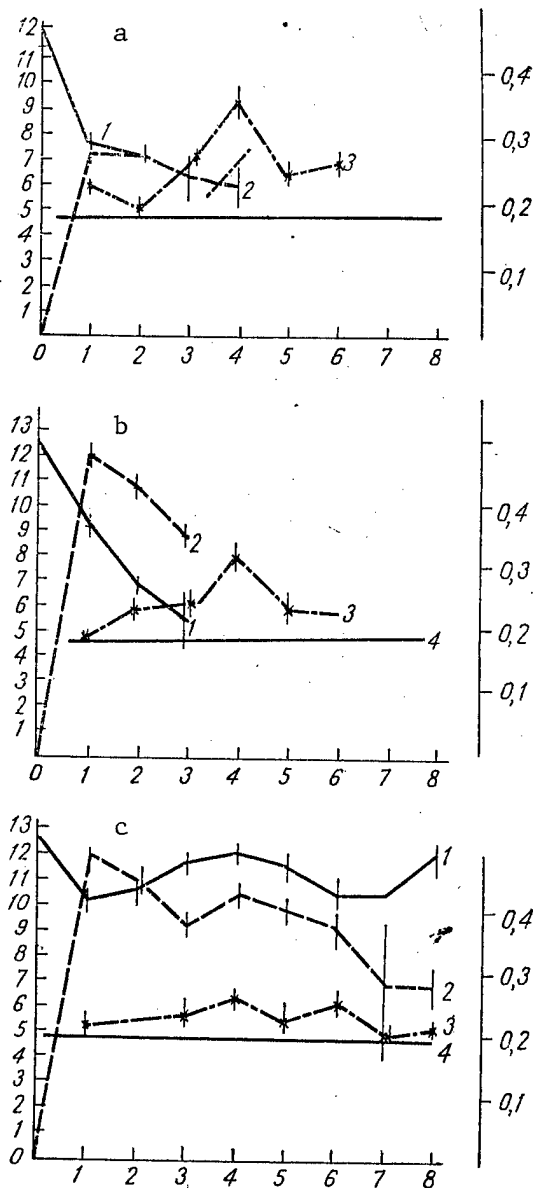


Figure 1.

Dynamics of concentration of soluble antigen and plasma cell reaction

a) capsular antigen (experiment No 20,

$$\ln \frac{D_0}{N} = 12.0; \text{ PHAR}$$

b) capsular antigen (experiment No 14,

$$\ln \frac{D_0}{N} = 12.6; \text{ ANR}$$

c) complete antigen (experiment No 9,

$$\ln \frac{D_0}{N} = 12.7; \text{ PHAR}$$

-
- 1) antigen concentration in abdominal cavity
 - 2) in blood
 - 3) probability of plasma cells in spleen of immunized animals (PC_0)
 - 4) nonimmunized animals (PC_k)

X-axis, time (days) after giving antigen; y-axis, left--natural logarithm of concentration of antigen molecules/nuclear spleen cell; right--probability of plasma cells among nuclears in spleen

capsular antigen was recovered from acetone-dried bacterial mass of vaccine strains EV and K1 (4, 16]. Complete *F. tularensis* antigen was recovered in dry form from standard strain 503/830 [12, 13]. After assaying protein according to Lowry and serological activity, solutions of these antigens were injected intraperitoneally to CBA mice in a volume of 0.25 ml, then we dissected 5 animals from each group given each dose, daily for 6-10 days (the dosage was usually reduced gradually to one-fourth). The method of examining the animals was described previously [9]. We used the antibody neutralization reaction (ANR) with stable antigenic diagnosticum and passive hemagglutination reaction (PHAR) with stable antibody diagnosticum [5, 11] by the micromethod (of Takacs) to determine antigen concentration in the entire volume of blood (mean serum volume was considered to be 3.5% of average mouse weight), after which we counted the average antigen molecules per nuclear cell in the spleen. We proceeded from the fact that the molecular weight of *P. pestis* antigen is 150,000 [1, 9, 10] and that of *F. tularensis* complete antigen 11,000,000. One minimum neutralizing dose of capsular antigen determined in the ANR was considered to equal 0.001 μ g protein of "pure," i.e., without significant impurities, capsular antigen, whereas 1 hemagglutinating unit (HU) of the same antigen, as determined in the PHAR, was estimated as 0.0001 μ g protein of "pure" capsular antigen [9]. We used

the following estimates for *F. tularensis* complete antigen: 1 minimum neutralizing unit equals 0.1 μg dry antigen, 1 HU = 0.01 μg dry complete antigen. The erythrocyte diagnosticum of about the same sensitivity were matched over the entire period of our tests.

We ground the spleen of each mouse in a homogenizer; a suspension was prepared in Hanks solution, after which we counted nuclear cells in a Goryayev chamber, as well as total plasmocyte class cells (mature and immature plasma cells, plasmoblasts and blasts) under an MBI-6 phase-contrast microscope at magnification of $90 \times 7 \times 2.5$ in 300-400 lymphoid cells encountered at random [7, 20].

As an example, we describe the dynamics of concentration of antigen in the abdominal cavity and blood, as well as concentration of plasma cells in several experiments only at the times when we succeeded in demonstrating it with the serological reactions (Figure 1). It is easy to see that the dynamics of the complete antigen differed considerably from the dynamics of capsular antigen.

Thus, the mathematical expression of modulus $\underline{a}$ acquires the following appearance:

$$\text{Modulus } \underline{a} = \frac{\int_1^{t+2} (PC_0 - PC_k) \Delta t}{\int_0^t \ln \frac{D_i}{N} \Delta t} \approx \frac{\sum_1^{t+2} (PC_0 - PC_k) \Delta t}{\sum_0^t \ln \frac{D_i}{N} \Delta t}, \quad (1)$$

where $\underline{a}$ is the modulus of change from antigenic function to accumulation of plasma cells; Δt is the time interval, which was 1 day in all of our experiments; D_i is the amount of antigen in molecules over the entire blood volume at time i ; N is the number of nuclear cells in the spleen; PC_0 is the probability of finding plasma cells among nuclears of the spleen at time i in immunized animals; PC_k is the probability of finding plasma cells in nonimmunized animals

$$\left[\sum_1^{t+2} PC_k \Delta t = (t+2) PC_k \right],$$

t is the time of recording demonstrable amounts of antigen in blood (days).

The scales of values were submitted previously [9].

It must be noted that most of the intraperitoneally injected soluble antigen was demonstrable in blood within 1-2 h, for which reason we equated the amount of antigen in blood at the moment of injection with the amount of antigen given (D_0). The second distinction of equation (1) is that the plasmocyte reaction was estimated at the time when antigen was demonstrated in blood plus 2 days.

This restriction was used in order to keep track of differentiation in plasma cells of the mobile cells that were recruited to the spleen from other lymphoid structures under the influence of antigen. Moreover, the peak plasmocyte reaction was usually observed 2 days after a maximum concentration of antigen in blood was reached (Table 1).

It was logical to assume that the greater the value characterizing the background (PC_k), the greater the plasmocyte reaction:

$$\sum_0^{t+2} (PC_0 - PC_k)$$

and, consequently, modulus $\underline{a}$. For this reason, we introduced a new concept, the plasmocyte index $PC_i = \underline{a}/PC_k$. Incidentally, in a mathematical model [9] modulus $\underline{a}$ is encountered in expressly such a combination; moreover, unlike modulus $\underline{a}$, PC_i is a dimensionless parameter. The value of PC_k is most probably related to the animals' average weight (Figure 2). Another factor that influences the plasmocyte reaction is the purity of the capsular antigen preparation:

$$I = \left(\ln \frac{d}{q} \right)^2 + 1,$$

where q is estimation of minimum neutralizing dose or hemagglutinating unit, as given above, and d is the weight characteristic of a specific antigen series. On the basis of the foregoing, we proposed that dimensionless parameter A be used as a constant:

$$\text{Parameter } A = \frac{a}{PC_k} \cdot \frac{1}{\left[\left(\ln \frac{d}{q} \right)^2 + 1 \right]}. \quad (2)$$

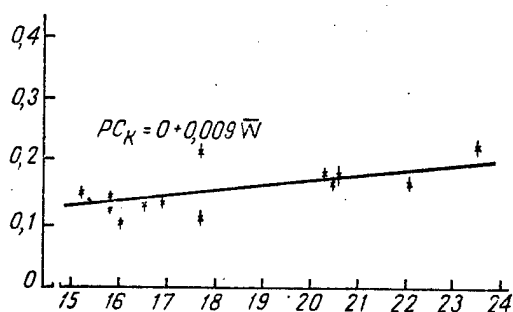


Figure 2.

Mean weight of CBA mice ($\bar{W}$) as a function of probability of finding plasma cells in spleen of nonimmunized animals (PC_k)

X-axis, mean mouse weight ($\bar{W}$) (g) in experiment; y-axis, probability of finding plasma cells in spleen of nonimmunized animals (PC_k). Arithmetic mean PC_k and error of means, as well as line of regression (regression equation $PC_k = 0 + 0.009 \bar{W}$)

Table 1. Values of modulus $\underline{a}$ in different experiments

Exper. No	Agent used for immunization	Mean mouse wt. (g)	PC_k	Sero- logical test	Antigen dose $\left(\ln \frac{D_0}{N}\right)$	Obtained modulus $\underline{a} \times 10^{-3}$
4	Fräction I P. pestis K1	16,1	0,107	ANR	14,5; 13,0; 11,8	3,3; 2,7; 2,1
10		23,6	0,234	PHAR	14,1; 12,7; 11,3	3,6; 3,0; 2,6
14		20,6	0,180	ANR	11,9; 10,5	4,8; 2,3
				PHAR	13,5; 12,1	5,3; 3,3
				ANR	14,0; 12,6	8,5; 7,0
				PHAR	12,6; 11,2	10,3; 7,3
20	Fraction I	20,6	0,187	ANR	12,3; 10,9	11,0; 8,0
	P. pestis K1 +			PHAR	12,0; 10,6	12,7; 10,8
	Fraction I			ANR	12,2; 10,8	7,7; 8,0
	P. pestis EV			PHAR	12,0; 10,6	9,5; 11,2
8	Fraction I P. pestis EV	17,0	0,162	PHAR	15,3; 13,9; 12,5	7,1; 4,6; 4,4
16		20,4	0,190	ANR	15,6; 15,8; 15,6	3,8; 7,1; 5,9
				PHAR	15,4; 15,4; 15,4	3,7; 7,1; 4,7
9	Complete antigen	20,8	0,190	ANR	13,4; 12,0; 10,6	9,6; 4,3; 2,4
6	F. tularensis	16,6	0,141	PHAR	14,1; 12,7; 11,3	7,0; 4,5; 3,2
				ANR	12,8; 11,4; 10,0	5,9; 11,3; 9,3
				PHAR	13,0; 11,6; 10,2	5,8; 6,8; 6,2

On the basis of the values of parameter A in different experiments (Table 2), we calculated the arithmetic means of modulus $\underline{a}$ and parameter A (Table 3), and both were compared according to coefficient of variation. For fraction 1 of P. pestis, the mean values of modulus $\underline{a}$ and parameter A had substantially different coefficients of variation when using the PHAR, but not ANR, which could be attributed to the longer demonstration of soluble antigen in blood and, for this reason, more accurate determination of the value of this parameter. Since we only had one series of complete antigen of F. tularensis at our disposal, the values listed in the table could merely serve to predict the plasmocyte reaction but not to compare variability thereof.

As it applies to consideration of concentration of soluble antigen in blood, the previously proposed [9] equation for the plasmocyte reaction can be written down in the following manner:

$$\frac{\sum_{i=1}^{t+2} PC_0 \Delta t}{\sum_{i=1}^{t+2} PC_k \Delta t} = 1 + \frac{a}{PC_k} \cdot \frac{\sum_{i=0}^t \ln \frac{D_i}{N} \Delta t}{t+2}. \quad (3)$$

Using equation (3) and mean values of parameter A for the entire set of experiments, we calculated the dimensionless variable in the left part of the equality, after which we compared the estimated value for each specific experiment to the one found experimentally, and we also calculated deviation of estimated value from experimental one (see Table 2). We found that the estimates did not deviate very significantly from experimental values.

Table 2. Determination of parameter A from data of different experiments and check for possibility of predicting plasmocyte reaction

Exper. No	Preparation used for immuniz.	Amount of protein or com- plete antigen in dose given (μg)	Serol. test	Para- meter Ax10 ⁻³	$\frac{\sum_{i=1}^{t+2} PC_0 \Delta t}{\sum_{i=1}^{t+2} PC_k \Delta t}$		Devia- tion of estimate from experim. values (%)	
					estim.	exper.		
4	Fraction I P. pestis K1	512	ANR	4,7	1,321*	1,329*	0,6***	
			PHAR	3,8	1,340	1,329	-0,8	
		128	ANR	4,0	1,266	1,329	4,7	
			PHAR	3,3	1,342	1,329	-1,0	
		32	ANR	3,1	1,266	1,167	-8,5	
			PHAR	2,8	1,233	1,167	-5,7	
10			250	ANR	1,7	1,404	1,273	-10,3
				PHAR	5,2	1,133	1,298	12,7
		62	ANR	0,8	1,361	1,174	-15,9	
			PHAR	3,3	1,141	1,204	5,2	
14			260	ANR	9,0	1,241	1,448	14,2
				PHAR	4,5	1,411	1,448	2,5
		65	ANR	7,2	1,226	1,310	6,4	
			PHAR	3,2	1,420	1,310	8,4	
20	Fraction I P. pestis K-1 + fraction I P. pestis EV	62	ANR	6,4	1,305	1,453	10,2	
			PHAR	6,1	1,278	1,453	12,0	
		16	ANR	4,3	1,287	1,137	13,2	
			PHAR	4,9	1,272	1,218	-4,4	
		64	ANR	4,1	1,314	1,303	0,8	
			PHAR	4,5	1,320	1,387	4,8	
16		ANR	4,3	1,287	1,137	13,2		
		PHAR	4,9	1,272	1,218	-4,4		
8	Fraction I P. pestis EV	420	PHAR	3,5	1,503	1,538	2,3	
		105	PHAR	2,3	1,453	1,538	5,5	
		26	PHAR	2,2	1,422	1,538	7,5	
9	Complete antigen F. tularensis	400	ANR	50,5	1,342	1,383	3,1	
			PHAR	36,8	1,340	1,357	1,3	
		100	ANR	22,6	1,347	1,172	-13,0	
			PHAR	23,8	1,321	1,224	-7,9	
		25	ANR	12,6	1,282	1,079	-15,8	
			PHAR	16,8	1,274	1,132	-12,5	
6			800	ANR	41,8	1,429	1,394	-2,4
				PHAR	41,1	1,337	1,394	4,3
		200	ANR	80,1	1,342	1,604	19,5	
			PHAR	48,2	1,274	1,376	8,0	
		40	ANR	66,0	1,269	1,390	9,5	
			PHAR	44,0	1,249	1,313	5,1	

*We used the mean parameter A (see Table 3); probability of plasma cells in nonimmunized animals was calculated with regression equation

$$PC_k = 0 + 0.009 \bar{W} \text{ (Figure 2).}$$

**Values of $\bar{w}$, $\ln N$, $\sum_{i=0}^t \ln \frac{D_i}{N} \Delta t$ and $\frac{\sum_{i=1}^{t+2} PC_0 \Delta t}{\sum_{i=1}^{t+2} PC_k \Delta t}$ were found in the experiments.

$$***\text{Deviation} = \frac{\text{experimental value} - \text{estimated value}}{\text{experimental value}} \times 100.$$

Table 3. Mean values of modulus a and parameter A, and variability thereof

Antigen	Compared signs	Serol. test	Number of tests (n)	Means (t)	Arithmet. mean ($\times 10^{-3}$)	Coefficient of variation (%)
Fraction I P. pestis	Modulus <u>a</u>	ANR	14	3,3	5,9 $\pm$ 0,73	46,4
		PHAR	17	4,0	6,5 $\pm$ 0,79	54,5
	Paramet. A	ANR	11	3,3	4,5 $\pm$ 0,72	53,3
		PHAR	14	4,0	3,9 $\pm$ 0,30	29,2
Complete antigen F. tularensis	Modulus <u>a</u>	ANR	6	2,5	7,1 $\pm$ 0,14	48,0
		PHAR	6	6,2	5,6 $\pm$ 0,60	26,2
	Paramet. A	ANR	6	2,5	45,6 $\pm$ 10,44	56,1
		PHAR	6	6,2	35,1 $\pm$ 4,98	31,7

Finally, we used the determined mean values of parameter A to predict accumulation of cells of the plasmocyte class in a special experiment, in which mice were given intraperitoneal injections of a mixture of different doses of P. pestis fraction I and F. tularensis complete antigen. We dissected 5 animals given each dose daily, for 8 days, in order to assay concentrations of both antigens and count plasmocyte elements. According to the previously established pattern for the variant of giving two different antigens [8, 21], we calculated accumulation of plasmocyte elements using the following equation:

$$\left[\frac{\sum_{i=1}^{t+2} PC_{oi} \Delta t}{\sum_{i=1}^{t+2} PC_{hi} \Delta t} \right]^{CA+FI} = 1 + \left[\frac{a}{PC_h} \cdot \frac{\sum_{i=0}^t \ln \frac{D_i}{N} \Delta t}{t+2} \right]^{CA} + \left[\frac{a}{PC_h} \cdot \frac{\sum_{i=0}^t \ln \frac{D_i}{N} \Delta t}{t+2} \right]^{FI} \quad (4)$$

The designations in this equation are referable to overall data for complete antigen (CA) or fraction I (FI), or for their combined effect (CA+FI). Since PC_h was determined in the experiment (and it was found to be virtually identical to the estimate), we compared the estimates to experimentally found values under more rigid conditions. However, our results indicated that there is a very real possibility of predicting the level of the plasmocyte reaction, and the demonstrated deviations of estimates from experimental values were not so considerable (Table 4).

Thus, the proposed equations, which describe accumulation of cells of the plasmocyte class in the mouse spleen as a function of dynamics of concentration of soluble antigen in blood, enable us to predict the level of the plasmocyte reaction. We must stress the need to consider the dynamics of antigen concentration in blood. However, there are direct indications to the effect that dynamics of antigen at the injection site are a concrete function of administered dose [6, 9]. Most probably, the dynamics of antigen in blood are also a function of given dose of antigen, which experiences the influence of certain other factors (accumulation of antibodies in blood, elimination of antigen from the organism, etc.).

Table 4. Accumulation of plasmocyte class cells in spleen of mice given a mixture of P. pestis capsular antigen and F. tularensis complete antigen

Dosage of antigen mixture*	Preparation used for immunization	Serol. test	$\ln \frac{D_0}{N}$	t	$\sum_{i=0}^t \ln \frac{D_i}{N} \Delta t$	$H_{t+2} - H_{t+1} = \frac{D_{t+2}}{N} - \frac{D_{t+1}}{N}$	$\frac{a}{PC_k} \times 10^{-3}$	$\frac{t+2}{\sum_{i=1}^{t+2} PC_k \Delta t} \frac{CA+FI}{\Delta t}$		Deviation of estim. from experimental value (%)****
								experimental***	estim.	
Large	Fraction I P. pestis	ANR	12,1	5	64,1	1,660	13,5	1,7449	1,5660	24,0
	Compl. antigen F. tularensis	PHAR	10,5	5	55,1	1,660	32,4	1,7449	1,6165	17,2
		ANR	15,1	6	77,2	1,844	45,6	1,7449	1,5660	24,0
		PHAR	15,1	6	82,7	1,844	35,1	1,7449	1,6165	17,2
Medium	Fraction I P. pestis	ANR	10,7	3	40,3	0,982	13,5	1,4670	1,5101	-9,2
	Compl. antigen F. tularensis	PHAR	9,1	3	33,8	0,982	32,4	1,4670	1,5174	-5,3
		ANR	13,8	4	53,1	1,180	45,6	1,4670	1,5101	-9,2
		PHAR	13,7	4	51,3	1,180	35,1	1,4670	1,5174	-5,3
Small	Fraction I P. pestis	ANR	9,3	1	16,0	0,597	13,5	1,4927	1,4277	13,2
	Compl. antigen F. tularensis	PHAR	7,7	3	27,2	1,005	32,4	1,5045	1,4536	10,1
		ANR	12,4	2	31,2	0,806	45,6	1,4927	1,4277	13,2
		PHAR	12,3	4	47,3	1,213	35,1	1,5045	1,4536	10,1

*Mean mouse weight ($\bar{W}$) = 15.9 g; $PC_k = 0.134$.

**Modulus a was calculated using parameter A and protein concentration in dose ($a = A \cdot I \cdot PC_k$).

***Since (t+2) for complete antigen and fraction I was found to be different, accumulation of plasma cells was estimated as the arithmetic mean for two times.

****Deviation = $\frac{\text{experimental value} - \text{estimated value}}{\text{experimental value}} \times 100\%$.

Conclusions

1. It was found that the concentration of antigen in blood and accumulation of cells of the plasmocyte class in CBA mice given intraperitoneal injections of capsular antigen (fraction I) of the pathogen of plague or complete antigen of the pathogen of tularemia were correlated, and the change from antigenic function to accumulation of plasma cells in a concrete experimental model could be described by a constant, parameter A.
2. A comparison of actual values of the plasmocyte reaction found in the experiment and estimates obtained by means of the average values of parameter A failed to demonstrate significant discrepancies.
3. The proposed equations and values for parameter A, which were obtained previously with administration of antigens separately, were tested in order to predict the level of the plasmocyte reaction in an experiment, where animals were given a mixture of different doses of soluble antigens; the estimates differed to some degree from experimental values, but these differences were relatively minor.

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ISOLATION OF FRANCISELLA TULARENSIS FROM SIBERIAN LEMMINGS IN EASTERN TAYMYR

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[Article by L. S. Yegorova, V. A. Il'in, I. P. Algazin and G. B. Mal'kov, Omsk Scientific Research Institute of Endemic Infections]

[Text] Cases of tularemia among humans were observed in the Eurasian subarctic region in several parts of the Extreme North [6, 8, 9, 11, 15, 17, 18, 21, 22]. The pathogen of this infection was isolated from the *Lepus timidus* L. mountain hare [21, 22], *Arvicola terrestris* L. water vole, *Microtus oeconomus* Pall. root vole and *Microtus middendorffi* Poljak vole [11]. In addition, antibodies to *F. tularensis* were detected in *Rangifer tarandus* L. reindeer [3]. Epidemic outbreaks of tularemia occurred against the background of a large population of subarctic rodents. Cases of tularemia among humans in Scandinavia and the Kola Peninsula were preceded by mass scale deaths of *Lemmus lemmus* L. Norway lemmings [18, 20-22].

Many authors have described, long ago, epizootics among lemmings in years where there was a large number of rodents; however, in most cases, the etiology of these cases was undetermined. It has been established that lemmings are susceptible to tundra rabies and tularemia. The susceptibility of lemmings to tundra rabies, which is a widespread disease among wild and domestic animals of the Extreme North, was proven by serological examination of the brain of tundra rodents using the complement fixation test [CFT] [7]. It was possible to isolate the pathogen of this infection from the brain of lemmings who died after experimental infection, after 3-7 blind passages with change in species composition of laboratory animals. Spontaneous tularemia infection among lemmings was found in Scandinavia [20, 21]; heretofore, *F. tularensis* had not been isolated from lemmings in the territory of the Soviet North. Experimental studies demonstrated susceptibility of lemmings to *F. tularensis* on the example of *L. lemmus*, which [19] is classified in the first group of mammals that are highly susceptible and highly sensitive to tularemia [13].

The accumulated information about the probable involvement of lemmings in the spread of tularemia in the Extreme North warranted the assumption that there are endemic sites of tularemia of the tundra (lemming) type [10] and inclusion of the tundra region in the nosological range of tularemia [14].

We investigated the incidence of endemic infections in the Arctic in 1972-1973, in the summer (July, August) on the territory of the Taymyr National Okrug, Krasnoyarsk Kray, in the combined "North" expedition of the Siberian Branch of the USSR Academy of Medical Sciences. We selected regions of West and East Taymyr in the moss-lichen tundra subzone (72-73° north latitude) for our field studies and gathering material. In 1972, the work was conducted on the route along the Pyasina River (from the mouth of the Yangoda River to Pura River, 300 km route) and in 1973, at a permanent base in the Khatanga River basin (in the vicinity of the Novorybnoye trading post).

In this period, we trapped 498 lemmings and 2000 gamasid ticks. We used Gero traps, cylinders and our hands to catch the rodents. The standard method of trap-lines was used to record the number of lemmings, and the traps were equipped with a caprone [nylon-6] trip cord [2].

We took blood (unadulterated and on discs) and excised the spleen from the trapped lemmings, which were placed in a mixture of vaseline and paraffin [1]; the test tubes with spleens were stored in the refrigerator.

The lemming population of the Taymyr tundra is represented by two species, Siberian (*Lemmus sibiricus* Kerr.) and Arctic (*Dicrostonyx torquatus* Pall.), which constituted 96 and 4%, respectively, in our collections. It is known that very drastic changes in population size, with 2- or 3-year periods of fluctuations, are inherent in lemmings. Our observations of populations revealed that, after a profound "depression" of rodents in 1971 (according to the data of the Taymyr "Service of Yield of Game"), the number of lemmings started to increase in 1972 to an average of 7 specimens (in some areas up to 15) per 100 l.s. [not further identified]. In 1973, because of increased summer reproduction of rodents, there was a further rise in number of lemmings (up to 20, and in northern parts of the tundra up to 35 specimens).

In 1972, serological examination of 98 samples of lemming blood serum for tularemia using the HANR [hemagglutination neutralization reaction] with erythrocytic tularemia diagnosticum in a titer of 1:20 (+++++) positive results were obtained in 4 cases (4.1%): in 2 Siberian (rodents caught near the mouth of Lyungfada River and Pura River, tributaries of Pyasina River) and 2 Arctic lemmings (caught along the Seliye River, tributary of Pura). Sero-positive results were noted in animals that had spent the winter there and young specimens that appeared healthy, as well as in material from fresh rodent carcasses (2 positive cases for tularemia in Arctic lemmings, whose carcasses were discovered in an Arctic fox hole next to the Seliye River). Examination of spleens by the biological method (26 biological tests) in 1972 yielded negative results. The positive serological data led us to assume that there is circulation of *F. tularensis* among lemmings in Taymyr.

In 1973, we examined the spleens of 400 lemmings in 41 biotests on white mice (2 mice for each analysis). Cultures of *F. tularensis* were isolated from Siberian lemmings in 6 biotests. White mouse death occurred on the 5th-7th day. Necropsy of laboratory animals revealed the typical pathoanatomical changes for tularemia. Impression smears of organs from mice used in the biotests showed large quantities of *F. tularensis*. Organs (lymph nodes, spleen, liver) and blood were inoculated on coagulated yolk medium and, for a control,

on beef-extract agar. After incubation for 24 h at 37° in the yolk medium, we observed growth inherent in *Francisella tularensis*; there was no growth of bacterial flora on agar. The culture showed the typical morphological signs of *F. tularensis* in smears stained according to Gram. All 6 strains were agglutinated by specific antitularemia serum (titer 1:3200) to one-half (++++) or a complete (++) titer.

Inoculation on medium with glycerin by the method of Yemel'yanova [5] revealed that the *F. tularensis* isolated from lemmings were referable to the Holarctic race; they did not ferment glycerin.

Table 1. Determination of virulence of *F. tularensis* isolated from organs of Siberian lemmings

Laboratory animals	Dosage (bacterial cells)	Numb. of exper. animals	Died within 3-15 days	Survived
Rabbits	1 billion	3	3	0
	100 million	3	0	3
	10 million	3	0	3
White rats	1 billion	5	5	0
	100 million	5	1	4
	10 million	5	0	5
Guinea pigs	10	3	3	0
	1	3	3	0
	0.1	3	1	2
White mice	10	5	5	0
	1	5	5	0
	0.1	5	2	3

The virulence of one of the cultures was tested on rabbits, white rats, guinea pigs and white mice (Table 1). *F. tularensis* isolated from Siberian lemmings did not differ from the *F. tularensis* strains isolated from other regions of the Soviet Union [4]. The other 5 strains were tested for virulence only on white rats and white mice, and they behaved similarly to the culture described above.

It must be noted that the tularemia culture was obtained from lemmings which appeared outwardly healthy. One strain of *F. tularensis* was isolated from material taken from a fresh Siberian lemming carcass.

The tularemia cultures were isolated from lemmings caught in the following habitats in Eastern Taymyr (Table 2): bottomland of Bludnaya River (5 km north of the Novorybnoye trading post) and lake-swamp troughs (4 km northeast of this inhabited center), which were low, very wet parts of the moss-lichen tundra.

In order to identify the possible vectors of *F. tularensis*, in 1973 we gathered and submitted to bacteriological examination gamasid ticks from lemming nests and from combing the animals.

Table 2. Location of lemmings and isolation of *F. tularensis* from them in different East Taymyr habitats

Habitat	Number of lemmings caught		L. sibiricus	D. torquatus	Number of isolated cultures
	absolute	%			
Regions near brooks	117	29.3	117	-	-
Bludnaya River floodplain	86	21.5	86	-	2
Lake-swamp regions	192	48.0	192	-	-
Ravines, "plakory" [turf-reinforced slopes?]	5	1.2	2	3	-
Totals	400		397	3	6 [sic]

The species composition of gamasid ticks was relatively sparse. The most numerous representatives of the nest-burrow complex are widespread species--*Hirstionyssus isabellinus* Oudem. (obligate bloodsucking tick), *Haemogasamus ambulans* Thorell (facultative bloodsucking)--and the specific parasite of lemmings, the *Laelaps lemni* Grube epizootic species.

We took for bacteriological tests gamasid ticks from the nests of lemmings--*H. isabellinus* (41%) and *H. ambulans* (33%). Before testing, the ticks were kept alive in the nests with maintenance of the proper microclimate. Rodent nests were collected in the same habitats where lemmings were trapped. The ticks were driven out by standard "thermoeclectors" [heat-jet emitters?].

A suspension was prepared from the gamasid ticks, which was given to two albino mice in a volume of 0.5 ml, subcutaneously. The mice died 6 days after infection. Necropsy revealed insignificant enlargement of regional lymph nodes, injected vessels in the subcutaneous cellular tissue, enlargement of the liver and spleen. We succeeded in detecting typical accumulations of *F. tularensis* in impression smears of the spleen stained according to Romanovsky-Giemsa, in addition to extraneous bacterial flora. When viscera were cultured on McCoy's yolk medium, no positive results were obtained due to depression of *F. tularensis* growth by the extraneous flora. We also failed to isolate a tularemia culture after the second and third passages on white mice.

Spontaneous tularemia infection of *H. isabellinus*, *H. ambulans* and *L. lemni* has not been demonstrated in the Soviet Union; it was proven experimentally that *H. isabellinus* ticks are capable of transmitting and storing the pathogen of tularemia.

Thus, isolation of *F. tularensis* from lemmings in East Taymyr is indicative of the existence of tundra (lemming) tularemia sites in the Soviet Union. Gamasid ticks are probably involved in circulation and preservation of *F. tularensis* in tundra rodent populations. The absence of other rodents in typical tundra regions (the rodent population of the typical tundra is represented only by lemmings) and presence of an extensive zone without *Ixodes* ticks are indicative of substantial distinctions of endemic tularemia sites in the Extreme North.

Conclusions

1. Tularemia in tundra rodents was demonstrated for the first time in the Soviet Union--bacteriologically in the Siberian lemming, serologically in the Siberian and Arctic lemmings.
2. The cultures of *F. tularensis* isolated from lemmings do not differ in properties from pathogens isolated in other regions of the Soviet Union.
3. Positive serological tests on Siberian and Arctic lemmings in a year of depressed rodent population and isolation of tularemia culture from Siberian lemmings in a year of growth in number of lemmings are indicative of continuous circulation of the pathogen of this infection in populations of tundra rodents.
4. Bacterioscopic detection of *F. tularensis* in gamasid ticks is indicative of the probable involvement of representatives of the nest-burrow complex in transmission of the pathogen of tularemia among lemmings in tundra sites of this infection.

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EXPERIMENTAL STUDY OF MIXED TULAREMIA INFECTION AND OMSK HEMORRHAGIC FEVER
IN MUSKRATS

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[Text] In 1963, mixed epizootics of Omsk hemorrhagic fever (OHF) and tularemia were discovered on some lakes of West Siberia (among muskrats) [3, 4 and others]. The data in the literature [1, 2, 6 and others] warrant the conclusion that some infections can, in certain combinations, cause deviations in the course of tularemia in wild and laboratory animals. From the standpoint of epizootiology and epidemiology, the possibility of aggravation of the infectious process, which would be instrumental in involving animals in the epizootiological process, as well as the possibility of manifestation of heterologous immunity to tularemia [2, 5], are of considerable interest.

We tried to track the course of each of these infections in animals under experimental conditions after combined infection with the pathogens of tularemia and OHF.

Before starting the experiments, the experimental animals (muskrats) were in the laboratory for 2 months. We did not observe any nonspecific deaths during this period. We took 18 muskrats for each experiment. The same number of control animals was infected separately by the same doses of pathogens of OHF and tularemia.

For infection, we used the Balangul' strain of OHF, which had been submitted to numerous passages (log LD₅₀/ml was 8.5 with intracerebral infection) and virulent *F. tularensis* strain No 503. OHF virus was injected subcutaneously in the inguinal region of the left hind leg in a dosage of 100 LD₅₀/ml, and *F. tularensis* subcutaneously in the right hind leg in a dosage of 1 bacterial cell (according to the optical standard of the State Institute for Standardization and Control of Medical Biologicals). We sacrificed 2 animals 6 h after infection, then the same number every other day to the end of the experiment, after having collected blood from the heart for virological, microbiological and serological tests. The animals were dissected, and cultures on yolk medium were made of lymph nodes and parenchymatous organs. In addition, we prepared impression smears for bacterioscopy and testing by the method of

fluorescent antibodies (MFA). Organs were used for biological tests on white mice (weighing 6-8 g), which were infected intracerebrally for demonstration of virus. We determined presence of antibodies to OHF virus in muskrat blood using the hemagglutination depression reaction and to *F. tularensis* with the agglutination reaction. The results of blood tests on the muskrats before the experiment were indicative of absence of antibodies to these pathogens.

Table 1.
Results of virological tests on muskrats when infected with pathogens of OHF and tularemia

Infection	Day of post-infection examination	Blood dilution at which virus was isolated	Titer of antibodies to OHF virus found in animals	
			№ 1	№ 2
Simultaneous	6 h	0	0	0
	1	10	0	0
	2	10 ⁻¹	1:20	1:20
	3	10 ⁻²	1:40	1:40
	4	10 ⁻³	1:80	1:80
	5	10 ⁻⁵	1:80	1:40
	6	10 ⁻⁵	1:20	1:40
	7	10 ⁻⁵	1:80	1:160
	8	10 ⁻³	—	1:40
Successive	3	10 ⁻⁶	—	1:10
	4	10 ⁻⁶	1:20	1:20
	5	10 ⁻⁶	1:20	1:10
	6	10 ⁻⁵	1:40	—
	7	10 ⁻⁴	1:10	1:20
	8	10 ⁻⁴	1:160	1:160
	9	10 ⁻³	1:80	—

Note: 0--no virus isolated from whole blood; no antibodies found in 1:10 serum dilution.

In the biotest on white mice, the pathogen of tularemia was demonstrable in the inguinal lymph node 6 h after infection, in the submaxillary lymph node after 1 day and in the spleen and blood after 2 days. Thereafter, we were able to demonstrate it in all examined organs. Examination of the preparations by the MFA confirmed the results of the biotests (Table 2). Serologically, antibodies to *F. tularensis* in a titer of 1:10 (++) were demonstrated 8 days after infection.

Dissection of animals 6 h after infection failed to reveal visible changes in organs; at the site of injection of infectious material, there was dilatation of blood vessels on both the left and right sides after 1 and 2 days; enlargement of the spleen was found after 3 days. Thereafter, there was progression of visceral changes. After 5 days, necrotic foci appeared in the spleen, the lymph nodes were considerably hyperemic and enlarged. Subsequently, necrotic foci also appeared in the liver, and the intestine showed petechial hemorrhages.

In the first experiment, the animals were infected with the above-mentioned pathogens. Clinical signs of disease appeared on the 6th-7th day. The animals became sluggish, inactive and presented suppuration of the eyes; some muskrats developed paresis of the extremities, and one died 8 days after infection.

The virus was demonstrated in whole blood 1 day after infection. Viremia reached a maximum (10⁻⁶) after 3 days. At the end of the experiment virus titer in blood constituted 10⁻³. Virus-specific antibodies were found in a titer of 1:20 2 days after infection. At the end of the experiment antibodies were demonstrable in a titer of 1:40 (Table 1).

Direct inoculation of blood and organs on coagulated yolk medium enabled us to isolate cultures of *F. tularensis* 3 days after infection from the right inguinal and submaxillary lymph nodes and spleen, but only after 6 days from blood. Thereafter, the pathogen was isolated regularly from these organs to the end of the experiment.

In addition to the above-mentioned changes in internal organs, 8 days after infection we demonstrated necrosis of the inguinal lymph node.

Table 2. Results of tests for tularemia after infection with pathogens of OHF and tularemia

Infection	Postinfect. day of testing	Presence(+) of <i>F. tularensis</i> when tested							
		bacteriolog.				biologically			
		1	2	3	4	1	2	3	4
Simultaneous	6 h					+			+
	1					+	+		+
	2					+	+		+
	3	+	+	+		+	+	+	+
	4	+	+	+		+	+	+	+
	5	+	+	+		+	+	+	+
	6	+	+	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+
Successive	6 h					+	+		+
	1					+	+		+
	2					+	+	+	+
	3	+				+	+	+	+
	4	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+

Key: 1) inguinal lymph node 3) spleen
 2) submaxillary lymph node 4) blood

A comparison of the results of virological, microbiological and serological tests on animals with single and mixed infection shows that viremia differed only in dynamics of development, but at the end of the experiment it was the same in both groups of animals (10^{-3}). Delayed production of antibodies to OHF virus was a distinction of mixed infection: after 8 days, the antibody titer was 1:40, whereas in animals infected only with OHF it was 1:160. There was more intensive development of bacteremia in the case of mixed infection: *F. tularensis* was demonstrated in the spleen 1 day sooner and in blood 2 days sooner than with monoinfection.

In comparing these data to the morphological changes in animal organs, we must mention the more serious course of mixed infection. Muskrats died of tularemia 2 days earlier and of OHF 3-5 days earlier. In our opinion, this was caused by the more serious course of OHF, rather than tularemia, although the opposite could have been assumed according to microbiological data (early appearance of bacteremia). Morphologically, this was indicated, first of all, by 2-day delay in appearance of specific signs of tularemia in animals infected with both pathogens. At the same time, there were more serious vascular lesions: earlier appearance of inflammation and involvement in the process not only of small, but large vessels. The slower production of antibodies to OHF virus in the case of mixed infection also confirmed the assumption of more severe course of OHF.

Thus, concurrent infection with pathogens of OHF and tularemia elicited parallel development of both infections in muskrats, a more severe course of

infection related to more intensive vascular involvement under the effect of OHF virus and inhibition of tularemia due to development of OHF.

In the second experiment, the muskrats were infected with OHF virus and, 3 days later, *F. tularensis*. Clinical symptoms of disease appeared in the animals 3 days after infection with *F. tularensis*, i.e., 6 days after infection with OHF virus, and they were similar to symptoms observed in the first experiment. One muskrat died 5 days after *F. tularensis* infection and 4 more 6 days after infection. The rest of the animals presented paresis and paralysis of the limbs on the same days.

Virological blood tests revealed that viral titer reached a maximum (10^{-6}) 3 days after OHF infection and held at this level for another 2 days, after which it dropped to 10^{-4} and remained on this level to the 8th day, dropping to 10^{-3} by the end of the experiment (see Table 1).

Bacteriological examination enabled us to isolate a culture of *F. tularensis* 3 days after infection from the inguinal lymph node. Thereafter, the pathogen of tularemia was regularly isolated from all organs examined (see Table 2).

With the biotest on white mice, we were able to detect *F. tularensis* in the inguinal and submaxillary lymph nodes 1 day after infection, in the spleen 2 days after and also in blood 3 days after. Thereafter, *F. tularensis* was consistently isolated from all mentioned organs and blood to the end of the experiment. The results of the test by the MFA essentially confirmed the results of the biological method (see Table 2).

The macroscopic changes in internal organs upon dissection 6 h after infecting the muskrats with *F. tularensis* were found to be the typical ones for the initial stage of development of OHF: hyperemia of the left inguinal lymph node, dilatation of intestinal blood vessels, petechial hemorrhages in the intestine and other organs. After 1 day, similar changes were demonstrated in the organs, whereas after 2 days we also found changes in lymph nodes on the right side, in the form of enlargement and hyperemia. In addition, the spleen was enlarged, fine necrotic sites appeared in the intestine, hepatization in the lungs and vasodilatation in the brain (brain tissue was plethoric on section). Thereafter, the visceral changes progressed, with prevalence of changes inherent in OHF in some muskrats, changes inherent in both OHF and tularemia in others.

Thus, our investigation revealed that infection of muskrats first with OHF virus and 3 days later with *F. tularensis* virtually failed to elicit deviations in development of viremia, as compared to the control; as with control infection of muskrats with OHF virus, we observed elevation of blood virus titer to maximum values 3-4 days after infection, after which it gradually declined. The difference between experimental and control titers was insignificant 9 days after infection.

Our study of development of the tularemia process enabled us to demonstrate some delay in its development on the 1st postinfection day, which was confirmed bacteriologically, biologically and MFA results. This is attributable

to the fact that, in this case, as in the first experiment, there was depression of the infectious process elicited by *F. tularensis* due to development of OHF. Macroscopically the visceral changes were typical of both OHF and tularemia.

Consequently, infection with the pathogen of tularemia 3 days after administration of OHF virus aggravated the course of the infectious process and led to death of animals due to mixed infection; the muskrats died 2 days earlier than in the control when infected with OHF virus alone and 4-5 days sooner than in the control when infected only with *F. tularensis*.

Conclusions

1. Muskrats developed mixed infection when infected with the pathogens of tularemia and Omsk hemorrhagic fever.
2. The more severe course of mixed infection was attributable to development of vascular lesions under the influence of OHF virus.

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DIAGNOSTIC PROPERTIES OF BRUCELLOSIS AND TULAREMIA ANTIGENIC ERYTHROCYTE
DIAGNOSTICUMS

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[Text] Among the serological methods used to diagnose infectious diseases and
examine the qualitative aspects of the immunological response, the passive
hemagglutination reaction (PHAR) and various modifications thereof have gained
deserved recognition. However, this test can be used in broad practice only
for infectious diseases, for the diagnosis of which commercial production of
standard erythrocyte diagnosticums has been set up. As for brucellosis and
tularemia, methods of recovering the appropriate preparations have not yet
gone beyond the stage of laboratory testing.

Vershilova et al. [1] demonstrated that antigens recovered from brucella by
different methods are not the same in either serological activity or capacity
to sensitize erythrocytes. The polysaccharide extracted from brucella by the
Boivin method and then submitted to alkaline hydrolysis is the most suitable
for sensitization of erythrocytes. Antigenic erythrocytic diagnosticum for
tularemia was also obtained by the Boivin method [4]. A test of the diagnos-
tic features of these diagnosticums was indicative of their high sensitivity,
specificity and stability. However, the relatively complicated and time-
consuming Boivin method of recovering specific antigens, as well as the insigni-
ficant yield obtained, render it unacceptable to use these methods for
production of brucellosis and tularemia antigenic erythrocyte diagnosticums
on a commercial scale.

For this reason, we have developed methods of obtaining antigenic erythrocyte
diagnosticums for brucellosis and tularemia, which can be recommended for
commercial production. We are submitting here the results of studying the
diagnostic qualities of these products.

For the PHAR for brucellosis, we used erythrocytes loaded with a lipopoly-
saccharide-protein complex [5] from Br. abortus vaccine strain 19 by means
of secondary sodium alkylsulfate ($R = OSO_3 = Na$). The titer in the PHAR of
different series of formalin-treated erythrocytes, which were sensitized with

the above antigen, with homologous hyperimmune agglutinating serum ranged from 1:51,200 to 1:102,400 (in the agglutination test the titer was 1:3200). The diagnosticum retained its activity for 8 months and, in dry form, up to 1 year (observation period). In all, we prepared 37 series of antigenic erythrocytic diagnosticum for brucellosis, and the total amount thereof was about 46 g. The quality of the produced series of diagnosticum was stable.

We tested the specificity of diagnosticum in the PHAR with hyperimmune heterologous sera and with serum from people and animals known to be in good health, as well as from people with various infectious diseases. A positive reaction in low titers was obtained with most standard hyperimmune heterologous sera (1:10-1:80), and it was positive up to a titer of 1:320 only with cholera and tularemia sera. In the latter case, the positive result could be attributed to the fact that the structure of surface antigenic determinants is the same in the pathogens of brucellosis, tularemia and cholera. In the PHAR with serum from people and animals immunized against tularemia and those who had had this infection, using brucellosis antigenic erythrocyte diagnosticum, the titer did not exceed a dilution of 1:80. In the PHAR with 646 sera from people known to be in good health, as well as animals, the results were negative in all cases. Testing of 122 blood serum samples from people with various infectious diseases revealed positive reactions in 52 cases, with titers of 1:10 to 1:80. All these data served as grounds to consider serum dilution of at least 1:100 to be a diagnostic titer for the PHAR.

We conduct the studies and evaluated the diagnostic properties of different series of brucellosis erythrocyte diagnosticum using the PHAR on blood serum of people and animals at different stages of the vaccinal and infectious brucellosis process. In order to compare the diagnostic value of the PHAR to conventional serological tests, as well as check the reliability of PHAR results, we ran concurrently the agglutination reaction (RA), complement fixation reaction (CFR) and reaction of prolonged complement fixation (RPCF) using conventional methods and standard commercial antigens. In all, we tested 8515 serum samples, 1136 of which were from humans, 1196 from guinea pigs and 6180 from cattle.

In all groups, the incidence of positive PHAR results was higher than with use of AR, CFR and RPCF, which was indicative of the greater sensitivity of PHAR (Table 1). At the same time, on the basis of the percentage of positive PHAR, CFR and RPCF, it can be stated that these reactions were virtually equivalent in diagnostic qualities; however, the appreciable difference in labor required to run these tests was in favor of the PHAR.

In the course of our study, we found that normal hemagglutins (in titers of 1:10 to 1:80) to unsensitized ram erythrocytes were encountered in blood serum from healthy, sick and immunized farm animals. Preliminary adsorption of such sera by erythrocytes eliminated completely the positive reactions starting with a dilution of 1:40. In order to reduce time and labor involved in the PHAR, one can test serum without preliminary adsorption, but with mandatory checking of the reaction with unsensitized erythrocytes and the tested serum. If control erythrocytes are agglutinated by the tested serum, it must be adsorbed and retested. In this regard, it should be noted that, even with a

negative check with unsensitized erythrocytes and unadsorbed tested serum, the result in the PHAR could be 1-2 dilutions higher due to the fact that, in this serum, normal hemagglutinins contained in subliminal doses emerge as an additional load to specific hemagglutinins and increase the reaction titer.

Table 1. Results of serological testing of different groups of animals for brucellosis

	Animal group	Number of animals tested	PHAR		AR		CFR	RPCF
			positive results, %	geometric mean of titers	positive results, %	geometric mean of titers	positive results, %	
At farms with no brucellosis problem	Cattle [large]							
	50 days after immunization	480	98,3	898	96,0	179	97,8	
	1 year after immunization	846	84,0	184	47,0	63	80,0	
	2 years after reimmunization	574	50,3	106	25,0	57	33,7	
Farms with brucellosis problem	Cattle [large]	1527	58,9	326	34,3	98	53,8	
	Goats and sheep	1960	69,4	364	57,2	116	62,3	62,9

In our tests, the mean PHAR titer without preliminary adsorption constituted 1:686 for 248 serum samples with negative checks with unsensitized erythrocytes. After adsorption of sera with 50% suspension of formalin-treated erythrocytes, the mean titer in the PHAR dropped to 1:296. These data indicate that, when running the PHAR, adsorption of serum by erythrocytes should be one of the mandatory factors that rules out nonspecific readings and yields more reliable data.

We then determined the diagnostic value of the PHAR with the above-mentioned diagnosticum in studying the immunological structure of people living in areas with a brucellosis problem. We screened 1026 people in all: 160 with chronic brucellosis, 574 who were immunized against brucellosis and 292 who were not immunized (Table 2). In 89% of those who were immunized against brucellosis, the Wright reaction was negative, whereas when these sera were tested in the PHAR, positive reactions were obtained with titers that were reliable in specificity (over 1:100) in 32% of the cases. We also demonstrated positive reactions among people who did not receive brucellosis inoculations. This indicated that the working and living conditions of these people had led to brucellosis infection. These data warrant recommending the PHAR for studies of immunological structure of the public in order to detect infection in groups of people who were exposed to different conditions of contact with sources of Brucella.

Testing by the PHAR of 160 samples of blood serum from people with chronic brucellosis yielded positive results in 64%, whereas the Wright reaction was positive in only 8%.

We also tested the antibody neutralization reaction (ANR). We used the method of Levi and Momot [2] to run this test. Specificity was checked with 5 species of heterologous microorganisms (pathogens of tularemia, cholera, plague, typhoid fever, dysentery). It was established that the brucellosis erythrocyte diagnosticum detected 2 million to 1 million homologous bacterial cells in the ANR. Positive results were not obtained in any cases in the tests with heterologous species of microorganisms, which was indicative of specificity of this diagnosticum.

Table 2. Results of serological testing of people living in areas with brucellosis problem

Groups screened	Quantity of samples tested	PHAR		AR	
		posit. %	titer geom. mean	posit. %	titer geom. mean
Livestock farmers:					
total	524	36,2	352	11,8	100
immunized	472	37,7	486	11,8	124
not immunized	52	23,0	217	11,5	78
People in other occupations:					
total	342	4,3	156	3,2	45
immunized	102	8,8	166	4,9	42
not immunized	240	2,5	147	2,5	47
Patients with chronic brucellosis	160	64,3	738	8,1	235

When we used the ANR to test organs of 246 guinea pigs, which were infected with different doses of *Brucella*, the results were positive in 23% of the cases, with negative results of bacteriological test, and in 48% of the cases a positive ANR coincided with culturing of *Brucella* from organs. The range of delay in the reaction was 1:10 to 1:160.

Thus, our testing of the brucellosis antigenic erythrocyte diagnosticum, which we prepared, in a mass screening of people and animals confirmed its stability, high sensitivity and specificity. A check of the diagnostic value of the diagnosticum using the ANR was also indicative of its high sensitivity and specificity. All this can serve as grounds for more extensive use of this product in practical brucellosis diagnostics.

To prepare the antigenic erythrocyte diagnosticum for tularemia, we used a specific antigen recovered by treating commercial tularemia diagnosticums with a surfactant--secondary sodium alkylsulfate. For this purpose, we added 2% of secondary sodium alkylsulfate to the tularemia diagnosticum in its original state and left it at room temperature for 1 h, mixing it periodically. The suspension was then centrifuged for 1.5-2 h at 6000 r/min; the supernatant was decanted and combined with an aliquot of 5% suspension of erythrocytes treated with formalin according to Vaynbakh in the modification of Men'shov and Shmutter [3]. After exposure to 37° for 16-18 h, the erythrocytes were eluted 3 times in saline, with centrifugation at 1500 r/min for 10 min. The sediment of erythrocytes was resuspended in saline to a 2.5% concentration and used in the PHAR. We added 5% formalin to the diagnosticum as preservative.

The hemagglutination reaction with erythrocytes sensitized by tularemia antigen as described above and standard agglutinating tularemia serum was positive in a titer of 1:204,800 (in the AR the titer was 1:1600). The original activity of the 26 series of antigenic erythrocyte diagnosticums for tularemia that we prepared did not diminish for 7-8 months and, in a dry state, for up to 1 year (duration of observation period).

We determined the diagnostic properties of the tularemia antigenic erythrocyte diagnosticum in a comparative test of serum obtained from 486 animals with experimental tularemia, using the AR and PHAR. In all cases, the serum titer was 7-8 dilutions higher in the PHAR than AR. The range of specificity of the diagnosticum was tested with the PHAR using blood serum from people known to be in good health, as well as with various infectious diseases (brucellosis, typhoid fever, dysentery, etc.). In all cases, the results of testing 236 serum samples by the PHAR with tularemia diagnosticum were negative.

We also checked the specificity of the diagnosticum in the ANR with homologous and different species of heterologous microorganisms (pathogens of brucellosis, cholera, plague, typhoid fever, dysentery). It was established that the tularemia erythrocyte diagnosticum detected 4 million to 1 million homologous bacterial cells in the ANR. At the same time, the results of the ANR with different species of heterologous microorganisms were negative in all cases, which was indicative of specificity of this diagnosticum.

Use of the ANR to test organs of 164 animals experimentally infected with tularemia yielded a positive result in 86 cases: a delayed reaction was observed in dilutions of 1:10 to 1:256, with negative results in the bacteriological test. Use of the ANR to test 524 pellets from birds collected in an endemic site for tularemia yielded positive reactions in 34 cases, in dilutions of 1:10 to 1:164.

Thus, the antigenic erythrocyte diagnosticum for tularemia is highly sensitive, specific and stable. A comparative study of diagnostic properties of diagnosticum prepared by our method and diagnosticum produced by Meshcheryakova revealed that these products are equal in sensitivity and specificity with the PHAR and ANR; however, as we have indicated above, extraction of antigen by the Boivin method is relatively difficult and time-consuming, whereas our method is simpler and yields much more specific antigen from bacterial mass, which ultimately lowers the cost of the product.

Conclusions

1. The antigenic erythrocytic preparations for diagnosing brucellosis and tularemia, which were produced by our method, were found to be highly sensitive, specific and stable in a mass-scale serological study of people and animals at different stages of the vaccinal and infectious process.
2. The simplicity of recovering specific antigens to be used for sensitization of formalin-treated erythrocytes and consistency of the results of using the methods of preparing antigenic erythrocyte diagnosticums enables us to recommend the described methods for use on an industrial [commercial] scale.

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DETECTION OF FRANCISELLA TULARENSIS IN ANIMAL ORGANS AT EARLY STAGES OF DEVELOPMENT OF INFECTION

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[Article by N. G. Olsuf'yev, Ye. V. Ananova, I. S. Meshcheryakova and R. A. Savel'yeva, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The existing test methods make it possible to detect the pathogen of tularemia in infected animals with adequate accuracy. The biological test on white mice is the most sensitive and reliable method of detecting *F. tularensis*. However, even with a large infective dose, the mice die only on the 3d-4th day, whereas with use of a small number of bacteria for infection they die on the 7th-9th day or later.

In order to speed up the answer, one can sacrifice tested animals at the early stage of development of the infectious process, rather than wait for them to expire, and examine their organs for the pathogen. In this case, the results can be obtained several days sooner. The method of fluorescent antibodies (FA), which was developed to apply to testing for *F. tularensis* by several authors [1, 2, 4, 5, 10, 11, 13], must be deemed particularly promising for this purpose. The advantage of this method is that it is quite sensitive and results are obtained quickly (within 1.5-2 h). It was previously shown [1] that with the FA method the bacterium can be detected in white mouse organs 4 days after being infected subcutaneously with 10 bacterial cells. The culture method can also be used to isolate the pathogen within the same time.

However, there are indications in the literature of earlier detection of *F. tularensis* in white mouse organs--3 days [11] and even 18 h [3] after infection with a minimal dose.

In recent years, serological reactions based on the use of erythrocyte diagnosticums have been used with success for demonstration of *F. tularensis* in animal organs. The antibody neutralization reaction (ANR) in the presence of antigenic erythrocyte diagnosticum was developed [6, 8], and it was shown that the minimum demonstrable dose of antigen constitutes 1 million bacterial cells. Use of an antibody erythrocytic diagnosticum [9] in the passive hemagglutination reaction (PHAR) permits demonstrations of thousands of bacterial cells.

Our objective here was to detect *F. tularensis* at the early stages of the infectious process (with minimal infection doses) using a set of bacteriological and serological methods.

We used 156 white mice weighing 16-18 g in the experiments. The animals were infected subcutaneously with strains No 503/834 (Holarctic race) and Schu (Nearctic race), which were given in 3 doses: 1, 10 and 100 bacterial cells according to the standard of the Institute imeni Tarasevich. The cultures to be used for infection were grown for 48 h on coagulated yolk medium. The animals were sacrificed 1, 2, 3 and 4 days after infection. At each tested time and for each infective dosage we used 5 (for the Schu strain) or 8 (for strain No 503/834) animals.

Upon dissection of sacrificed animals, we recorded the condition of internal organs and examined tissue at the site of infection, including regional lymph nodes and the spleen. We used the following methods: inoculation on nutrient media, immunofluorescence test, PHAR with antibody and ANR with antigenic erythrocyte diagnosticums. Cultures were made on coagulated yolk medium and Yemel'yanova's medium (fish-yeast agar with cystine, glucose and 10% defibrinated horse blood).

The cultures were kept at 37° for 10 days, observing growth and time of its appearance.

For the immunofluorescence study, we prepared impressions of a tissue section from the site of infection, together with regional lymph nodes and spleen, on a thoroughly defatted slide. In addition to the impressions, we prepared smears from homogenates of the same tissues. The preparations were air-dried at room temperature, then fixed in 96° alcohol for 20 min. Preparations from mice infected with different doses and at different times were fixed separately to preclude the possibility of accidental transfer of some bacteria from one slide to another. The fixed preparations were treated with a mixture of fluorescent tularemia serum and bovine albumin labeled with rhodamine sulfofluoride, used in one dilution less than the working dilutions (both are produced by the Institute of Epidemiology and Microbiology imeni Gamaleya). This mixture was applied to a slide with an impression and it was "stained" for 20 min in a humid chamber at room temperature. Subsequent treatment of the preparation and examination were performed by conventional methods. The presence of a few fluorescent bacteria in several fields of vision was considered as the minimal criterion of a positive result.

For testing in the PHAR and ANR, tissue from the infection site and the spleen were ground in a porcelain mortar, with 2 ml saline added; we arbitrarily considered the initial suspension to be a 1:40 dilution for tissue from the infection site and 1:20 for the spleen. For the purpose of decontamination and fuller extraction of antigen, the obtained suspensions were boiled in a water bath for 10-15 min.

The supernatant was filtered through an asbestos filter, and for removal of heterologous antibodies to ram erythrocytes the filtrates were adsorbed, adding 50% formalin-treated erythrocyte suspension at the rate of 0.1 ml/ml test-tube contents. After leaving it to stand (for at least 1 day), the supernatant was tested using the ANR with antigenic diagnosticum and PHAR with antibody diagnosticum using conventional methods.

We failed to demonstrate differences in time of detection of *F. tularensis* in mice between the experiments with No 503/834 and the Schu strain (see Table); for this reason, the results submitted below were summated.

Results of testing white mice for *Francisella tularensis*

Time of testing	Infective dose (quantity of bacterial cells)	Strain No 503/834								Schu strain							
		tissue from site of infection				spleen				tissue from site of infection				spleen			
		culture	FA	PHAR	ANR	culture	FA	PHAR	ANR	culture	FA	PHAR	ANR	culture	FA	PHAR	ANR
1st day	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2d day	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3d day	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4th day	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key: +) positive results in all cases
 ±) positive results in some of the cases
 -) negative results in all cases

The pathogen was not demonstrable in any of the cases 1 day after infection with 1 bacterial cell. After infection with 10 bacteria, the pathogen was found in only 2 out of 13 cases and after infection with 100 bacteria, in 6 out of 13, mainly in culture and partially by the FA method. There was considerable rise in incidence of demonstration of the pathogen after 2 days, but it constituted 100% only after infection with 100 bacterial cells, whereas with use of 1 bacterial cell there were 5 findings out of 13 cases and with use of 10 bacterial cells 12 findings out of 13. At this time, the pathogen was also demonstrated mainly in culture, much less by the FA method and in only 2 cases (out of 13) was the PHAR positive after infection with 100 bacteria. After 3 days, the pathogen was demonstrated in all tested mice infected with 10 and 100 bacterial cells (13 for each dose) and in 11 out of 13 mice infected with 1 bacterial cell. There was an increase not only in number of positive findings, but in amount of pathogen in the examined tissue.

After 4 days, the pathogen was found in all mice, regardless of infective dosage (total of 38 mice tested) and by virtually all of the methods used. Very high titers were demonstrable with the PHAR and ANR, up to 1:320,000 and 1:5120, respectively. At this time, many fluorescent bacteria were demonstrated by the FA method.

In most cases, *F. tularensis* was found both at the site of infection and in the spleen, particularly at the late testing times. But, in some cases, the pathogen was isolated only from tissue at the site of infection and seldom only from the spleen.

The largest number of positive results were referable to cultures, and growth was demonstrated slightly more often on Yemel'yanova's medium than coagulated yolk medium. In all, we obtained 354 cultures, 195 in the former medium and 159 in the latter. Growth was usually observed after 2-3 days, but in a number of cases later and, occasionally, as early as 1 day after inoculation. The FA method is in second place according to incidence of demonstration of the pathogen; with it, *F. tularensis* was found in 101 cases. Positive results with the PHAR and ANR were observed from the 3d day on (very seldom after 2 days), and the most consistently when large doses--10 and 100 bacterial cells--were used for infection.

It should be mentioned that when the mice were infected with the strain of the Nearctic race we observed somewhat less accumulation of the pathogen in them, as compared to infection with strain No 503/834 of the Holarctic race. This was manifested by a smaller quantity of bacteria according to the FA method, as well as results of PHAR and ANR.

Examination of material from mice infected with 10 and 100 bacteria of the Schu strain showed positive reactions only in some cases after 4 days, and the titers were considerably lower than in mice infected with No 503/834. Thus, the PHAR with tissue from the infection site was positive in less than half the mice in titers of 1:80-1:640, and with spleen tissue in titers of 1:40-1:2560. The ANR with tissue from the site of infection was positive in titers of 1:60-1:320. These findings could be related to the distinction of pathogenesis of infection when using *F. tularensis* of different geographic races [7].

As we know, corticosteroids lower natural resistance, creating the conditions for rapid reproduction and massive contamination of organs and tissues with a pathogen. In view of this, we conducted an additional experiment on animals infected with the same strains, but after preliminary hydrocortisone treatment. We used the Hungarian product of the Richter Firm in a dosage of 5 mg per white mouse, giving it once hypodermically 5 h before infecting them with *F. tularensis*.

In this experiment, we used two infective doses, 1 and 10 bacterial cells, since with 100 bacteria there was intensive development of the pathological process with massive contamination of organs and tissues with the pathogen even in mice untreated with hydrocortisone.

Otherwise, we used all of the above-described methods in this experiment.

As shown by the findings, there was no appreciable difference between mice treated with hydrocortisone and intact animals with regard to time of demonstration of the pathogen. Our results coincide with data in the literature [11]. Some differences were noted only in the group of animals infected with the Schu strain, as compared to the analogous group that was not given hydrocortisone. Thus, the FA method demonstrated more accumulation of the pathogen than in animals not treated with hydrocortisone. This was also reflected in the PHAR and ANR results. These reactions were positive in all experimental mice on the 3d and 4th days, and in high titers: with tissue from infection site ANR in titers of 1:160-1:1280 and PHAR 1:1280-1:20,000, with spleen tissue 1:160-1:2560 and 1:320-1:80,000, respectively.

Analysis of our data shows that, with hypodermic infection of white mice with minimal doses of 1 and 10 bacterial cells (according to State Control Institute of Medical Biologicals imeni Tarasevich) of Holarctic or Nearctic race, the pathogen was consistently demonstrable in animals with all methods used 3 days after infection, positive findings were irregular after 2 days and isolated after 1 day. With use of the method of FA of impression smears, a larger number of positive results was obtained than with testing of smears of organ homogenates.

Thus, our data concerning the time of detection of the bacterium in white mouse organs in the case of low infective doses are consistent with the results of Franek [12] and do not confirm the data of Gulida [3], who recommended that mice be tested in analogous cases 1 day after infection.

Conclusions

1. Bacteriological and serological tests make it possible to consistently demonstrate *F. tularensis* in white mouse organs 3 days after hypodermic infection with minimum doses; earlier detection of the pathogen is possible in isolated cases and only by methods of culture and FA.
2. Use of serological tests with erythrocytic diagnosticums provides a quantitative characteristic: accumulation of bacteria in animal organs.
3. We failed to demonstrate appreciable differences between infection with Holarctic and Nearctic strains of *F. tularensis*, as well as pretreatment of mice with hydrocortisone, with regard to time of detection of the pathogen.

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USE OF IMMUNOFLUORESCENCE METHOD FOR DETECTION OF F. TULARENSIS IN DEVELOPING CHICK EMBRYOS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 1, Oct 75 (manuscript received 5 Aug 74) pp 18-22

[Article by R. I. Kudelina and Ye. V. Ananova, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] In recent years, the immunofluorescence method has been considered rather promising as a method of high-speed laboratory diagnosis, which permits rather reliable identification of pathogens of many infectious diseases.

The feasibility of using the immunofluorescence method for detection of F. tularensis has been demonstrated in a number of works, by both Soviet and foreign authors [1-5, 7, 10, 11, 14, 15, 17]. With this method, F. tularensis can be demonstrated in impression smears, histological sections of organs and tissues from animals infected with F. tularensis, smears of different dilutions of pure and mixed cultures, and in other preparations. The lowest concentration of F. tularensis consistently demonstrable by the immunofluorescence method is 1 million bacterial cells (according to the standard of the State Control Institute of Medical Biologicals imeni L. A. Tarasevich--GKI) per ml tested suspension. In lower concentrations, the pathogen is demonstrable but not in 100% of the cases. The sensitivity of this technique is close to that of the method of making cultures of animal organs on nutrient media [3].

Our objective here was to determine whether it is possible to use the immunofluorescence method for detection of F. tularensis in yolk sacs of developing chick embryos. The need for such studies is attributable to the fact that the usual staining methods according to Gram, Romanovsky-Giemsa and Zdrodovskiy are not very suitable for this purpose. This is related to the fact that F. tularensis does not have an elective color, whereas the presence in yolk sacs of significant amounts of lipids, phosphoproteins and pigment groups makes it difficult to differentiate between F. tularensis and tissular elements. Most researchers concerned with cultivation of F. tularensis in chick embryos assessed bacterial reproduction according to titration of a suspension of yolk sacs in mice or by inoculation on solid nutrient medium [13, 18]. These methods are rather sensitive, but they do not permit rapid determination of presence of F. tularensis in chick embryos.

Using the immunofluorescence method (we were unable to find any works dealing with use thereof for detection of *F. tularensis* in chick embryos in the literature available to us), we tried to identify the tissues and fluids, in which the pathogen of tularemia multiplies, track the dynamics of its accumulation as related to day of embryo death and magnitude of infective dose.

In our work, we used standard virulent strain No 503 (Holarctic race) and Schu (Nearctic race). We conducted the studies on developing chick embryos incubated for 8-10 days. Preliminary experiments revealed that embryos of this age are the most suitable model for cultivation of *F. tularensis*. Infection was produced in the cavity of the yolk sac by the conventional method [12]. For infection, we used bacterial suspensions prepared by suspending in saline 24-h cultures of *F. tularensis* grown on solid medium of fish hydrolysate with cystine, glucose and blood. The cultures were standardized to 10^9 bacterial cells per ml (according to GKI standard). With the obtained suspension, we prepared successive 10-fold dilutions (from 10^9 to 1 bacterial cell/ml). Each dilution, in a volume of 0.5 ml, was injected in 6-7 embryos. Concurrently, we checked the material for *F. tularensis* by means of culturing 0.1 ml of each dilution on a medium of fish hydrolysate with cystine, glucose and blood, or coagulated yolk medium.

After infection, the embryos were incubated at 37° at high relative humidity. The eggs were examined daily. Dead embryos were dissected the same day. We prepared impressions simultaneously on two thoroughly defatted slides, which were air-dried at room temperature, of pieces of yolk sacs slightly pressed down on filter paper. One of the impressions, without prior fixing, was stained with a mixture of Giemsa stain and methyl alcohol taken in equal parts [6, 9]. With this staining method, *F. tularensis* presented a violet color and could be clearly distinguished against a light lilac background. The other impression was fixed for 20 min in 96° ethyl alcohol, then stained with fluorescent equine tularemia serum (series No 859 produced by the Institute of Epidemiology and Microbiology imeni N. F. Gamaleya). For staining, we used the conventional direct method of fluorescent antibodies [2]. In order to "quench" nonspecific fluorescence, the preparations were treated with a mixture of fluorescent serum and bovine albumin labeled with rhodamine sulfo-fluoride, which were taken in 1 dilution lower than the working dilution, and applied to the preparations. Staining was continued for 20 min, after which the preparations were washed in 0.15 M NaCl for 10 min at a time, twice, changing the solution. Before examination, a drop of solution containing 1 part 0.15 M NaCl and 9 parts glycerin was applied to the preparation, and it was covered with a cover glass.

The preparations were examined in the incident light of an ML-2 fluorescence microscope using an immersion objective and system of light filters.

In positive cases, when the preparation contained *F. tularensis*, bacteria with specific bright green-yellow fluorescence were demonstrable on a dark or reddish (from rhodamine) background. Most often, there was bright fluorescence of the entire bacterial cell, rather than only the rim, as in most bacteria. This is related to the fact that *F. tularensis* has a mucous capsule-like sheath that adsorbs fluorescent antibodies on its surface, which prevents distinct visibility of the fluorescent rim and dark center of the

cell. We failed to demonstrate any specific fluorescence in any impressions of yolk sacs from a normal chick embryo of the same age taken as a control.

A study by the immunofluorescence method of accumulation of *F. tularensis* in tissues and fluids of an embryo (9-days old) that perished on the 4th day after infection with 10^9 bacterial cells of virulent strain No 503 revealed that a significant quantity of bacteria was contained in all fields of vision of the yolk sac impression smear, irregular amount of bacteria (ranging from considerable to isolated cells) in the chorioallantois membrane, isolated bacteria in allantois fluid and a significant number of bacteria in the extra-embryonic fluid after dissection of the yolk sac. Analogous findings had been reported by other researchers with regard to accumulation of *F. tularensis* in yolk sacs [13, 16].

Studies of the dynamics of accumulation of *F. tularensis* in yolk sacs as a function of time of death of embryos infected with virulent strain No 503 (10^8 - 10^7 bacterial cells) revealed the following: on the first day of deaths, bacteria were not demonstrable or else there were isolated bacteria in isolated fields in half the cases; on the 2d day, the quantity of bacteria increased, and they were demonstrable in all fields of vision, from isolated ones to a considerable number in different preparations. On the 3d day, we observed considerable quantities in all preparations. As a rule, the embryos did not survive to the 4th day. The surviving ones were dissected, and isolated bacteria were demonstrable in isolated fields. The latter is apparently related to individual embryo sensitivity to *F. tularensis*.

Results of using luminescence and light microscopy for demonstration of *F. tularensis* in chick embryo yolk sacs

Strain	Infective dose (bacter. cells)	Embryo lethality (deaths/inf)	Average day of embryo death	Culture		Microscopy results					
				Yolk sac	Blood-film agar	Fluorescence (f)		light (l)		coincidence	
						+	-	+	-	+	-
Virulent strain No 503	10^9	7/7	2,4	+	+	7	0	6	1	6	1
	10^8	7/7	3,1	+	+	7	0	7	0	7	
	10^7	7/7	4,3	+	+	7	0	7	0	7	
	10^6	7/7	4,0	+	+	6	1	6	1	6	1
	10^5	7/7	4,0	+	+	6	1	5	2	5	1
	10^4	7/7	4,6	+	+	7	0	7	0	7	
	10^3	4/7	5,0	+	+	7	0	5	2	5	2
	10^2	4/6	6,0	-	+	6	0	2	4	2	4
	10^1	4/6	7,4	-	+	5	1	4	2	3	1
	1	3/5	6/3	-	-	3	2	2	3	2	1
Totals: absolute %		57/66				61 92,3	5 7,7	51 77,3	15 22,7	50 83,3	5 11 16,7

Studies of dynamics of accumulation of *F. tularensis* in yolk sacs as a function of magnitude of infective dose revealed (see Table) that both the percentage and time of embryo death depended on the size of the infective

dose. For example, with administration of 10^9 - 10^4 bacterial cells, embryo death was observed in 100% of the cases on the 3d-4th postinfection day, whereas a dosage of 10^3 or less elicited partial death on the 5th-7th postinfection day. Positive results for *F. tularensis* were observed in 92.3% of the cases according to fluorescence microscopy and 77.3% with light microscopy. The findings coincided in 83.3% of the cases and differed in 16.7% (positive results with fluorescence microscopy with negative data from light microscopy). There was particularly graphic manifestation of the advantage of the fluorescence method with use of doses of 10^3 or less for infection, when positive results were noted in only 50% of the cases with light microscopy and 85% with fluorescence microscopy.

The specificity of the obtained data is also confirmed by the positive results we obtained when culturing material on yolk medium and medium of fish hydrolysate with cystine, glucose and blood. As a rule, the bacterial content thereof was lower by 1-2 dilutions than in embryos. The latter was indicative of reproduction of virulent strain No 503 in embryos.

An analogous pattern was observed with cultivation of the Schu strain in embryos. After giving 10^7 - 10^3 bacterial cells, the embryos died on the 2d-3d postinfection day, with considerable accumulation of the pathogen. With use of 10^2 bacterial cells or less, the embryos survived in a higher percentage of cases. Dissection of live embryos on the 8th postinfection day or later revealed isolated bacteria in the smears, in all or some fields of vision. Fluorescence of Schu bacteria was just as bright as that of strain No 503.

Use of the fluorescence and serological method for demonstration in yolk sacs of avirulent strain No 21/400 and attenuated strain No 503 failed to yield positive results. This is attributable to the fact that the specificity of fluorescence is due to interaction between labeled antiserum and Vi antigen substances of *F. tularensis* [8]. Consequently, with fluorescent antibodies obtained for the virulent strain, one can succeed in demonstrating virulent strains of *F. tularensis*, which have a rich antigen complex, or vaccinal strains, since they also contain a certain amount of Vi antigen. Completely avirulent or markedly attenuated strains deprived of the Vi antigen complex lose the capacity for specifically reacting with ordinary antitularemia fluorescent serum. Thus, the immunofluorescence method, which has high specificity and rather high sensitivity, with no equal in speed of obtaining results, can be recommended for demonstration of *F. tularensis* when examining tissues of chick embryos infected with the pathogen of tularemia.

Conclusions

1. The immunofluorescence method can be recommended for detection of *F. tularensis* in chick embryo yolk sacs.
2. The immunofluorescence method is more sensitive than light microscopy; it permits detection of *F. tularemia* in smears of yolk sacs infected with minimum doses.

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PHAGOCYTIC ACTIVITY OF BLOOD NEUTROPHILS IN THE PRESENCE OF TULAREMIA IN ANIMALS DIFFERING IN SENSITIVITY TO INFECTION

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[Article by T. N. Dunayeva and K. N. Shlygina, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] In the case of tularemia, increased ingestive activity of blood neutrophils is observed in people and laboratory animals who have been inoculated or had this disease [1, 3, 4, 11]. Studies of tissue cultures revealed that there was an increase in digestive activity of peritoneal monocytes and polynuclear leukocytes in immune guinea pigs and rabbits [12, 13].

Our work involved a comparative study of ingestive and digestive capacity of polymorphonuclear leukocytes with reference to *F. tularensis* in animals differing in sensitivity to infection. We tested the opsonophagocytic reaction (OPR) of intact animals and animals infected in the course of the infectious process. We used the standard technique for the OPR: to a specific volume of citrated blood we added live virulent culture, obtaining 500 million bacteria/ml in the suspension to be tested. Fixed smears were stained according to Romanovsky-Giemsa diluting the stain in acetone [7]. We estimated the ingestion index of OPR in smears by the method that is used for brucellosis. We determined that phagocytosis was completed on the basis of the ratio of indexes found after 60 min and 2 h to the initial index, estimated after 30 min. An increase in quantity of bacteria in neutrophils, as well as increase in percentage of phagocytic cells, was indicative of mild digestive capacity of leukocytes [2]. We determined the intensity of OPR in intact animals: white mice, common voles, golden hamsters and guinea pigs, which are highly sensitive to tularemia, and albino rats and rabbits, which are not sensitive species.

Ingestion of *F. tularensis* by polymorphonuclear leukocytes was demonstrated in all animal species. After 30 min, the OPR index varied in different experiments, regardless of degree of sensitivity to tularemia infection of the animals (Table 1).

As shown by determination of digestive capacity of neutrophils, after 60 min, the OPR index exceeds the initial value by an average of 2 or more times in highly sensitive animals, and by 4-6 times in some white mice and guinea pigs. In some guinea pigs, the ingestion index rose insignificantly, while some animals showed a lower level than initially.

Table 1. OPR with *F. tularensis* in intact animals

Animal species	Number of experim.	Number of animals	Ingestion index after				Index ratio (60/30)
			30 min		60 min		
			range	mean	range	mean	
White mice	4	19	4—13,2	8,2	11,7—28,7	19,9	2,4
Common voles	2	8	4,5—6	5,0	14,3—16	15,1	2,8
Golden hamsters	1	3	3—6	4	14—16	15	3,7
Guinea pigs	5	19	3—12,8	7,7	11,6—25,6	17,2	2,2
Albino rats	3	12	6—21,3	11,3	15,2—21,6	18	1,5
Rabbits	7	19	4—20	12,4	4—25	13,8	1,1

In insensitive animals, the ingestion index increased to a lesser extent after 60 min than in highly sensitive ones. Many rabbits presented a marked digestive capacity of leukocytes. Mean ratios of indexes in different experiments ranged from 0.5 to 2.1 in rabbits and 1.1 to 2.8 in albino rats.

After 30 min, the quantity of phagocytic cells ranged from 16 to 53% in white mice, 13 to 51% in guinea pigs, 24 to 30% in white rats and 16 to 68% in rabbits. After 60 min, there was an increase in number of active neutrophils in all highly sensitive animals and albino rats. In some rabbits, the percentage of phagocytic cells diminished, or the increase was insignificant, which was indicative of manifestation of a bactericidal or bacteriostatic effect.

Changes in OPR were tracked on 30 white mice and 16 rats infected hypodermically with virulent strain No 503 in a dosage of 10 bacterial cells according to the optical cloudiness standard. At each time, we tested 4 animals. White mice were sacrificed; we took blood in vivo from the tail or heart of the albino rats. Reactions were run simultaneously on all animals tested from the 1st to 8th experimental days, and in rats on the 15th-20th postinfection day.

The ingestion index with 30-min exposure rose consistently in mice from the 2d to 6th day. Mice died on the 6th-7th day, whereas in rats the ingestion index decline on subsequent days and, on the 15th-20th day, did not differ from the index of intact animals. On the first postinfection day, there was minimal digestive function of neutrophils: in both mice and rats, the ingestion index was 2.6-2.9 times higher after 60 min than the initial level, which corresponded to the parameters of intact animals (Table 2). Thereafter, there was chronographic demonstration of an increase in ingestion index and percentage of phagocytic cells in mice. Consequently, phagocytosis had not terminated.

Upon examining blood smears of mice, we were impressed by localization of *F. tularensis* on small collections of thrombocytes. This interaction of blood platelets and bacteria increased from the 1st to 5th postinfection day and was minimal in intact mice. On the 6th day of disease, there was a reduction in accumulations of thrombocytes, and they virtually disappeared in some

animals. At this time, the smears showed an increase in quantity of freely arranged bacteria, which were not fixed on thrombocytes or in a state of attraction to leukocytes. There were degenerative changes in neutrophils: vacuolization of protoplasm, pyknosis and phagolysis. Vacuoles with undigested bacteria were visible in destroyed cells. On the 4th-5th day of disease, plasmocytes and, occasionally, macrophages appeared in blood. Phagocytic monocytes were seldom encountered. Mice seldom presented agglomerates of neutrophils and lymphocytes against the background of accumulations of platelets and bacteria.

Unlike mice, the rats clearly showed greater completion of phagocytosis, according to both the index ratio and decline in percentage of phagocytic neutrophils with increase in reaction time (see Table 2). Already on the 2d postinfection day, OPR index after 60 min and 2 h were only negligibly higher than after 30 min. Thereafter, there was a consistent decline of the index, as well as quantity of phagocytic neutrophils with increase in reaction time. This intensification of digestive activity of neutrophils persisted over the entire observation period (up to the 20th day of the experiment), and it persisted even with decline of ingestion index. Thus, chronographic determination of the OPR index revealed qualitative new distinctions in correlations between phagocytic cells and the pathogen, which cannot be demonstrated by single determination of phagocytotic activity.

Microscopically, the reaction of rat blood elements differed from that of mice in that there were larger accumulations of *F. tularensis* on blood platelets, more frequent agglomerates of such collections with neutrophils and connected lymphocytes and monocytes. The latter phagocytized bacteria somewhat more often. The neutrophils showed distinct digestion of bacteria; some of them were faintly stained. There were many lysing leukocytes, and in the residues of destroyed cells there were digestive vacuoles, usually empty or containing insignificant amounts of bacteria.

Phagolysis of neutrophils in citrated blood with addition of bacteria causes extracellular death of bacteria as has been demonstrated, for example, with plague, by releasing bactericidal lysosomal proteins. Immunization increases lysability of neutrophils and release of lysosomes from them [6]. The decline of the ingestion index in our experiments coincided with appearance in rat blood serum of antibodies to *F. tularensis* in titers of 1:10-1:320, and change of infection to the extinction phase [10]. This shows that the decline of the index could be due to more intensive digestion of *F. tularensis* and faster phagolysis, rather than decline of ingestive activity of cells. At this time, infected rats showed a decrease in intensity of organ contamination with the pathogen and transition of infection to the extinction phase [10].

Unlike mice, rats showed neutrophils with degenerative changes only on the 1st postinfection day and only after 2-h contact with the pathogen. On subsequent days, along with significant phagolysis, the smears showed more neutrophils without degenerative changes in the nucleus, but they had large vacuoles with *F. tularensis*. On the 7th-8th day and later, the neutrophils reverted to normal, the vacuoles were small and there was well-marked granularity. At this time, the smears showed an appreciable decrease in quantity of *F. tularensis*, lying freely or bound with thrombocytes.

Table 2. Dynamics of OPR on different postinfection days

Object examined	Reaction time (min)	Postinfection day																	
		1		2		4		6		7		8		15		20			
		ingest. index	active phago-cytes, %	ingest. index	active phag., %	ingest. index	active phag., %	ingest. index	active phag., %	ingest. index	active phag., %	ingest. index	active phag., %	ingest. index	active phag., %	ingest. index	active phag., %		
Infected mice	30	3	12	5	20	8,7	35	14,2	67	Died									
	60	8	32	12	49	9,7	39	26,5	83										
	120	12,5	46	17,7	71	19,2	77	22,2	89										
	Index ratio:																		
	60/30	2,6		2,4		1,1		1,8		—		—		—		—			
	120/30	4,1		3,5		2,2		1,5											
Infected rats	30	5,7	23	12	51	21,7	83	22,2	81	14	57	9,2	37	6	24	6	24		
	60	17	67	13	54	20,5	81	20,2	77	10	40	6,2	25	8,5	34	5	20		
	120	14,7	59	15,5	62	10,7	43	15,2	61	9,5	38	6,7	27	3,7	15	3,2	13		
	Index ratio:																		
	60/30	2,9		1,08		0,9		0,9		0,7		0,6		1,4		0,8			
	120/30	2,5		1,2		0,4		0,6		0,6		0,6		0,6		0,5			
Intact rats	30	6	24	—	—	—	—	—	—	—	—	—	—	7,5	30	—	—		
	60	17,3	62	—	—	—	—	—	—	—	—	—	—	15,2	61	—	—		
	120	19	72	—	—	—	—	—	—	—	—	—	—	20,2	83	—	—		
	Index ratio:																		
	60/30	2,8		—		—		—		—		—		2,0		—			
	120/30	3,1		—		—		—		—		—		2,5		—			

We observed adhesion of bacteria to thrombocytes in experimental animals by using the OPR with other pathogens--*Listeria*, *Salmonella*, pseudotuberculosis bacteria. This phenomenon was described in 1917 by Rickenberg (quoted in [8]) with reference to parasitic diseases. Subsequently, the reaction was also demonstrated in bacterial infections. Some researchers interpret clumping of blood platelets with bacteria as the first phase of phagocytosis [5]. According to current conceptions, aggregations of thrombocytes, bacteria and leukocytes are formed in the body under the influence of immune processes, with involvement of the antigen-antibody complex [9].

Conclusions

1. The ingestive capacity of phagocytic cells of intact animals fluctuates over a wide range, without reflecting the level of sensitivity to tularemia infection.
2. In the course of development of infection, the ingestion index after 30-min exposure to the reaction increases in mice and rats, reaching a maximum on the 5th-6th postinfection day and ending with death of mice. In rats, the ingestion index declines after the 7th day of infection and corresponds to the index of intact animals on the 15th-20th day.
3. Substantial differences in digestive activity of mouse and rat neutrophils are demonstrable already on the 2d postinfection day. Phagocytosis is incomplete in mice, whereas in rats completion of phagocytosis and decrease in quantity of free bacteria in citrated blood are observed 4-20 days after infection.

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NEW DATA ON INCIDENCE OF ENDEMIC INFECTIONS IN ARCTIC REGION OF EAST SIBERIA

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[Article by G. V. Kornilova, M. I. Raykhlin, V. K. Yastrebov, M. S. Shayman, L. S. Yegorova, F. F. Busygin, I. I. Bogdanov and N. A. Rogatykh, Omsk Institute of Endemic Infections]

[Text] Several biomedical problems arise in connection with the prospects of economic development of northern Siberia, among which investigation of the incidence of endemic diseases is of special importance. Studies of endemic sites for anthroponoses in regions of the Extreme North is a new and important aspect of development of teaching on endemicity of human diseases, which should elucidate the complicated aspects of the epidemiological process at high geographic latitudes, mechanism of formation and evolution of endemic sites and ecology of pathogens. These studies are also of definite practical value, primarily for planning and implementing measures for the prevention of endemic infections in the Arctic region.

In accordance with the plan for work on the problem of "Endemic Diseases of Man," for the purpose of detecting anthroponotic sites in the Extreme North (arbovirus infections, endemic rickettsiosis, tularemia, leptospirosis, toxoplasmosis), in 1972-1974, we conducted a combined epidemiological, virological, bacteriological and zooparasitological study on the territory of Taymyr National Okrug.

Of the above-mentioned infections only tularemia in this territory has been described in the literature [4], data being submitted on incidence of the disease among the inhabitants and isolation in the suburbs of Norilsk of the pathogen of infection from the root vole and Middendorff vole, whereas involvement of the typical tundra rodent species, lemmings (*Lemmus Lemmus* L.) in tularemia epizootics remained undetermined. For this reason, we formulated a specific task: to confirm bacteriologically tularemia epizootics among lemmings and, in this way, to obtain due data on endemicity of this infection. Concurrently, we investigated nest-burrow biocenoses in view of the possibility of having arboviruses carried in by migratory birds nesting in Taymyr and wintering in countries of the subequatorial, tropical and subtropical climate belts [5, 6].

In all, we submitted 1900 gamasid ticks from 77 nests of Siberian lemmings to virological examination, 398 spleens from lemmings and Middendorff voles

to bacteriological examination, 1064 blood serum samples from the indigenous inhabitants, 1990 from reindeer, 466 from lemmings and 113 from Arctic foxes in captivity ["kept in cages"] to serological testing.

The results of serological studies using the hemagglutination extinction reaction (HAER) with arbovirus antigens revealed that antibodies were present to viruses of the tick-borne encephalitis complex and West Nile fever among the indigenous population who had never left Taymyr (Table 1), as well as the reindeer. Positive responses were obtained in several different places located both in the western and eastern parts of the peninsula, which was indicative of viral activity over an extensive territory, rather than specific sites, i.e., probability of widespread sources of infection in nature (the studies were conducted in 9 populated centers).

Table 1. Results of serological tests on indigenous population (aborigenes) and reindeer of Taymyr Peninsula for demonstration of tick-borne encephalitis and West Nile viruses

Region of study	Object tested	Number of serum samples	Demonstration of antibodies to virus, % (M±m)		Reciprocals of titers of antibodies to virus	
			TBE	WNF	TBE	WNF
Western Taymyr	Indigenous population	367	2.2±0.7	0.8±0.4	10-40	10-80
	Reindeer	1088	0.7±0.2	0.7±0.2	10-80	10-160
Eastern Taymyr	Indigenous population	274	1.1±0.6	-	10-40	-

Key: TBE) tick-borne encephalitis WNF) West Nile fever

In the tundra regions, lemmings could play a part in the widespread sources of infection, since bloodsucking arthropods inhabit their nests, among which there is prevalence, according to our data, of parasitic gamasid ticks; *Haemogamasus ambulans* Thorel. and *Hirstionyssus isabellinus* Oudms. (over 70%). In the absence of ixodes ticks, it should be assumed that virtually the only probable factor in transmission of arboviruses to man and reindeer, with positive serological tests, could be mosquitoes.

The nests of lemmings were collected for examination in August 1973, in the vicinity of the Novorybnaya trading post in Khatangskiy Rayon, on the right bank of the Khatanga River (73-74° north latitude). We extracted gamasid ticks from the nest using electric "thermoeclectors," 41% of them being referable to *Hg. ambulans* and 32.6% to *Hi. isabellinus*.

From a sample containing 836 *Hg. ambulans* and *Hi. isabellinus* gamasid ticks, used to infect guinea pigs intraperitoneally with 10% suspension thereof in 1973, a strain of tick-borne encephalitis was isolated.*

Isolation of the virus of tick-borne encephalitis in the subarctic tundra was an utterly new finding, which indicated that, in the absence of ixodes ticks

*Additional identification was made at the Institute of Virology imeni Ivanovskiy, USSR Academy of Medical Sciences.

Table 2. Incidence of demonstration of antibodies to pathogens of leptospirosis, endemic rickettsiosis and toxoplasmosis among indigenous inhabitants and some animals on Taymyr peninsula in 1972-1974 (Mim)

Object examined	To leptospirosis virus		To Asian tick-b. encephalitis virus		Q fever		Toxoplasmosis	
	serum samples tested	incid. of antibod.	serum samples tested	incid. of antibod.	serum samples tested	incid. of antibod.	serum samples tested	incidence of antibody demonstration
Indigenous population	945	10,4±1,2	1064	2,1±0,4	1064	1,3±0,3	584	5,5±0,9
Reindeer	1698	11,6±0,9	1990	2,6±0,4	1990	1,3±0,3	659	4,1±0,8
Lemmings	397	0,7±0,2	466	4,1±0,9	466	1,7±0,6	58	—
Arctic foxes in captivity	113	16,8±4,3	69	3,0±0,9	69	—	113	—
								9,8±1,4
								13,8±4,5
								7,1±4,5

Note: Serological testing for leptospirosis was performed using the lysis agglutination reaction, for rickettsiosis using the CFT [complement fixation test], for toxoplasmosis using the CFT and immunofluorescence (IF) test.

there could be arbovirus sites in the nest-burrow biocenoses of lemmings. The obtained data were also interesting in that heretofore the existence of arbovirus sites in the Extreme North was known only in biocenoses of bird rookeries, where tick-borne encephalitis virus was isolated in the northern part of European USSR, the Far East and United States [1], as well as arboviruses new to science--Tyuleniy, Okhotskiy, Sakhalin and others [2, 3, 7, 8]--from Ixodes ticks (*Ceratixodes putus* Pick-Cambr, which is a parasite of aquatic birds, mainly guillemots [or murre]).

Endemic sites for tularemia in Taymyr were studied after preliminary serological testing. In 1972, when the lemming population was small, we obtained positive reactions with tularemia antigen in the indirect hemagglutination test in 5 out of 94 animals collected along the bank of the Pyasina River (West Taymyr). This could be indicative of the probability of local scattered epizootic outbreaks of tularemia among lemmings in a period of depression in their number. In 1973, the lemming population grew drastically, and isolated lemming carcasses were found in the vicinity of the Novorybnaya trading post, which was indicative of an incipient epizootic. Bacteriological examination of lemming spleens resulted in isolation and identification of six cultures of *F. tularensis*. Thus, there was bacteriological confirmation of the existence in Taymyr of endemic tundra (lemming) sites of tularemia. Full-fledged epizootics involving large territories were observed during periods of mass reproduction of lemmings. Since this type of tularemia site had not been examined in Taymyr, investigation thereof at the present time should be viewed as one of the first and foremost tasks in the area of regional pathology.

In 1974, there was a severe depression of the lemming population throughout Taymyr, for which reason we conducted studies to determine whether the pathogen of tularemia could be retained in the environment. We submitted to bacteriological examination 60 samples of moist substrate in the vicinity of nests, which had been gathered from the same lake-swamp region of the tundra. Cultures of *F. tularensis* were isolated from 2 samples of moist substrate. Studies of morphological and cultural properties, as well as virulence of the cultures, were indicative of their belonging to the Holarctic race. Examination of washings from 9 Middendorff vole nests revealed a culture of *Listeria* in one of them (in the vicinity of the Kresta trading post, 72° north latitude).

The results of serological tests on the public and animals for leptospirosis indicated (Table 2) that the etiological structure of these diseases among the inhabitants and reindeer virtually coincided, and that it was characterized by marked prevalence of the Australis and Ballum serogroups. Evidently, adaptation of *Leptospira* to arctic conditions is associated with elimination of certain serogroups that prevail at moderate latitudes of Siberia. Nor can we rule out the existence of lemming sites of leptospirosis, as indicated by serological data obtained in Taymyr. However, the incidence of seropositive results in tests with *Leptospira* referable to different serological groups is 1/12th-1/18th in lemmings of the level in humans and reindeer.

Together with veterinary workers, we found an outbreak of leptospirosis among blue Arctic foxes of the animal farm at the Sovetskiy Taymyr kolkhoz (72° north latitude). In the summer of 1973, fox deaths were observed on this farm. The leptospirous etiology of animal diseases was confirmed by pathological and serological studies. Antibodies to *Leptospira*, of the Grippotyphosa, Bataviae, Pomona and Ballum serogroups (15.8%), were demonstrated, and Grippotyphosa and Bataviae accounted for over 60% of the seropositive findings. No more deaths occurred after the foxes were immunized with polyvalent serum against leptospirosis of farm animals.

The positive results of serological tests on indigenous inhabitants, reindeer and lemmings for tick-borne Asian rickettsiosis and Q fever (see Table 2) were indicative of circulation of the pathogens of these infections in the Arctic.

Antitoxoplasma antibodies were demonstrated not only in the CFT, but by the immunofluorescence method. We should mention the relatively high titers of serological reactions (1:10-1:20) in one-third of the cases. In view of the nonsterile nature of immunity to toxoplasmosis, as well as data indicative of specificity and sensitivity of the serological reactions we used, we believe that toxoplasmosis is rather widespread in the northern part of East Taymyr. We demonstrated for the first time that the population of the Taymyr National Okrug was stricken by toxoplasmosis. Since there were virtually no house cats in most of the villages we screened, and they are the final hosts of *Toxoplasma*, reindeer could be a possible source of this infection in man, and 4.1% of them were found to be stricken in this region.

Thus, as a result of our combined studies of anthroponotic sites in the subarctic tundra of Taymyr, the existence of tick-borne encephalitis virus was demonstrated in the nest-burrow biocenoses of lemmings, as well as the

existence of infection with *F. tularensis* in this typical and widespread rodent of the tundra. At the same time, data were obtained on the presence of combined sites of these two infections, which are ecologically linked to lemmings, at high latitudes. The appearance of sites of tick-borne encephalitis virus in Taymyr could be related to its being brought in by migratory birds. In addition, we should mention the ecological flexibility of pathogens of anthroponoses and the theoretical possibility of their adaptation to conditions in the Arctic where, with all the paucity of fauna, there is retention of species of vertebrates and arthropods that are close to the known hosts and vectors of these infections.

Conclusions

1. For the first time, in 1973, tick-borne encephalitis virus was isolated from *Haemogamasus ambulans* and *Hirstionyssus isabellinus* gamasid ticks beyond the range of their habitat, in the subarctic tundra on Taymyr Peninsula out of nests of the Siberian lemming, and antihemagglutinins to this virus and West Nile fever virus were demonstrated in the indigenous population and reindeer.
2. In the same location in Taymyr, cultures of *F. tularensis* were isolated for the first time from lemmings, which proves the existence of northern tundra sites of tularemia in the Arctic and also confirms the etiology of the tularemia epizootic among lemmings, which occurred in 1973; in the period between epizootics, when there was a drastic depression of the lemming population and none at all in the region of our study, the pathogen of tularemia persisted in the tundra, in the environment.
3. *Listeria* was isolated from nests of Middendorff voles, which was indicative of the fact that this species of small mammals is stricken by the pathogen of listeriosis.
4. The results of serological tests on the inhabitants and animals were indicative of circulation of pathogens of leptospirosis, toxoplasmosis and endemic rickettsiosis in the arctic region of Eastern Siberia.

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CULTIVATION OF *F. TULARENSIS* IN DEVELOPING CHICK EMBRYOS

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[Article by R. I. Kudelina and V. L. Popov, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The studies of a number of authors [1, 6, 8, 9, 12] have shown that developing chick embryos are highly sensitive to the pathogen of tularemia infection. This pathogen, when injected in the chorioallantois or cavity of the yolk sac, multiplies intensively in ectodermal and mesodermal cells of membranes, and it causes a lethal disease in the embryo. The authors determined development of tularemia infection on the basis of time of embryo death, pathological findings, as well as titration of a suspension of membranes and embryo tissues on glucose-cystine blood agar or white mice. The authors did not pursue morphological studies for demonstration of the pathogen by light or electron microscopy, with the exception of Larson [9], who used the Wayson method to stain smears. Unfortunately, he does not submit the results of these studies, so that we cannot evaluate the suitability of this staining method for demonstration of *F. tularensis* in embryos.

The absence of studies of this aspect is attributable to the fact that, because of an underdeveloped mucopeptide layer, *F. tularensis* has a thin and brittle [delicate] cell wall, which is difficult to stain and readily injured during various manipulations. The presence in yolk mass of significant amounts of lipids, phosphoproteins and pigment groups makes it difficult to differentiate between *F. tularensis* and various cellular inclusions. For this reason, the usual staining methods are unsuitable for detection of *F. tularensis* in chick embryo yolk sacs.

We investigated the possibility of using developing chick embryos for cultivation of both virulent and avirulent strains of *F. tularensis*. We used light and electron microscopy for detection of *F. tularensis* in embryos. We devoted special attention to determination of possibly different *F. tularensis* with respect to supramolecular organization, depending on virulence of the strains.

The studies were performed on 2000 chick embryos after 8-10 days of incubation. We failed to demonstrate any differences in sensitivity to infection in embryos of this age. Embryos incubated for 5-7 days were found to be less

resistant and perished within 24-84 h after infection. Embryos incubated for 12-15 days were also unsuitable for experiments, since the time of their death due to infection with vaccinal or attenuated strains could coincide with the time of death occasionally observed in the period preceding hatching, which makes it difficult to interpret results.

The conventional Cox method [7] was used to infect the embryos in the yolk sac. For infection, we used strains of different geographic races and with different virulence: Schu (virulent), No 38 attenuated (Nearctic race), No 503 virulent, No 503 attenuated, No 15/10 vaccinal, No 21/400 entirely avirulent (Holarctic race). All of the strains were typical in morphological cultural and biochemical properties. In the experiments, we used day-old cultures of *F. tularensis* grown on solid agar medium of fresh fish hydrolysate with cystine, glucose and blood. The cultures were suspended in saline to 10^9 bacterial cells/ml (by the standard of the State Institute of Standardization and Control of Medical Biologicals imeni Tarasevich); from the obtained suspension we then made 10-fold dilutions. Each dilution, in a volume of 0.5 ml, was injected in the yolk sac of 5-6 embryos, 0.1 ml was plated on agar medium of the same composition for demonstration of *F. tularensis* and extraneous flora. As a control, we used embryos of the same age in whose yolk sac 0.5 ml saline was injected instead of culture. The embryos were incubated at 37° and high relative humidity, which was produced by placing a tray with water in the incubator. The eggs were examined daily to determine time of embryo death. Dead embryos were dissected on the same day. We used 10% yolk sac suspension in saline for serial passages; it was injected in amounts of 0.5 ml in the yolk sac of the next batch of embryos.

Impressions were made of pieces of yolk sacs from dead embryos, which were air-dried, then stained with a mixture of Giemsa stain and methyl alcohol taken in equal parts [2, 3]. With this staining method, *F. tularensis* turned violet and cellular elements a light lilac. The advantage of this method over the usual Romanovsky-Giemsa staining method is that it is fast, simple and there is no preliminary fixation, as well as that it differentiates between bacteria and cellular inclusions.

The stained smears were viewed under a light microscope. Accumulation of bacteria was evaluated on a 4-point system: isolated--up to 10 bacteria (-), 10-20 bacteria (+), 50-100 (++) and over 100 per field (+++). To calculate the specific accumulation of bacteria, we arbitrarily took a parameter of 0.5 for presence of isolated bacteria, 1 for + accumulation, 2 for ++ and 3 for +++; we divided the total number by the number of embryos that died, in which *F. tularensis* was found.

For electron microscopy, we usually dissected live or agonal embryos, and in only some cases some that had died. Pieces of yolk sac membranes from chick embryos infected with *F. tularensis* were fixed in cold 1% glutaric aldehyde on phosphate buffer at pH 7.2 for 20-30 min. The material was then additionally fixed in 1% OsO₄ on phosphate buffer for 2 h at room temperature, with contrasting in 3% uranyl acetate and 30% ethanol for stabilization of bacterial nucleoid [5]; dehydration was performed in ascending concentrations of ethanol and absolute acetone; it was soaked in mixtures of acetone and epoxy resin and imbedded in araldite M [10] or a mixture of epon and araldite [11]. Ultrafine

sections were made with an LKB-4800 ultramicrotome and contrasted with 5% aqueous solution of uranyl acetate. We used IEM -7 and IEM-100B microscopes with instrument magnification of 3000, 30,000 and 50,000 $\times$.

Examination of the cultural distinctions of *F. tularemia* in chick embryos revealed that virulent strains, Schu and No 503, multiplied intensively in yolk sacs causing embryo death on the 3d-4th postinfection days, even with administration of 10^4 - 10^2 bacterial cells; vaccinal strain No 15/10 caused embryo death when given in a dosage of 10^7 - 10^6 , whereas with use of attenuated strain No 503 and completely avirulent strain No 21/400 embryo death occurred only with 10^9 - 10^8 bacterial cells.

We demonstrated some correlation between virulence of strains and average time of embryo death (see Table). Thus, with administration of large doses of pathogen (10^7 - 10^9) average time of embryo death after infection with virulent strains Schu and No 503 was at 3.1-3.5 days, and with strain No 21/400 6.2 days. The percentage of embryos with positive bacterioscopic results, as well as the coefficient of specific accumulation of bacteria, were also considerably higher in embryos infected with virulent strains, as compared to avirulent ones (86-82.9% and 1.5 in the former case, 35.4% and 0.7 in the latter).

Results of cultivating *F. tularensis* in chick embryos

Geographic race	Strain	Number of embryos infected	Embryo deaths ¹	Bacterioscopically positive tests ²	Specific accumulation of bacteria	Average time of embryo death (days)
Nearctic	Schu	71	71/100	60/86	1.5	3.1
Holarctic	No 503 (virulent)	264	263/99.7	237/82.9	1.8	3.5
	No 15/10 (vaccinal)	121	115/95	114/70	1.2	4.0
	No 503 (attenuated)	255	251/98.4	120/47.8	0.9	5.1
	No 21/400	271	254/91.8	90/35.4	0.7	6.2
Totals		982	954			

¹Denominator--% deaths in relation to number of infected embryos.

²Denominator--% deaths with positive bacterioscopic results.

Note: Infective dose was 10^7 - 10^{10} bacterial cells.

Data about cultivation of strain No 38 are not listed in the table, since the embryos survived after being infected with these strains in a dosage of 10^7 bacterial cells and there was a low percentage of deaths within the first 1-2 days with a dosage of 10^8 bacterial cells. We failed to demonstrate *F. tularensis* in impression smears of pieces of yolk sac membranes from both dead and live embryos dissected on the 6th-12th postinfection day. We failed in our effort to adapt this strain to chick embryos by means of two blind passages:

bacterioscopy was negative, and no growth was demonstrable when a suspension of yolk sacs was plated on solid nutrient medium of fish hydrolysate with cystine, glucose and blood.

It was interesting to find out whether *F. tularensis* multiplies in chick embryos or is merely preserved there. To answer this question, we made a quantitative assay of *F. tularensis* in chick embryo yolk sacs. For this purpose, we prepared 10-fold dilutions from a suspension of yolk sacs (1 g/ml saline) and 0.1 ml of each dilution was cultured on medium of fish hydrolysate with cystine, glucose and blood. As shown by a comparison of culturing results to magnitude of infecting dosage, there was intensive reproduction of the pathogen of tularemia after infection with virulent strain No 503: there were several billion bacteria per gram yolk sac in an embryo infected with 10^7 bacterial cells, which died on the 3d day. Attenuated strain No 503 did not multiply as profusely as the virulent one: there were 10^5 bacterial cells per gram yolk sac. Strain No 21/400 did not multiply, but was merely preserved in the embryos: there were about 10^3 bacterial cells per gram yolk sac, and when this strain was submitted to passages in embryos there was no increase in number of bacteria, on the contrary, it decreased and by the 3d passage no bacteria were demonstrable in the smears.

Examination of ultrafine sections of yolk sac membranes from embryos infected with *F. tularensis* revealed that bacterial cells usually presented extracellular localization. Occasionally, we find cells among the cellular debris that were invested in an additional three-layer membrane, apparently the membrane of an intracellular vacuole (see Figure, e [photo not reproduced]). On this basis, it can be assumed that the bacteria may also be situated within the epithelial cells of the yolk sac membrane, in cytoplasmic vacuoles.

As a rule, the cells of virulent strain No 503 were surrounded by a light [or clear] zone, apparently formed as a result of interaction between the mucoid bacterial sheath and imbedding medium. Such a zone was seen much less often around cells of other strains.

Variation of dimensions was inherent in bacteria of all tested strains: along with cells of the usual size for a given strain, we encountered larger ones in the population; this was particularly typical of strain No 21/400. *F. tularensis* cells were round or oval, with the following ranges of size: 500-700x400-600 nm (virulent strain No 503), 400-600x300-400 nm (attenuated strain No 503) and 500-800x300-700 nm (strain 21/400). The cells were invested in a cell wall with smooth surfaces. The three-layer membrane of the cell wall 60-90 Å in thickness presented a symmetrical profile. In cells of avirulent strain No 21/400 and attenuated strain No 503, we occasionally demonstrated a more dense external layer of cell wall membrane, which was somewhat thicker, perhaps due to close adherence of microcapsular material. There were clusters of capsular material on the surface of such cells (see Figure, δ). The inner layer of the cell wall had moderate electron density, its thickness ranged from 2-3 to 5-7 nm in some places. The cytoplasmic membrane presented the usual three-layer profile for Gram-negative bacteria, and thickness of 6-7 nm. A large nucleoid, which is inherent in these bacteria [4], occupied the center of the bacterial cell; the cytoplasm of moderate electron density occupied the periphery of the cell. Ribosomes 15-20 nm in diameter were demonstrable in the cytoplasm.

We failed to demonstrate basic difference in ultrastructure of virulent and avirulent strains of *F. tularensis* in chick embryo yolk sacs.

In noting the high sensitivity of chick embryos to tularemia infection, we must also mention the negative aspects of this method. In the first place, it is not standard, apparently due to the dissimilar resistance of embryos to infection, as a result of which some embryos die within the first post-infection days and others at a later time, in spite of being given the same infective dosage. We observed 100% embryo death only when infected with large doses. In the second place, the time of embryo death depends on the diet of fowl that produced them. As we know, some antibiotics, for example, tetracycline and grisin [derived from *Actinomyces griseus*], are used as biogenic stimulators and added to fowl feed. Traces of these agents in embryos could have an adverse effect on efforts to adapt *F. tularensis* to embryos, since this pathogen is highly sensitive to them. Yet, it is not deemed possible to determine with reliability whether fowl were on an antibiotic diet, since we generally used embryos referable to different batches, sometimes obtained from different poultry farms. The alternation, "without cause," of periods of profuse accumulation of *F. tularensis* and periods of minimal accumulation, which was unrelated to season and other factors, is indicative of the existence of such a diet. This must be taken into consideration when culturing *F. tularensis* in embryos.

Thus, the results we obtained from cultivating *F. tularensis* in developing chick embryos revealed that this biological model can be used to grow the pathogen of tularemia. It was learned that virulent strains multiplied, while attenuated and completely avirulent ones merely were preserved in chick embryos, and some, for example strain No 38, disappeared when passed in embryos. The difficulty of adaptation of strain No 38 to embryos is apparently related to its metabolic distinctions as a result of prolonged cultivation in U. S. laboratories on glucose and cystine blood agar. It should be noted that Downs et al. [8] also reported inconsistent results when this strain was cultivated in embryos. Embryo deaths were observed only with administration of 400 million bacterial cells, on the 15th-18th postinfection day.

In this case, use of a high-speed staining method using a mixture of Giemsa stain and methyl alcohol for demonstration of *F. tularensis* in smears of yolk sac membranes justified itself. This technique enabled us to record accumulation of the pathogen of tularemia without using the time-consuming process of counting colonies in dishes inoculated with different dilutions of yolk sac suspension.

Our study of ultrafine structure of the pathogen of tularemia in chick embryo yolk sacs failed to demonstrate any basic differences between the structure of virulent and avirulent strains. When cultivated in this model, the structure of *F. tularensis* is similar to their ultrastructure when grown on solid nutrient medium [4].

Conclusions

1. Chick embryos are highly sensitive to tularemia infection, and virulent strains of *F. tularensis* multiply intensively in chick embryo yolk sacs,

causing death thereof on the 3d-4th postinfection day, whereas virulent strains are merely preserved in them.

2. One can use a high-speed method of staining with a mixture of Giemsa stain and methyl alcohol or acetone for demonstration of *F. tularensis* in chick embryo yolk sacs.

3. We failed to demonstrate differences in ultrafine structure of virulent and avirulent strains of *F. tularensis*.

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PRESERVATION OF *F. TULARENSIS* IN THE ENVIRONMENT IN EASTERN TAYMYR

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 2, Feb 76 (manuscript received 24 Jul 75) pp 73-75

[Article by I. P. Algazin, V. A. Il'in and L. S. Yegorova, Omsk Institute of Endemic Infections]

[Text] Investigation of the possibility of existence and wide distribution of a number of endemic diseases, including tularemia, merits special attention in view of the ever increasing economic development of some regions in the Extreme North. There are sparse data in the literature concerning tularemia in the Arctic, and they pertain essentially to detection of this infection in the northern part of European USSR (Kola Peninsula, Arkhangelsk Oblast) [5-7]. The tundra zone of East Taymyr has been the least studied in this respect, although conditions exist there for existence of active endemic tularemia sites [1].

In 1973, we demonstrated, for the first time in the USSR, spontaneous infection of Siberian lemmings with *F. tularensis* in this region. Studies conducted the following year coincided with a severe depression of the population of this species--not a single lemming was trapped in natural biotopes in July-August 1974. For this reason, we concentrated mainly on a search for sites where the pathogen is preserved in nature. In view of the complete absence of *Ixodes* ticks in the Taymyr fauna, we assumed that *F. tularensis* could be preserved in water, the substrate of unoccupied nests and gamasid mites [ticks] that inhabit these nests during a period of lemming depression. The possibility of prolonged survival of *F. tularensis* in the environment was the subject of investigation by many Soviet and foreign researchers [2, 3, 8, 9]. It was established, in the field and experimentally, that low temperature, absence of direct insolation and high ambient humidity are the chief factors that are instrumental in preservation of this pathogen [2, 3]. The distinctions of the tundra landscape (small lakes formed in permafrost craters, numerous swamps, the water of which is in contact with the permafrost layer that is in the immediate vicinity of the ground cover) provide, in our opinion, optimum conditions in this respect.

We gathered material for bacteriological testing in the vicinity of the village of Novorybnoye (Khatangskiy Rayon of Taymyr National Okrug), in a region of moss-lichen tundra, at permanent observation posts. We collected

water samples for analysis from exposed sources--glacier run-off, brooks, tundra lakes (7 samples) and moist substrate in the vicinity of nests in the summer habitats of lemmings (60 samples) which, according to absence of fresh traces of vital functions, had not been visited by the animals since the previous fall. Water samples were put in sterile vials, 150-250 ml in each. The method of collecting samples of the substrate near nests consisted of the following: we took the nest from a lemming burrow, then carefully collected the moist layer of soil around it, squeezed it over a vial and used the obtained suspension for biological tests. We took one water sample from each substrate. The nests gathered from burrows (63 nests) were also subsequently examined for tularemia. The obtained material was delivered to a field laboratory, where 0.5 ml of tested water was given by hypodermic injection under sterile conditions (in the right inguinal region) to white mice (2 mice per sample). Animals infected with each specimen were kept separately. We used a vaseline and paraffin mixture [4] to preserve first-passage viscera. Subsequent examination was made in the laboratory of the Omsk Institute of Endemic Infections. The animal organs were submitted to a second passage on white mice. We used coagulated yolk medium for demonstration of *F. tularensis* and beef-extract agar as a control. We identified the isolated strains using the complete agglutination test with specific tularemia serum (series No 17, titer 1:3200, shelf life up to November 1975).

In the first passage, 7 mice died on the 7th-8th day. Necropsy revealed an infiltrate at the injection site and considerable enlargement of the spleen in all cases. In addition to bacillary forms, we examined coccus bacteria suspect for tularemia in impression smears of viscera from three mice. The other animals were sacrificed after 10 days. We examined the spleen of dead (individually) and sacrificed (5-10 at a time) animals a second time by the biological method.

We succeeded in isolating two cultures of *F. tularensis* as a result of a second passage of white mouse organs infected with water from two different samples of substrate near nests. It must be noted that the cultures were isolated in a test of material from one of the regions where spontaneous tularemia infection had been demonstrated in 1973 among Siberian lemmings. The distance between sites of collection of water samples from the substrate near nests constituted about 100-150 m.

Death of second-passage animals infected with water samples occurred on the 5th-7th day. Necropsy revealed typical changes in internal organs: enlargement and hyperemia of vessels in the subcutaneous cellular tissue, enlargement and consolidation of the spleen and liver. Impression smears showed the morphological forms that were typical of *F. tularensis*. Typical mucoid ("pebbled leather" type) growth, which did not differ in color from the nutrient medium, was observed when lymph nodes, spleen, liver and blood were cultured on yolk medium, after 24-h incubation at 37°. There was no growth on agar. In smears of cultures submitted to Gram staining, we demonstrated Gram-negative coccobacteria. Both cultures were agglutinated to titer by diagnostic serum. The virulent properties of the isolated strains of *F. tularensis* did not differ from the strains isolated from Siberian lemmings in 1973: the completely lethal dose constituted 1 bacterial cell for white mice and 1 billion bacterial cells for albino rats (according to the standard of the State Institute of Standardization and Control of Medical Biologicals imeni Tarasevich).

Biological testing of washings from nests gathered in lemming burrows failed to yield positive results.

In view of these data, it is important to continue with investigations of the role of abiotic factors in preservation and distribution of tularemia, as it applies to the specific conditions of endemic sites in the Arctic).

Conclusions

1. The possibility of preservation of *F. tularensis* in the moist substrate near nests in summer habitats of lemmings during a period of depression in their number was demonstrated for the first time for high latitudes (73° north latitude).
2. The biological properties were retained, including virulence, in strains that were found under natural conditions in the near-nest substrate.

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ESTABLISHMENT OF ENDEMICITY OF TULAREMIA IN TUVA ASSR

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[Article by M. I. Antsiferov, N. A. Zykina, L. V. Sizykh, T. G. Charnaya, V. R. Zharov, N. I. Yel'shanskaya and A. V. Yevdokimov, Irkutsk Scientific Research Plague-Control Institute of Siberia and the Far East, Tuva Republic Sanitary and Epidemiological Station, Tuva Plague-Control Station]

[Text] Tularemia had not been detected in Tuva ASSR up to 1974. However, the existence of endemic sites of this infection in neighboring republics, krays and oblasts (Buryat ASSR, Altay and Krasnoyarsk Krays, Kemerovo and Irkutsk Oblasts) warranted the belief that tularemia did exist in Tuva.

Examination of field material (rodents, ectoparasites, including Ixodes ticks, flying bloodsucking insects) for tularemia, which was conducted in 1968 in the laboratories of the Tuva Plague-Control Station and Department of Particularly Dangerous Infections of the Tuva Sanitary and Epidemiological Station was not so significant in volume, and the negative results it yielded could not warrant the conclusion that there were no endemic tularemia sites on the territory of Tuva ASSR.

It is now time to obtain a definitive answer to the question of presence or absence of endemic sites for tularemia in Tuva and, institute preventive measures on this basis in view of the rapid economic development and influx of people to this republic.

In 1974, the Irkutsk Plague-Control Institute, together with the Tuva Plague-Control Station and Department of Particularly Dangerous Infections of the sanitary and epidemiological station, conducted a survey to answer this question. Attention was focused mainly on gathering Ixodes ticks and submitting them to bacteriological examination.

A total of 20,099 Ixodes ticks were gathered between 16 and 29 April 1974 at 7 points in 4 rayons of this republic (Tandinskiy, Ulug-Khemskiy, Mongun-Tayginskiy, suburbs of Kyzyl). The main collections of ticks were made in the vicinity of Lake Khadyn (Tandinskiy Rayon) in a narrow coastal strip, where there is prevalence of Siberian pea-shrubs [Caragana arborescens Lam], water sedge short-grass meadows and chee grass. In this strip, there are

numerous settlements in strips [bands] of the narrow-skulled vole, active burrows of the Daurian pika [*Ochotona daurica* Pallas] are common; it is also inhabited by the midday gerbil and long-tailed Siberian suslik. Lake Khadyn is used to water cattle and sheep. There, the number of ticks constituted about 300 per flag-h (flag 1×1.2 m in size made of flannel).^{*} All of the collected ticks were referable to the same species, *Dermacentor nuttalli*.

The ticks were examined by the biotest method on white mice in the Tularemia Department of Irkutsk Plague-Control Institute (7011 specimens) and in the laboratory of the Tuva Plague-Control Station (13,088 specimens).

Bacteriological testing of 7011 ticks resulted in isolation of 11 strains of *F. tularensis*. Examination of 13,088 ticks yielded negative results. Ten strains were isolated from ticks gathered in the vicinity of Lake Khadyn and 1 from ticks (247) removed from cows at the Ulug-Khaya Sovkhoz in Ulug-Khemskiy Rayon. In addition, 4 strains were isolated somewhat later from ticks (5144) collected in the same rayon (by G. S. Letov) and tested in the laboratory of the Department of Particularly Dangerous Infections of the Tuva Republic Sanitary and Epidemiological Station. The territorial distance between the points where tularemia-infected ticks were found leads to the conclusion that there are scattered endemic sites of this infection in this republic.

Biotested mice died of tularemia on the 7th (1), 8th (2), 9th (3), 10th (1), 12th (2), 14th (2) and 19th (1) days, presenting the typical pathoanatomical signs of this infection. Liver and spleen *F. tularensis* content in these mice was given a score of 4.

Studies of morphological, cultural, tinctorial, serological and virulent properties confirmed that the isolated strains were referable to *F. tularensis*: bacteria did not grow on ordinary nutrient media; there was delicate, ciliated growth on yolk medium with no motility; all strains were agglutinated by specific serum in a titer of 1:3200 (1 to the serum titer and 1 to 1:6400). The virulence of all of the cultures was the same when determined in white mice---Dclm [clinically lethal median dose?] constituted 1 bacterial cell. Isolation of *F. tularensis* from ticks confirmed conclusively the presence of endemic sites of this infection in Tuva ASSR.

Another confirmation is referable to the results of our studies of retrospective detection of tularemia and a natural immune stratum among the population. Thus, in 1974, we found two people who had suffered from tularemia in 1972 and were infected in Tuva ASSR. They had been in the tayga, in the upper reaches of the Yenisey River from late August to 8 September, where they gathered field material for work on their diploma. They were infected by four muskrat carcasses, which the students found on lakes in the tayga and which they had skinned. Three days later, both students experienced malaise. This served as grounds to stop their work prematurely and leave the tayga to return to their homes (one in Irkutsk and the other in the village Kyren, Buryat ASSR). There, they became sick (on 8 and 10 September). Their

^{*}Zoologists from the Tuva Plague-Control Station participated in gathering ticks: G. K. Yevteyev, V. K. Melkova, Yu. V. Nikiforov, P. S. Obukhov, L. A. Os'kina and I. M. Ustyuzhina

illness was associated with high fever (for 6 days in one of them and 9 days in the other), headache, loss of appetite, severe weakness, nocturnal perspiration and axillary bubos. One of the patients spent 2 weeks in a hospital in the region of his residence with the diagnosis of axillary lymphadenitis, the other stayed at home and did not seek medical attention. The result of the epicutaneous allergy test with tularin, which we did for retrospective confirmation of tularemia, was positive for the student who lived in Irkutsk (infiltrate 2x2 cm in size). The expanded agglutination test for tularemia was positive in a titer of 1:100.

We additionally screened by the tularin test the naturally immune stratum of the population living permanently in this republic. In all, we tested 1733 people from 7 populated centers (Kyzyl--368 people, Tandinskiy Rayon--442, Piy-Khemskiy Rayon--413, Kungurtug village--510). The epicutaneous allergy test with tularin was positive in 2 cases after 48 h. One of them, a professional hunter 42 years of age, lives in the village of Ust-Elegest, but every year he hunts for muskrats in the vicinity of Kungurtug; the other, a rural co-op salesman, is a resident of Kungurtug and is an amateur muskrat hunter. They had not been inoculated against tularemia. We were unable to establish the existence of prior illness that could be suspected of being tularemia. Nevertheless, we tend to believe that the positive skin test with tularin in these cases resulted from prior disease that had gone unnoticed.

By agreement with the Tuva Republic Veterinary Bacteriological Laboratory, we collected for serological testing for tularemia 240 samples of serum from cattle [large horned cattle] from 4 rayons (Kaa-Khemskiy--107, Kyzylskiy--52, Tandinskiy--52, Ulug-Khemskiy--26). Agglutination test was positive (in low titers) with 4 serum samples from animals in Kaa-Khemskiy Rayon (1:80 in 1 case, 1:40 in 1, 1:20 in 2), 2 samples from animals in Tandinskiy Rayon (1:20 in 1 and 1:10 in the other) and 2 in Ulug-Khemskiy Rayon (1:20 in both).

Thus, the results of studies conducted in 1974 on the territory of Tuva ASSR confirmed the existence of endemic sites of tularemia there.

Our future studies should direct themselves to epidemiological and epizootiological distinctions of tularemia in Tuva ASSR, identification of types of endemic sites, their biocenosis and biocenotic links, epidemiological types of morbidity and necessary preventive measures to assure a good epidemiological situation.

Conclusion

The results of studies conducted in 1974 confirmed the existence of endemic sites for tularemia on the territory of Tuva ASSR.

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STUDY OF CATALASE ACTIVITY IN *F. TULARENSIS*

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[Article by I. V. Rodionova, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The existing information about the link between catalase activity and virulence of *Francisella tularensis* is contradictory: Mizuhara reported that there was a direct correlation between these features [6]. Avi-Dor and Janiv [4] failed to demonstrate any correlation in their study of subcultures differing in virulence. A comparison of *Yersinia pestis* to various strains of microorganisms referable to 23 genera of aerobes showed that lowest catalase activity was referable to *F. tularensis* and *Pasteurella septica* [5]. This is virtually the extent of existing studies of this enzymatic system of *F. tularensis*, and it prompted us to conduct an extensive study of cultures differing in virulence and referable to three geographic races.

We used highly virulent cultures, of which strains No 503/834 and 503/840, 85 and 61 isolated in the USSR, KF, B-300 and O-458B isolated in the United States, as well as 5 strains of the Japanese variety--Jama, Kosho, Ichijo, Jasoe and Miura--were referable to the Holarctic race. The group referable to the Nearctic race consisted of Schu, strains O-284, No 8859, Cole and E-261, while the Central Asian strains were No 122, 55, 117 and 543. The minimum lethal dose of these cultures when given hypodermically to white mice constituted 1 bacterial cell according to the standard of the State Institute of Standardization and Control imeni Tarasevich.

In addition, we studied Holarctic vaccine strain No 15/10, which has residual virulence for laboratory animals and immunogenicity, and, lastly, avirulent cultures of Holarctic strains No 503 attenuated, No 15 attenuated and No 21/400, Nearctic No 38, R variants produced by Kudelina [2] using erythromycin from Schu and No 543. All of the avirulent strains were nonpathogenic for white mice when given in a dosage of 1 billion bacterial cells, and they were non-immunogenic.

Most strains were plated from vials on yolk medium, then a 2-day culture was grown on blood agar of fresh fish hydrolysate, with cystine and glucose. The strains were cultivated for 24 h at 37°. In order to recover the required

amount of working suspension, cultures of each strain were reinoculated on the same medium, but without blood, and grown for 1 day at 37°. The only exception was referable to the Central Asian strains, for which addition of blood to the above medium was required. We established in preliminary experiments that presence of blood in the media did not affect catalase activity. Several strains were submitted to preliminary passages in guinea pigs: No 503/834 and Schu once, No 85, 55 and 122 three times, No 503/840 and 543 for 6 successive times.

At least two experiments with three concurrent assays of the enzyme in each were conducted with most strains.

Presence of catalase was recorded by the Jolles iodometric method [3]. We used unpurified extracts of bacteria suspensions as the enzyme, after lysis of cells with Tween-20 in saline. To obtain extracts, the *F. tularensis* cultures were eluted twice in saline with centrifugation (6000 r/min at 4°). We added an aliquot of 1% Tween-20 to a suspension containing 40 billion cells per ml, kept the mixture at room temperature for 2 h and allowed it to stand overnight in the refrigerator at 4°. On the following day, we assayed protein according to Lowry in the mildly opalescent supernatant obtained after separation of cells by means of centrifugation at 6000 r/min. When necessary, the extract was diluted to a concentration equaling about 500 µg protein/ml.

Specific activity of the enzyme was expressed in micromoles H_2O_2 dissociated in 1 min under optimum conditions (pH 6.8), with saturating concentration of substrate (0.005 M H_2O_2) in 0.006 M phosphate buffer and temperature of 0°, scaled to 1 mg protein. On the basis of assaying H_2O_2 concentration, we calculated the initial rate of the reaction V_0 [1] at different intervals (1-3 min) using a graphic method. The results are submitted in units corresponding to 1 µM H_2O_2 /mg protein/min. Presence of insignificant amounts of Tween-20 in the reaction medium did not affect demonstration of the enzyme.

In analyzing the data obtained, it should be noted, first of all, that all of the *F. tularensis* cultures examined contained catalase and that the V_0 reaction, calculated in parallel experiments for each strain, were identical or differed insignificantly (see Table).

In the experiments, we noted a correlation between catalase activity and the geographic race to which the strains belonged. Maximum V_0 were demonstrated in virulent Nearctic strains, for which this parameter was in the range of 44.2-84.6 units of activity. The initial rate of the catalase reaction constituted only 17.1-29.9 in typical Holarctic strains, including the American representatives thereof. It is expedient to single out in a special group the Japanese variety of the Holarctic race, which had activity of 34.3-38.7. With the exception of strain No 543, the virulent Central Asian strains had V_0 of 44.0-53.1 units, thus being between the Japanese variety and the Nearctic race.

Consequently, our results were not in contradiction with the conception of more intensive energy metabolism in the Nearctic race and, probably, the Central Asian one, which ensues from analysis of data in the literature.

Initial rate of decomposition of H_2O_2 by catalase of different strains of *F. tularensis*

Race	Strain No	Year iso- lated	V ₀ (activity units/g protein)	
			First	Second
			experiments	
Holarctic: typical	503/840***	1949	29,3	29,9
	503 attenuated		3,4	3,1
	85***	1974	24,6	25,1
	61*	1974	24,0	—
	KF	1958	26,6	—
	B-300	1960	17,1	—
	O-458B	1968	23,5	—
	15/10 vaccinal	1937	11,4	8,1
	15 attenuated		7,9	9,1
	21/400 »	1949	14,9	9,8
Japanese variety	Jama	1957	34,3	—
	Kosho	1965	36,0	40,4
	Ichijo	1975	38,7	—
	Jasoe	1975	38,2	—
	Miura	1975	36,3	—
Central Asian	117	1960	44,3	44,0
	543***	1965	35,9	35,3
	543 attenuated		10,5	10,8
	122**	1973	53,1	—
Nearctic	55**	1973	44,6	—
	Schu *	1941	58,7	59,5
	Schu attenuated		15,9	15,2
	O-284	1959	54,6	50,8
	8859	1959	49,1	44,2
	Cole	1972	67,0	—
	E-261	1972	84,6	—
	38 attenuated	1920	13,2	15,7

*Culture after one passage in guinea pig.

**After 3 passages.

***After 6 passages.

It is also significant that there is some correlation between level of catalase activity and virulence of cultures. For example, the initial reaction rate in vaccinal Holarctic strain No 15/10 and avirulent strains of this race did not exceed 14.9, i.e., it was almost one-half the mean value for typical virulent cultures. Nearctic avirulent strain No 38, which had a V₀ averaging 14.45 units, differed from virulent strains in that catalase activity was one-third to one-sixth of the values for them.

This correlation was particularly well-demonstrable when we compared virulent strains Schu, No 503/840 and 543 to genetically related avirulent subcultures. Also to consider is the fact that the culture of Schu strain used in the first experiments, with some deficiency in virulence that apparently occurred in the course of frequent subcultures while working out optimum conditions for the next experiments, presented significant increase in catalase activity after a single passage in a guinea pig (59.5 versus 15.2 units in the first experiment).

In assessing the experimental data, however, we cannot fail to mention the significant fluctuations of catalase activity in strains within the race groups, which were probably related to the time that had elapsed from the moment a strain was isolated, duration and conditions of storage thereof, frequency of subculturing on nutrient media. Thus, Nearctic strains Cole and E-261, which were isolated in 1972, had higher V_0 (67.0 and 84.6, respectively) than strains No 8859 and 0-284, which were isolated in 1959 and had catalase activity of 46.6 and 52.7 units, respectively. Holarctic standard strain No 503/834 and Central Asian No 543, which had undergone numerous subcultures on media and periodic passages in sensitive animals, initially presented low activity of this enzyme (10.3 and 22.1, respectively), without appreciable change in virulence. We succeeded in raising this catalase level to 29.9 and 35.9 after 6 passages in guinea pigs. But already after 3 successive subcultures from yolk medium to agar of fresh fish hydrolysate, activity of catalase dropped to 27.5 in passage strain No 543.

Thus, we demonstrated appreciable differences in catalase activity of *F. tularensis* strains, which were related to geographic race and virulence thereof. At the same time, we demonstrated fluctuations of levels of this enzyme in cultures of the same strain, which were related to its age and condition.

Conclusions

1. Catalase was demonstrated in all *F. tularensis* strains, regardless of their virulence and geographic race.
2. Lowest catalase activity was found in typical Holarctic strains and highest in Nearctic ones; the Japanese variety of the Holarctic race and the Central Asian race occupied an intermediate place according to this parameter.
3. Attenuation of *F. tularensis* strains led to drastic reduction of catalase activity.

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QUANTITATIVE LEVELS OF IMMUNOGLOBULINS (A, G, M) IN THE PRESENCE OF
DIFFERENT FORMS OF TYPHOID FEVER

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[Article by V. Ya. Lashin, Ye. A. Markova, L. L. Khundanov and L. V.
Pogorel'skaya, Second Moscow Medical Institute imeni Pirogov]

[Text] At the present time, much importance is attributed to serum masses of immunoglobulins (Ig), since quantitative changes therein could affect the course and outcome of disease. For this reason, we felt it would be interesting to demonstrate quantitative changes in Ig among patients with typhoid fever, as well as carriers, in order to gain deeper understanding of some aspects of its pathogenesis. There has been insufficient coverage of this question in the literature.

We submit here the results of assays of total Ig in patients suffering from typhoid fever, those who recovered from it, were immunized and essentially healthy subjects, as well as carriers. We tested serum from 309 people (Table 1).

We used the method of simple radial immunodiffusion in gel with monoreceptor antisera against human globulins (IgA, IgG and IgM), produced by the Institute of Epidemiology and Immunology imeni Gamaleya and titrated by the international standard [5], for quantitative assays of different Ig classes.

Of the 96 cases of typhoid fever, 80 were men and 16 women. A mild course was observed in 10 patients (10.6%), moderately severe in 80 (84%) and severe in 6 (6.4%).

The diagnosis of typhoid fever was confirmed bacteriologically in 46 (48%) patients, serologically in 35 (36.4%), and in 15 cases (15.6%) it was determined on the basis of typical clinical signs and confirmed (in 11 out of 12 cases) by the positive result of the intracutaneous test with eberthin (the test was not performed on 3 patients).

At all stages of illness, the level of all classes was higher, with statistical reliability, in blood serum of patients with typhoid fever than in the control ($P < 0.05-0.001$). During the early convalescence period (up to the 10th day of normal temperature), the quantity thereof increased ($P < 0.05$), as compared to

to the acute period; prior to discharge it decreased, but still remained considerably greater than in healthy subjects ($P<0.05-0.001$).

Table 1. Levels of serum Ig (mg/ml) in different groups of tested people

Group tested	Number tested	Total tests	Immunoglobulin class		
			IgA	IgG	IgM
Typhoid fever cases	96	197	4.06 ± 0.13	20.40 ± 0.53	1.84 ± 0.08
Typhoid fever recurrence	9	21	4.67 ± 0.22	22.84 ± 1.46	1.96 ± 0.19
acute carriers	9	30	4.86 ± 0.29	24.85 ± 1.68	1.34 ± 0.13
chronic carriers	61	123	4.68 ± 0.12	31.5 ± 1.01	1.16 ± 0.03
a) phase of isolation	40	82	4.86 ± 0.13	33.55 ± 1.25	1.17 ± 0.09
b) latency phase	11	21	4.06 ± 0.22	28.0 ± 2.09	1.21 ± 0.06
c) transient	10	20	4.69 ± 0.3	26.19 ± 1.65	1.14 ± 0.07
With history of typhoid fever	43	43	2.95 ± 0.13	16.84 ± 1.29	1.12 ± 0.06
Immunized	59	59	2.75 ± 0.09	15.67 ± 0.6	1.35 ± 0.03
Healthy	32	32	2.34 ± 0.07	12.05 ± 0.51	1.16 ± 0.03
Totals	309	505			

Before being discharged from the hospital, 72 (75%) patients showed virtually no difference in IgM content, as compared to essentially healthy subjects, whereas it was elevated in 24 (25%) cases, which was most often associated with nonspecific bacteremia (in 16 out of 24). IgG and IgA content remained elevated in most patients with typhoid fever, and the increase was quite significant in 19 (19.8%) of them.

We failed to demonstrate an appreciable difference in levels of serum Ig in typhoid fever cases related to methods of laboratory confirmation and severity of the process. IgA level was somewhat higher only with mild course of the disease, as compared to severe and moderately severe.

IgA and IgG of individuals who subsequently became acute carriers were high at all stages of disease, until they were discharged from the hospital. In these cases, we demonstrated more often positive results with the intracutaneous test with Vi typhin, which is inherent in chronic carriers.

Since high levels of IgA and IgG were inherent in acute carriers, which also persisted prior to discharge from the hospital, we deemed it necessary to analyze the case histories of patients who presented higher IgA and IgG levels (19 out of 96) at the stage of late convalescence. Clinical analysis revealed that these patients did not differ in amounts of all Ig classes from acute carriers throughout the illness.

Thus, as shown by the results of our studies, one can detect, already at the acute stage of the disease, patients with high IgA and IgG levels, who could subsequently develop into chronic carriers.

With recurrence of typhoid fever (9 people), we demonstrated high levels of all classes of Ig at all stages of illness, but they dropped virtually to normal in the prerecidivation period.

In chronic typhoid carriers (see Table 1), we observed high levels of IgA and IgG, whereas their IgM content did not differ from that of healthy subjects.

These data confirm once more the previously expounded [2] thesis, according to which the carrier state develops against a background of proper immunological processes.

Table 2. Serum Ig content (mg/ml) in patients with typhoid fever (according to stages of illness)

Stage of illness	Number of patients tested	IgA	IgG	IgM
Acute	48	$3,12 \pm 0,11$	$17,47 \pm 0,65$	$1,68 \pm 0,74$
Early convalescence (up to 10th day of normal temperature)	55	$4,52 \pm 0,16$	$21,23 \pm 0,16$	$1,77 \pm 0,09$
Late convalescence (before discharge from hospital)	60	$4,42 \pm 0,13$	$19,73 \pm 0,7$	$1,52 \pm 0,9$
Healthy subjects	32	$2,34 \pm 0,07$	$12,05 \pm 0,51$	$1,16 \pm 0,03$
<i>P</i>		$<0,01$	$<0,001$	$<0,05$

Note: The data were submitted to statistical processing on a Minsk-22 computer

In our opinion, the data on Ig levels in so called transient carriers (see Table 1) are of special interest, since the existence of transient carriers has been the subject of debate heretofore. Quantitative levels of the Ig group and incidence of positive intracutaneous tests with Vi typhin, titers of O and Vi antibodies demonstrated in the passive hemagglutination reaction, morphological and histochemical changes in bone marrow [6] of subjects in this group differed insignificantly from the same parameters in latent carriers, for which reason the existence of transient carrier state is questioned.

At the active phase, IgG content was somewhat higher than in the latent phase, which is apparently related to isolation in most cases of the typical form of pathogen, which has high antigenic activity. The demonstrated identity of changes in quantitative levels of Ig in chronic carriers at the isolation [production] phase and latent stage is also indicative of the fact that these two processes are closely interrelated and that they are of some interest to determination of some aspects of pathogenesis of the chronic carrier state [2].

The set of tests we performed (intracutaneous test with Vi typhin, passive hemagglutination with separate demonstration of O and Vi antibodies, bacteriological and immunofluorescence tests of bone marrow, quantitative assays of Ig) enabled us to confirm the opinion we had advanced previously, i.e., that the carrier state is a phasic process, which could be present in the form of latent infection for some time [2]. On the basis of these data, it can be

concluded that among individuals who have had typhoid fever there should be some in whom typhoid infection is at the latency phase. For this reason, all subjects who had had typhoid fever were divided into two groups, depending on the results of the intracutaneous Vi typhin test. IgA and IgG content was considerably greater in individuals who reacted positively to Vi typhin than in individuals who had had typhoid fever and did not react to Vi typhin, as well as healthy subjects. A comparison of Ig content in individuals who had had typhoid fever and reacted positively to Vi typhin to chronic carriers in the latent phase failed to demonstrate any differences.

Thus, the results of our studies revealed that the high levels of IgG and IgA in both groups of patients are probably attributable to the same factor, intracellular persistence of the pathogen. This was also indicated by fluorescence in the presence of typhoid antigen in our immunofluorescence study of bone marrow, blood, feces and urine, as well as presence of Vi antibodies in blood serum (passive hemagglutination test), which is what gave us grounds to evaluate patients with positive intracutaneous tests as chronic carriers at a latent phase. It can be assumed that after having had typhoid fever, some of these individuals developed physiological equilibrium between the macro-organism and microorganism--latency phase, which changed to a period of positive findings [isolation] in certain conflict situations, which was demonstrated in surveys of typhoid fever sites or testing of autopsy material [1, 3, 4].

The levels of Ig of the classes in question were higher in individuals immunized against typhoid and paratyphoid than in healthy subjects ($P < 0.05$), but lower than in patients with typhoid fever.

Thus, our studies enabled us to determine that the chronic carrier state is a dynamic process, which is characterized by high IgA and IgG levels, that start to form already in the acute period. The set of laboratory tests enabled us to demonstrate 28% among those who had a history of typhoid fever who presented the latent phase of the carrier state, which confirms the previously voiced opinion that the actual number of carriers is considerably higher than presently recorded by bacteriological examination of people with a history of typhoid fever.

Conclusions

1. Clinical and laboratory findings in a study of quantitative levels of immunoglobulins (IgA, IgG and IgM) revealed that the chronic typhoid carrier state is characterized by high levels of IgA and IgG antibodies, whereas chronic carriers did not differ from essentially healthy subjects in levels of macroglobulin antibodies.
2. Chronic (phase of isolation and latency), transient carriers and those with history of typhoid fever who reacted positively to Vi typhin did not differ in IgA and IgG content, and it was high in them. Individuals who had had typhoid fever and did not react to Vi typhin did not differ from essentially healthy subjects with regard to levels of antibodies of the classes in question.
3. Demonstration of high levels of IgA and IgG antibodies, positive intracutaneous tests with Vi typhin, Vi antibodies and specific fluorescence with

typhoid antigen in the immunofluorescence analysis of blood, feces and urine of transient carriers raises doubt as to their existence.

4. The serum Ig content of patients with typhoid fever was higher, with statistical reliability, than in healthy and immunized subjects, and it was unrelated to either severity of illness or laboratory method by which it was confirmed.

5. The acute typhoid carrier state is characterized by high levels of IgA, IgG and IgM, and formation thereof occurs against a background of rather marked immunological reactivity in the patients, with overt lack of "shortage" of macroglobulin (IgM) antibodies.

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EXPERIMENTAL STUDY OF SUSCEPTIBILITY AND SENSITIVITY TO TULAREMIA INFECTION OF THE ARCTIC LEMMING (*DICROSTONYX TORQUATUS* PALL.)

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 7, Jul 76 (manuscript received 25 Nov 75) pp 110-112

[Article by I. P. Algazin and V. F. Marenko, Omsk Institute of Endemic Infections and Omsk Medical Institute imeni Kalinin]

[Text] It is generally recognized that the intensity of distribution of tularemia infection in wild animal populations is determined by the degree of their susceptibility and sensitivity to infection, as well as population size [4]. With regard to tularemia, only 1-2 groups of animals are significant as sources of infection and hold a leading place in the process of circulation of the pathogen in endemic sites [3].

In the Soviet Union, tularemia was first demonstrated in 1972-1973 among tundra rodents,--lemmings: in the Siberian lemming bacteriologically and serologically, and in the Arctic lemming serologically [2]. The obtained data prompted us to pursue an experimental study of susceptibility and sensitivity to infection of one of these two species, the Arctic lemming.

We used one of the strains previously isolated from lemmings, No 3917, for experimental infection of the animals. Studies of the main biological properties, which were pursued in the Tularemia Laboratory of the Institute of Epidemiology and Microbiology imeni Gamaleya, revealed that this strain is referable to the Holarctic race (*Francisella tularensis holarctica* Ols.) and is highly virulent for laboratory animals: minimum lethal dose for white mice and guinea pigs is 1 bacterial cell.

In the experiment, we used 15 adult Arctic lemmings weighing 58-60 g. We used each dose on three animals. Concurrently, as a control, we infected white mice (in the same quantities). The bacterial suspension was injected hypodermically in the right inguinal region in doses of 100, 10, 1, 0.1 and 0.01 bacterial cells (according to the standard of the State Institute of Standardization and Control of Medical Biologicals imeni Tarasevich). Rodents that died in the course of the experiment were dissected, their lymph nodes, viscera (spleen, liver, brain) and blood were cultured on McCoy's coagulated yolk medium, blood agar with fish hydrolysate and, as a control, beef-extract agar.

Regional and peripheral lymph nodes, in relation to injection site, spleen, liver, lungs, kidneys, heart and brain were submitted to microscopic examination, using conventional methods for histological treatment of tissues and the ordinary pan-optic stains (hematoxylin-eosin, picrofuchsin, Nissl and others).

Time of animal death as related to infective dose

Dose (bacterial cells)	Number of animals used in experiment	Number of deaths (on following days)				Survivals
		5th	6th	7th	8th	
100	3	1	2	0	0	0
10	3	0	3	0	0	0
1	3	0	3	0	0	0
0.1	3	0	0	2	0	1
0.01	3	0	0	0	0	3

The studies revealed (see Table) that time of rodent death after infection was related to magnitude of infective dose: with doses of 100, 10 and 1 bacterial cells lemming death occurred on the 5th-6th day, with 0.1 bacterial cell only 2 out of 3 animals died. Analogous findings were made on white mice: 0.1 bacterial cell elicited death of 1 out of 3 infected mice. Dissection of dead lemmings revealed a solid infiltrate at the site of injection of the bacterial suspension, in some places with suppurative dissolution of tissues, enlargement and hyperemia of regional lymph nodes, severe hyperemia of vessels in the surrounding cellular tissue, enlargement and consolidation of the spleen and liver. Cultivation of viscera, lymph nodes and blood on nutrient media (McCoy's and blood agar with fish hydrolysate) revealed that the tissues were strongly contaminated with *F. tularensis*. Analogous changes in internal organs and results of cultures were observed in the control group of animals.

The changes demonstrated in lemming organs by microscopic analysis were virtually the same as lesions demonstrated in control tests on white mice. Regional lymph nodes presented complete disappearance of usual structural pattern, vessels of both the follicular and paracortical, as well as medullary, zones were dilated showing signs of erythrocyte and plasma stasis. Massive necrotic sites involving the capsule were found against the background of extensive infiltration of lymph node tissue and capsules by neutrophil leukocytes. Analogous necrotic foci were demonstrable in peripheral lymph nodes and the spleen. In the liver, the alterations appeared more diffuse, due to severe and extensive large-droplet fatty dystrophy of hepatocytes with lysis of nuclei. Multiple bacterial embolisms were found in the hepatic sinusoids. The large vessels presented friable walls infiltrated by round-cell elements. The kidneys revealed severe hemodynamic disorders against the background of extensive granulation dystrophy of the epithelium of the convoluted tubules. Productive vasculitis was encountered in the myocardium, mainly of fine vessels, as well as microsites of necrobiosis and necrosis. The changes in the lungs consisted of dyscirculatory disorders with hemorrhages in the alveolar lumen. There was prevalence of vasocirculatory disturbances in the brain.

Absence of granulomatous reaction in organs was a distinctive feature of the morphological changes demonstrated in lemmings and white mice. Such changes

are typical of tularemia infection of white mice, and from the morphological point of view this determines their high susceptibility and sensitivity to *F. tularensis* [1]. It should also be noted that we were unable to demonstrate differences in morphological manifestations of tularemia in animals infected with different doses of the pathogen at the final stage of the process. Thus, we established that the Arctic lemming is highly susceptible to tularemia, which determines the importance of this species in epizootiology and epidemiology of tularemia in endemic sites of the tundra type.

Conclusions

A combined experimental study demonstrated that the Arctic lemming is highly susceptible and sensitive to infection by *F. tularensis*, and that it is referable to the first animal group (according to Olsuf'yev).

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INFLUENCE OF SITE OF APPLICATION OF BACTERIAL AEROSOL ON PATHOGENESIS AND SYMPTOMATOLOGY OF AEROSOL INFECTION

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[Article by K. G. Gapochko, Military Medical Academy imeni Kirov, Leningrad]

[Text] We previously demonstrated [2] that the dispersed fraction composition of bacterial aerosol is the principal factor determining retention and primary distribution of aspirated particles in respiratory organs. At the same time, it was established that different species of microorganisms have their own inherent most sensitive regions of the respiratory tract, which are the optimum portal of entry for aerosol infection [3, 9].

As a result, in the course of phylogenesis, determination was made for each infectious disease with an aerosol mechanism of infection of the portals of infection that caused development of a specific clinical pattern. Relatively limited segments of respiratory organs, through which the pathogen penetrates into the body, are inherent in some of them, whereas for others the entire respiratory tract could be the portal of entry of infection.

Diphtheria, scarlet fever and ornithosis (psittacosis) are among the few diseases with phylogenetically limited portals of infection.

Without mentioning the wound variants of disease, clinically marked forms of diphtheria (throat, nose, larynx, conjunctiva) and scarlet fever, in the case of aerosol infection, develop only if the pathogens are applied and accumulate in the upper respiratory tract, conjunctiva and trachea, where the principal pathological focus is formed, which determines the symptomatology of disease. Considering the data on retention and primary distribution of bacterial aerosol in respiratory organs, it can be concluded that infection with diphtheria and scarlet fever occurs by relatively large-particle aerosol with particles of more than 5-10 μm , 80-100% of which are retained and localized in the upper respiratory tract and trachea; the trachea is stricken when even larger particles, over 25 μm in size are impacted on it.

Ornithosis (psittacosis), on the contrary, is characterized by portals of infection situated in the deep parts of the lung, and lung tissue is the site of primary localization and accumulation of the pathogen [12]. McGavran [28] made a comprehensive study of the pathogenesis of psittacosis on *M. mulatta* monkeys,

which were infected with highly dispersed (1-1.5 μ m) liquid aerosol of the Borg strain. The infective dose constituted 4000-5000 LD₅₀ for mice with intracerebral infection and 100 LD₅₀ for monkeys.

Pathohistological examination of the lungs revealed that aerosol infection of monkeys was consistently associated with development of microfocal pneumonia, the foci of which were similar and associated with hyperplasia of tracheo-bronchial nodes. The pathological process in the lungs started with involvement of respiratory bronchioles equipped with rudimentary alveoli, into which highly dispersed aerosol penetrated. In the second postinfection week, the inflammatory process spread concentrically to peripheral alveoli. On the 10th-16th day, the center of the lesion was localized in respiratory bronchioles and adjacent alveoli, filled with exudate consisting of mononuclears and neutrophils with a small amount of fibrin.

Thus, it can be concluded that, unlike diphtheria and scarlet fever, in the case of ornithosis the portals of infection are only the respiratory parts of the lungs, in particular the respiratory bronchioles, into which highly dispersed (1-2 μ m) aerosol particles penetrate and 50-60% retained, causing the typical clinical pattern with localization of the process in the lung parenchyma.

In addition to diseases for which the portals of infection are relatively limited, there are numerous nosological forms, for which although there is a most sensitive zone in respiratory organs, the pathogen is capable to invade the body over the entire respiratory tract, thus eliciting a specific disease. This applies to influenza, measles, smallpox, plague, tularemia, anthrax, brucellosis, glanders, melioidosis, Q fever and deep mycosis, for which the aerosol route of infection is the only one, while the localization of portals of infection has an appreciable influence on the initial phase of pathogenesis and course of disease, often determining its clinical form.

As we know, the clinical course of tularemia, as reflected in the generally recognized classification of Rudnev [10], is closely related to mechanism of infection. Later on, it was determined that, when *M. mulatta* monkeys are infected with highly dispersed aerosol (1-8 μ m), the primary pathological process and bacterial reproduction are demonstrable, as in psittacosis, in the region of the respiratory bronchioles, where specific bronchiolitis develops, which spreads to adjacent lung tissue and involves the alveolar spaces causing development of microfocal pneumonia, which is confirmed histologically and roentgenologically. At the same time, when the animal inhaled monodispersed tularemia aerosol with particles ~18 μ m in diameter no lung changes were demonstrable, while the portals of infection were the nasopharynx with involvement of regional lymph nodes, from which the pathogen "broke through" into the blood stream causing hematogenic dissemination and animal death [23].

It must be assumed that the oculobubonic form of tularemia can develop when the conjunctiva is impacted by relatively large (≥ 25 μ m) particles of dust aerosol. The angina-bubonic form, though it is related to water and food outbreaks, can also occur by aerosol infection by dust aerosol, with particles over 5-10 μ m in size, which are retained in the nasopharynx and, in a sufficient dosage, can infect the tonsils.

The pneumonic variant of the pulmonary form develops as a result of penetration of particles no more than 1-2 μm in size into respiratory bronchioles and alveoli. Since 40-50% of them are retained upon aspiration, they penetrate expressly into these deep parts of the respiratory tract, causing a specific process in the lung parenchyma.

Rather similar findings are made in the case of aerosol-caused plague. As we know, for a long time the question of pathogenesis of the pulmonary form of this disease was the subject of bitter debates. Bazarov [18] succeeded in producing pneumonic plague by means of insertion of infected cotton sponges in the nasopharynx of guinea pigs, which resembled pneumonic plague in man; however, this experiment could not always be reproduced. At the same time, intratracheal infection of guinea pigs [1, 17, 22, 27] and monkeys consistently produced primary pneumonic plague in these animals.

In addition, when a suspension of *Y. pestis* was packed into the nose, throat and conjunctiva, as well as with infection of animals (mice, guinea pigs, monkeys) by aspiration of liquid aerosols, the degree of dispersion of which was not determined in the experiments, the experimenters obtained rather contradictory results. Primary pneumonic plague developed only in an insignificant part of the animals, whereas most of them presented the bubonic form of the disease, with involvement of cervical lymph nodes, acute tracheobronchitis and resulting in fatal septicemia; in some animals, septicemia developed without local lesions in the region of the portals of infection [8, 25, 29, 30, 35].

Numerous experimental data, as well as pathoanatomical findings made at autopsy on patients who expired of plague, enabled Kulesha [6, 7] to formulate his theory of pathogenesis, according to which the portals of entry of infection are the tonsils and lymphoid [tonsillar] ring, from where the pathogen penetrates hematogenically into the lungs and elicits secondary pneumonic plague. The experimental work of Druett et al. [21] provided conclusive clarity to the question of pathogenesis of aerosol-caused plague and pneumonic plague [21]. Primary bronchopneumonia, followed by fatal septicemia, developed in guinea pigs after infection with highly dispersed aerosol, with particles on the order of 1 μm in diameter. At the same time, infection of guinea pigs with aerosol consisting of 12 μm particles led to involvement of lymph nodes of the head and neck, which also ended with fatal septicemia, which developed even faster than in the presence of pneumonia. Infection of monkeys (*M. rhesus*) with liquid aerosol, with 1-12 μm particles, led to development of primary lobular pneumonia.

Thus, it was demonstrated that after aerosol plague infection, the pathogenesis and clinical signs of disease depended, like tularemia, on localization of portals of infection.

An analogous conclusion is also valid for anthrax. After aspiration of a sufficient dose of highly dispersed aerosol ($\leq 2 \mu\text{m}$) by monkeys, *B. anthracis* spores penetrate into the deep parts of the lungs, invading the lymphatic system on the level of the respiratory bronchioles. This route of infection is not associated with development of primary pathological changes in the lung parenchyma and deep respiratory tract which, in this case, are merely intact portals of infection. Nevertheless, under such conditions, against the background of

subsequent sepsis, there is development of signs of disease, the distinctive feature of which is an intrathoracic complex: edema of the lungs and mediastinum, hemorrhagic inflammation of hilum, tracheobronchial and mediastinal lymph nodes (mediastinitis), hemorrhagic pleurisy. When monkeys are infected with a sufficient dose of coarsely dispersed ($\geq 12 \mu\text{m}$) aerosol, particles of which are retained in the upper respiratory tract, expressly these parts of the respiratory tract become the portals of infection. When relatively small doses are used for infection, the process may start with the lymphogenic phase, which is clinically associated with lymphadenitis of regional nodes and edema of the face and head; however, it ends with typical hematogenic dissemination, with involvement of internal organs, sepsis and intoxication. With massive infection doses that penetrate into any part of the respiratory tract, or if there is attenuation of the patient's immunobiological resistance, there may not be a lymphogenic phase of anthrax infection and bacteremia can develop right away, evolving into sepsis. Finally, if there is some injury in the respiratory tract of an infected person or animal, or else functional deficiency is present, conditions appear for infection of expressly these regions and for development of primary pulmonary [pneumonic] anthrax [13, 14-16, 19-21, 32].

These data offer convincing evidence of the fact that localization of portals of infection and, consequently, the particulate dispersion of an aerosol have a substantial effect on the clinical course of numerous infectious diseases. Although this question has been studied experimentally for a narrow range of forms of infection, clinical observations convince us that this pattern is much more widespread.

Influenza virus, which is pneumo-epitheliotropic, is always localized in respiratory organs after aspiration. It is believed that expressly the respiratory tract serves as the site of viral reproduction, as well as development of diverse local pathological processes specific to influenza. However, it is assumed that, in some cases, the process could move to the lung parenchyma, causing development of pneumonia differing in form, localization and severity. It is stressed that primary influenzal pneumonia can also develop [4]. Should it not be assumed that, by analogy to previously described diseases, infection by highly dispersed droplet aerosol creates optimum conditions for penetration of virus into deep parts of the lungs and development of primary influenzal pneumonia, whereas coarsely particulate dust aerosol is fixed in the upper respiratory tract, eliciting a less severe form of infection.

Analogous considerations are also valid for measles and primary measles pneumonia.

Adenoviral diseases, for which the aerosol route is the main route of infection, occur in three clinical forms: pharyngoconjunctival fever, acute respiratory disease and pneumonia [5]. We cannot rule out the possibility that, in this case too, along with the distinction of pathogenic properties of different strains of adenoviruses and condition of the macroorganism, primary application and site of pathogen invasion play a substantial role in development of a particular form of the disease.

The portals of entry of infection in the case of aerogenic Q fever infection are primarily the respiratory organs, conjunctiva, as well as gastrointestinal

tract into which the aerosol can penetrate. The typical distinction of the disease is the absence of primary pathological changes at the site of penetration of the pathogen in the organism. At the present time, it is believed that Burnet's *Coxiella* [rickettsia] does not have any definite tropism for different organs and tissues; nevertheless, according to the latest observations, patients with pulmonary lesions in the form of interstitial pneumonia still constitute 5-10% of all clinically marked cases [12]. For this reason, it is again valid to assume that coarsely dispersed aerosol is retained in the upper respiratory tract without eliciting pathological changes in the lung parenchyma, whereas highly dispersed aerosol penetrates into respiratory regions causing development of pneumonia. In view of the fact that spontaneous aerosol infection of man with Q fever occurs chiefly by inhalation of coarsely dispersed aerosol dust, we can understand the relative scarcity of pneumonic forms of the disease.

Thus, we have examined a rather representative group of infectious diseases, for which the localization of portals of entry of aerosol infection had a more or less appreciable effect on the clinical course of illness, causing even development of independent clinical forms in a number of cases.

However, there are nosological forms, for which localization of the portals of entry of infection in the region of the respiratory organs does not affect the clinical course of disease, and as a rule there are no pathological changes at the site of invasion. This applies to transmissible diseases reproduced by experimental aerosol infection: Venezuela and North American equine encephalomyelitis, Rocky Mountain fever and yellow fever. The same group includes typhoid fever (experiments on chimpanzees), botulism (poisoning), as well as many diseases which are not transmitted by the aerosol route in nature. In all these cases, the pathological process is characterized by absence of primary changes in the respiratory system; however, it reproduces entirely the specific clinical signs inherent in these infections in the case of natural routes of infection [24, 26, 31, 33, 36-38].

In conclusion, it should be stressed that there have been particularly comprehensive studies to data of the pathogenesis of aerosol infections caused by highly dispersed aerosols. It was established that the region where infectious particles settle has a significant influence on the outcome of host and micro-organism interaction. As we know, there are no purely physical mechanisms for removal of settled particles in the region of the respiratory bronchioles, since the ciliate and mucous layer ends in the region of connection of terminal and respiratory bronchioles. At the same time, it is expressly here that micro-organisms are subject to phagocytosis by alveolar macrophages. The bronchiolar region has a particular abundance of blood and lymphatic vessels, as well as lymphoid tissue and rudimentary alveoli along the course of the respiratory bronchus. Numerous experimental studies have shown that highly dispersed aerosols are retained in expressly this region, where development of the pathological process begins [34].

The general patterns pertaining to particle and dispersion of bacterial aerosols, portals of infection and clinicopathogenetic distinctions of disease are not applicable to each specific case. Thus, in particular, when there is a very high concentration of finely dispersed aerosol, infection may occur through the upper respiratory tract, rather than the lungs, or else simultaneously

through both these parts of the respiratory tract. At the same time, relatively large aerosol particles, particularly in the case of high concentration and prolonged exposure, could penetrate into the deep parts of the lungs and elicit a pathological process there. It is also known that large aerosol particles can disintegrate in respiratory organs, become finer and thus penetrate deep into the respiratory tract. Finally, one must also take into consideration the active migration of aerosol particles, which occurs due to the activity of the ciliate epithelium and phagocytes.

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EXPERIMENTAL MIXED TULAREMIA AND OMSK HEMORRHAGIC FEVER INFECTION OF
WATER VOLES

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[Article by N. B. Dunayev, Omsk Scientific Research Institute of Endemic
Infections]

[Text] With each year, the natural resources of West Siberia and the Extreme North are being developed more and more intensively, for which reason there is more contact between the public and natural sites of zoonoses, to which man is susceptible. Tularemia and Omsk hemorrhagic fever (OHF) are among the most widespread diseases among animals in West Siberia. Although these diseases have been studied rather well, many aspects of their endemicity are still unclear. Thus, there is a need for further investigation of the question of mixed tularemia and OHF infections, epizootics of which had been repeatedly recorded in previous years in populations of muskrats and water voles in West Siberia [5]. No doubt, the possibility of infection of the local inhabitants engaged in agricultural work and other groups is important during epizootic periods. Thus, Sizemova [9] observed concurrent OHF and tularemia in 23 people in an endemic site.

Continuing the studies we started previously [2-4, 6], we investigated mixed tularemia and OHF infection in water voles who, according to our data [1] and those of other authors [7, 8, 10, 11], are not only the principal sources of tularemia but carriers of the OHF virus.

We used a total of 70 water voles in experiments and as a control. They were infected with the Balangul strain of OHF virus, subcutaneously in the internal aspect of the left hind thigh (in a dosage of 100 LD₅₀/ml) and strain No 503 of *F. tularensis*, also subcutaneously in the right hind thigh (in a dosage of 1 bacterial cell according to the standard of the Institute imeni Tarasevich). In order to track development of the infectious process, presence and nature of viremia, distribution of viral antigen and *F. tularensis* in organs and tissues, as well as distinctions of pathomorphological changes in organs, we collected material at different postinfection times, and for this purpose animals were sacrificed, dissected, placing lymph nodes and pieces of organs in 10% neutral formalin and Carnoy solution for histochemical examination.*

*The morphological examination was made by V. F. Marenko, assistant in the Department of Pathological Anatomy, Omsk Medical Institute.

Concurrently, we prepared impression smears for fluorescence microscopy. We used the bacteriological (McCoy medium) and biological (on white mice) methods of demonstrating and isolating *F. tularensis*. Virological tests were made on mongrel white mice weighing 6-8 g and on a culture of chick embryo fibroblast tissue (CEF).

The first experimental group of water voles was infected with OHF virus and *F. tularensis* at a 3-day interval in order to track the course of mixed infection at the height of development of both infections, since with this interval one observes a coincidence of time of maximum development of clinical manifestations of both tularemia and OHF.

Table 1.
Results of virological examination of
water vole organs

Interval between giving OHF virus and <i>F. tularensis</i>	Day, after <i>F. tularensis</i> infection, of testing	Virus titer in blood	Demonstration of viral antigen by method of fluorescent antibodies				
			brain	spleen	liver	kidney	blood
3 days	4	10^{-4}	+	+	+	+	+
	6	10^{-5}	++	++	++	++	++
	7	10^{-4}	++	++	++	++	++
	11	10^{-3}	+	+	+	+	+
7 days	8	10^{-2}	+	+	+	+	+
	10	10^{-3}	+	++	++	++	++
	12	10^{-5}	+	++	++	++	++
	14	10^{-4}	+	+	+	+	+
	15	10^{-2}	+	-	+	+	+

Note: 10^{-2} - 10^{-5} --blood dilutions of
1:100-1:100,000

Visible symptoms of disease appeared in the water voles 3 days after administration of *F. tularensis* (6th day after inoculation of OHF virus), and they consisted of decrease in motor activity and refusal of feed. The animals died 8 days after inoculation of *F. tularensis*.

The results of the virological examination revealed that the titer of OHF virus in blood constituted 10^{-4} 4 days after infection and reached a maximum on the 6th day (10^{-5}). We then observed a decline of viremia, and on the 11th observation day viral titer constituted 10^{-3} (Table 1). The method of fluorescent antibodies revealed viral antigen on the 4th, 6th, 7th and 11th days in the brain, spleen, liver, kidneys and blood (see Table 1).

Bacteriological investigation of development of the tularemia process enabled us to isolate *F. tularensis* cultures 3 days after infection from a regional and peripheral lymph node, as well as spleen; after 4 days from the liver and after 8 days from blood. Analogous results were obtained on subsequent days up to the end of the observation period (Table 2). Using the method of fluorescent antibodies, *F. tularensis* was demonstrated in a regional lymph node 1 day after infection. *F. tularensis* was demonstrated in all organs examined from the 3d day after infection to the end of the observation period, and in blood starting on the 4th day (see Table 2).

The biological method yielded analogous data: *F. tularensis* was demonstrable 1 day after infection in regional and peripheral lymph nodes; in addition to lymph nodes, it was also demonstrated in the spleen and liver after 3 days and in blood after 4 days. Similar results were obtained on subsequent days (see Table 2).

Table 2. Results of testing water vole organs for tularemia

Interval between giving OHF virus and F. tularensis	Day of test after giving F. tularensis	Demonstration of F. tularensis														
		bacteriological method					biological method					fluorescent antibody method				
		regional lymph node	peripheral lymph node	spleen	liver	blood	regional lymph node	peripheral lymph node	spleen	liver	blood	regional lymph node	peripheral lymph node	spleen	liver	blood
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Macroscopically, 1 day after giving F. tularensis (4th day after inoculation of OHF virus), regional and peripheral lymph nodes on the side of OHF inoculation were enlarged and invested in a hyperemic capsule. Dilatation of blood vessels was observed at the site of injection of F. tularensis. We must also mention that there was some enlargement of the spleen and dilatation of blood vessels of the meninges and intestine. Dissection after 3 days revealed more significant changes in lymph nodes on the side of administration of OHF virus, considerable enlargement of the spleen, necrotic foci in the liver and areas of hepatization in the lungs. The meningeal blood vessels were dilated, while brain tissue appeared plethoric on sections. The macroscopic changes on the 4th day after giving F. tularensis were all of the same type, unlike those demonstrated previously, with progression of signs of hemorrhagic diathesis and appearance of petechial hemorrhages in the intestinal mucosa, meninges, liver and lungs. Analogous changes, which were associated with appearance of numerous necrotic sites, were observed upon dissection on the 8th day after administration of F. tularensis.

Morphological examination of animal organs 1 day after infection with F. tularensis failed to demonstrate any changes that were typical for either infection. After 3-4 days, signs of tularemia infection appeared in the liver and spleen: granuloma, with necrosis in some of the latter. Appreciable hemocirculatory disturbances were found in other organs. Against this background, structural disturbances of the walls of some small vessels, which are typical of OHF, were noted in brain tissue. This was associated with separation of fibers and plasma saturation. The endothelium proliferated and flaked off, edematous areas appeared around the vessels. We were unable to detect diapedesis of erythrocytes or neuronal damage at this time. But, on the 8th day, there was prevalence of signs of tularemia infection in organs, with predominant

involvement in the process of lymph nodes, spleen and liver. There was a drastic increase in number of granulomas subject to breakdown. The vascular disorders were characterized by circulatory disturbances in all areas. The lungs presented severe interstitial-hemorrhagic pneumonia. There was prevalence of circulatory disturbances in brain tissue, and signs of wall lesions inherent in OHF were noted only in some of the fine vessels.

The second experimental group of water voles was infected with OHF virus and 7 days later, when viremia reached a maximum, with *F. tularensis*. Visible signs of disease appeared on the 6th day after giving OHF virus, whereas deaths were observed 7 and 8 days after inoculation of *F. tularensis*.

Virologically, OHF virus was demonstrable in blood in a titer of 10^{-2} 8 days after administration. The titer rose somewhat, to 10^{-3} , after 10 days. Viremia reached a maximum (10^{-5}) on the 12th day. Thereafter, viral titer dropped: 10^{-4} on the 14th day and 10^{-2} on the 15th (see Table 1).

By the method of fluorescent antibodies, viral antigen was demonstrable starting on the 8th day after administration of OHF virus in all organs examined; there was none in the liver on the 14th postinfection day and in the spleen on the 15th postinfection day (see Table 1).

We tracked development of the tularemia process for 8 days, since all of the water voles died by this time. Bacteriologically, we succeeded in isolating *F. tularensis* cultures only on the 5th postinfection day, from regional and peripheral lymph nodes, liver, spleen and blood. Analogous findings were made on subsequent observation days (see Table 2).

We succeeded in demonstrating *F. tularensis* by the method of fluorescent antibodies in regional and peripheral lymph nodes one day after infection, additionally also in the spleen and liver after 3 days and blood after 5 days. On subsequent days, *F. tularensis* was consistently demonstrable in all examined organs. Data obtained by the method of fluorescent antibodies were identical to the results of studies by the biological method (see Table 2).

Macroscopically, the changes in organs 1 day after infection with *F. tularensis* (8th day after infection with OHF virus) were identical to changes observed in animals infected with OHF virus alone, which were examined at the same time; hemodynamic disorders and changes in lymph nodes, both regional to the site of administration of OHF virus and peripheral, on the side of inoculation of virus, were the most prominent. There was hyperemia and enlargement of lymph nodes, considerable plethora of internal organs and enlargement thereof. In the intestine we observed numerous petechial hemorrhages. Examination after 3 days revealed the following, in addition to the above-mentioned changes: consolidation of the lungs, which assumed a dark red color, intensification of plethora of meninges and brain matter with appearance of some hemorrhages. These macroscopic changes were also found in animals 5 days after infection with *F. tularensis*, whereas 7 and 8 days later we additionally found considerable changes in lymph nodes situated on the side of injection of *F. tularensis*, which consisted of enlargement, hyperemia and necrosis.

Morphologically, 1-2 days after administering *F. tularensis* the animals presented only circulatory disturbances in all organs, whereas lymph nodes,

mainly on the side of administration of OHF virus, presented hyperplastic reactions in the form of diffuse proliferation of reticuloendothelium with appearance of "sinus catarrh." The lungs demonstrated lymphohistiocytic infiltration of interalveolar septa. After 5 days, severe dystrophic changes in the liver, which consisted of diffuse granular dystrophy of hepatocytes, were added to these changes. No specific tularemia granulomas were demonstrated. However, on the 7th day, tularemia granulomas of typical structure were found in the lymph nodes, liver and particularly the spleen, which underwent necrosis on the 8th day, with fusion into extensive necrotic fields. Other organs showed circulatory disturbances, to which were added in brain tissue lesions to the walls of fine vessels, which are typical of OHF.

Conclusions

1. Infection of water voles with pathogens of OHF and tularemia at a 3-day interval elicited development of both infections, without appreciable increase in severity of the pathological process.
2. Administration of *F. tularensis* 7 days after infection with OHF virus aggravated the course of mixed infection which, according to morphological data, is attributable to intensive progression of the tularemia process at the final stage of the observation period.

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FIFTIETH ANNIVERSARY OF DISCOVERY OF TULAREMIA IN THE USSR

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[Article by N. G. Olsuf'yev, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences]

[Text] 1976 marked the 50th anniversary of the discovery of tularemia in the USSR. In view of this, it is justified to list in brief the main results of studying and controlling this infection in our country.

Tularemia was first diagnosed in 1926 near Astrakhan by Suvorov, Vol'ferts and Voronkova, physicians in the plague-control organization. The outbreak involved about 200 cases, and the diagnosis was confirmed by isolation of the pathogen from 3 patients. In the course of the laboratory work, 4 workers contracted the disease and their serum agglutinated the isolated cultures. The correct diagnosis of tularemia was instrumental in general and intensive development of medical science in the young Soviet republic, development of a network of scientific research institutes dealing with microbiology in our country, in particular the Mikrob Plague-Control Institute, which was staffed by highly qualified specialists.

There is every reason to believe that tularemia had been encountered in our country even prior to 1926, but was diagnosed as some other disease. Thus, there is no question that the outbreak of diseases in 1877 near Astrakhan, which struck about 200 people diagnosed as having a mild case of plague was referable to tularemia.

Already in the early years after discovering tularemia, the medical service attracted attention because of the considerable epidemic outbreaks, which struck hundreds and thousands of people. To date, tularemia is known in 14 Union republics (with the exception of Kirghiz SSR). The distribution of endemic tularemia sites has been established from the western frontiers of the USSR (Kola Peninsula, Karelian ASSR, Baltic region, Kaliningrad Oblast, western Belorussia, the Ukraine and Moldavia) east to the Chukotsk Peninsula, Kamchatka, Sakhalin and Maritime Kray. Tularemia sites to the north are distributed in some areas up to the polar circle and even partially beyond (Gremikha in Murmansk Oblast, Pechora River delta, mouth of the Ob River, Taymyr, northern Yakutia), whereas in the south they are encountered in Transcaucasia and some republics of Central Asia.

The numerous outbreaks of the disease among people, duration of illness and slow convalescence, as well as the wide distribution of endemic sites of this infection in our country, made it necessary to pursue a comprehensive study of tularemia and work out measures to control it.

It is opportune to recall on the 50th anniversary of the discovery of tularemia in the USSR the names of those with whom the main stages of investigation of this infection in the USSR are closely linked: Suvorov, Vol'ferts, Zarkhi, Golov, Khatenever, Sinay, Tumanskiy, Tiflov, El'bert, Gayskiy, Stradomskiy, Rudnev, Bilibin, Drobinskiy, Berinskaya, Karpov, Somov, Pokrovskaya, Vereninova, Novikova, Gromashevskiy, Mayskiy, Yelkin, Faybich, Burgasov, Saltykov, Tinker, Drozhevskina, Popov, Shmuter, Yemel'yanova, Dunayeva, Tsvetkova, Savel'yeva, Uglov, Ananova, Meshcheryakova, Rodionova, Dobrokhotoy, Kudelina, Pronina, Mashkov, Sil'chenko, Myasnikov, Borodin, Nekipelov, Bozhenko, Petrov, Maksimov, Naumov, Kucheruk, Domaradskiy, Pilipenko, Aykimbayev, Akhundov, Kondrashkin, Ravdonikas, Antsiferov, Brikman and a number of other researchers.

With respect to institutions, in which tularemia was investigated, we must mention, first of all, the All-Union Scientific Research "Mikrob" Plague-Control Institute, Institute of Experimental Medicine, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Central Institute for Advanced Training of Physicians, the plague-control institutes of Irkutsk, Stavropol, Rostov-na-Donu, Central Asia, as well as Tomsk Scientific Research Institute of Vaccines and Sera, Rostov Institute of Epidemiology, Microbiology and Hygiene and Omsk Institute of Endemic Infections. The network of oblast, kray and republic-level plague-control stations, which were reorganized in 1955 into departments of particularly dangerous infections of sanitary and epidemiological stations, played a major role in the study and control of tularemia.

In the period in question, Soviet researchers accumulated basic information about the etiology, epidemiology, epizootiology, laboratory diagnosis, pathology, symptomatology, treatment, immunology and prevention of this disease. This knowledge, along with many years of experience in the control of tularemia, served as the foundation for succeeding in lowering drastically the incidence of this infection in our country.

With respect to etiology, comprehensive studies were made of morphological tinctorial, cultural, biochemical, antigenic and pathogenic properties of the pathogen of tularemia. For the first time, by means of electron microscopy, structural details of the cell were identified, determination was made of some enzymatic properties, presence of endotoxin and certain other factors of virulence, nutrient requirements, conditions of in vitro and in vivo reproduction, etc. Sensitivity of the pathogen to exogenous factors and disinfectants was determined. A substantial contribution of Soviet researchers to the study of tularemia was classification of its pathogen as an independent genus, Francisella, and establishment of three geographic races--Holarctic, Central Asian and Nearctic (American)--which differ in pathogenicity and other properties. Variants have been distinguished within the races, in particular, strains of the Holarctic race were found to differ in sensitivity to erythromycin and other antibiotics.

Comprehensive and in-depth investigation of endemicity and epidemiology of tularemia is one of the most important achievements in the study of this

infection in the USSR. This was aided by the teaching of Academician Pavlovskiy on endemicity of human diseases. Extensive data on the geography of sites endemic to tularemia were accumulated and submitted to generalization. The natural sources of tularemia infection--water rats, common voles, house mice, hares, muskrats, etc.--that are the main ones for the USSR were identified and studied comprehensively. Secondary infection carriers were defined. In all, 82 species of wild vertebrates were found to contract tularemia in the USSR. Studies were pursued of the patterns of the epizootic process among rodents. Experimentally, the infection was modeled in a significant number of wild mammals and separation thereof into three groups was validated on the basis of their susceptibility and sensitivity to infection, which is important to definition of their role in maintaining endemic tularemia sites. Demonstration was effected of the great importance of Ixodes ticks in transmission and prolonged preservation of tularemia infection in nature. It was also proven that blood-sucking Diptera (mosquitoes, horseflies and clegs [or gadflies]) played an important role as mechanical vectors of this infection.

Spontaneous infection with *F. tularensis* has been established in the USSR for a total of 74 species of bloodsucking arthropods. Determination was made of the important role of water in transmission of infection among amphibian rodent species, as well as of spontaneous infection of hydrobionts--mollusks, caddis flies and other invertebrates (total of 14 species) in aquatic tularemia sites.

A classification of endemic sites of tularemia based on landform was compiled as a result of accumulation of information about these sites, which included seven main types; the distribution of different types was determined, as well as routes of circulation of infection in them, etc. Methods were developed for forecasting fluctuations in rodent population size, since they are the main sources of infection, and this is important to planning of preventive measures. Views have been formulated on paleogenesis of *F. tularensis*.

Comprehensive studies have been pursued of conditions of infection and routes of transmission of infection to man. A correlation was established between mechanism of infection and clinical forms of disease, which made epidemiological analysis much easier. The patterns of the epidemic process were identified, and the correlation in it between biological and social factors was shown. A classification was developed of epidemiological types of disease (outbreaks), which comprises nine main types and several variants. Most types and variants were described for the first time by Soviet authors, and the classification is the only one in the world to be so complete.

A substantial contribution has been made to the study of pathogenesis and symptomatology of tularemia. Experimental studies have been made of the stages of spreading of *F. tularensis* in the macroorganism, from the site of invasion to penetration of internal organs; determination was made of intensity of reproduction and level of accumulation of the pathogen in different organs and tissues; morphological, immunological and, in part, pathophysiological macroorganism reactions have been defined. A distinction has been made of phases of the infectious process and a pathogenetic description thereof furnished. The localization of primary lesions and subsequent course of illness were shown

in relation to portals of entry of infection. The routes and intensity of discharge of the pathogen in the environment were tracked. A comprehensive study was made of symptoms of tularemia in man and the fullest new clinical classification was prepared of this infection, which includes six main forms (according to localization of primary lesions). The latter have been confirmed experimentally on laboratory animals. Wide studies were pursued of therapy methods, and the high efficacy of antibiotics--streptomycin, chlorotetracycline, etc.--was demonstrated.

In the area of laboratory diagnosis of tularemia, methods of demonstrating this infection in man, as well as wild and domestic animals, bloodsucking arthropods, in water, grain, fodder and other objects, were successfully developed. Determination was made of the high sensitivity and reliability of the biological test on laboratory animals (white mice, guinea pigs) for demonstration of pathogen in material under study. Immunological reactions were investigated comprehensively, and their high specificity was demonstrated. The tularin skin test, which was proposed by Soviet researchers, found wide use in combination with serological tests for diagnosing tularemia in man, whereas at the present time it is used extensively (in the epicutaneous variant) as well for determining the intensity of postvaccinal immunity. A method of immunofluorescence was developed for detection of pathogens in tested material or antibodies in serum. Much attention was devoted to the study of the highly sensitive hemagglutination test in serological diagnosis of tularemia. A new and rather effective method of detecting tularemia epizootics was proposed on the basis of this reaction and introduced to practice, involving demonstration of antigen in bird pellets, droppings of predatory mammals, as well as decayed or mummified rodent carcasses.

As a result of investigation of the antigenic structure of *F. tularensis*, it was established that it has two antigen complexes: membrane or "superficial-somatic" (Vi) and "somatic" (O). A link was established between virulent and immunogenic properties of the pathogen with the membrane complex. The chemical structure of different antigenic substances was studied. On the basis of comprehensive investigation of variability of *F. tularensis*, methods were developed for attenuating it and obtaining highly immunogenic vaccine strains that are safe to man and provide a high level of protection against any natural route of infection. Development of a live tularemia vaccine was a major achievement of Soviet scientists, and such a vaccine was not developed abroad. Strain No 15 of Gayskiy turned out to be the most valuable, and it has been used in our country to immunize the public for over 30 years. Comprehensive studies were made of the patterns of formation of immunity to tularemia due to immunization or infection. Determination was made of the mechanisms of immunity related to cellular and humoral factors. Methods were successfully developed for mass cultivation of tularemia vaccine on artificial nutrient media, particularly with use of aeration, lyophilization of vaccine, which assures prolonged preservation, and finally an epicutaneous method of administering the vaccine, which made it much easier to immunize people. In the last few years, a highly effective method of administering the vaccine with a jet injector was developed. It was shown possible to combine immunization against tularemia and brucellosis, plague, smallpox and other infections, without attenuating the immunizing effect of each constituent.

The high efficacy of live tularemia vaccine made it possible to use it as the principal means of preventing tularemia. It was learned that immunization of the public rapidly arrests an outbreak of any origin. The duration of post-vaccinal immunity made it possible to move in tularemia sites to scheduled inoculation of the public, with reimmunization every 5 years. It was learned that by immunization alone, one could reliably prevent epidemic outbreaks (but not sporadic cases) in sites of intensive tularemia epizootics among rodents, but for this it is necessary that 90% or more of the public exposed to the risk of infection consist of immune individuals, as ascertained by the tularin skin test. By means of mass scale immunization of the public inhabiting in regions that are enzootic for tularemia, as well as urban residents who travel there, it is possible by means of vaccination combined with other measures to achieve a stable decline of incidence of tularemia among people to isolated cases per year within an oblast, kray or republic. Vaccination is supplemented with such methods as eradication of rodents, prevention of their access to sources of water, foodstuffs, etc. Methods have been developed for eradication of the water rat, common vole and other rodents, which are the principal sources of infection, as well as Ixodes ticks, in order to depress endemic tularemia sites. However, the difficulty of these measures limits their use. Eradication of tularemia sites in some places was the result of developing large reservoirs, with flooding of the habitat of the water rat, thorough drainage [filling] of swamps and other reservoirs, overall plowing of land covering large areas, etc.

The incidence of tularemia among humans in the USSR varied considerably in different years. In the prewar period (1936-1940), when there was no mandatory record of this disease, according to the far from complete data there were an average of 6000 cases of tularemia per year (maximum of 11,000 in 1940). During the Great Patriotic War and the first few postwar years, 40,000-65,000 cases on the average were recorded annually (maximum 136,000 in 1945). Mass scale immunization of the public was instituted in several oblasts starting in 1946, and by 1950 immunizations were used in virtually all regions of the USSR enzootic for tularemia. Since that year, there began a drastic decline in incidence of tularemia among people, and in the last decade (1965-1975) it has held at low levels, an average of about 100 cases per year (maximum of 271 in 1967 and minimum of 22 cases in 1970). Only sporadic cases were noted in most administrative territories, and minor outbreaks much less often. A significant decline in morbidity was achieved, in spite of the tularemia epizootics among rodents, which were observed annually in some republics and oblasts. It can be considered that immunization combined with other measures prevents an average of at least 10,000 cases per year in our country.

The brilliant results obtained in the USSR in control of tularemia require reinforcement in the future, by means of thorough immunization and other measures. Tularemia is an endemic infection, and lowering morbidity among people as a result of mainly immunization does not mean that the endemic sites have been suppressed, since they are widely distributed, quite persistent and, in a number of cases, highly active.

We can advance the following main problems of investigation of tularemia for the next few years: 1) further accumulation of data on nosogeography, with more precise delineation of territories with problem sites and determination of

properties of pathogen distributed there; definition of epidemiological breakdown [zoning] of territories; 2) distinctions of the epidemic process and preventive measures during periods of low human morbidity; 3) conditions of existence, epizootiological and epidemiological forecasting, and means of eradicating different types of endemic [enzootic] sites; 4) genetic distinctions factors of immunity and patterns of variability of *F. tularensis*; providing for stability of vaccine strains stored under laboratory conditions; 5) taxonomy, antigenic structure and biochemical properties; 6) further investigation of pathogenesis and mechanisms of immunity; 7) refinement of laboratory diagnostic methods.

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EXPERIMENTAL STUDY OF PNEUMONIC FORMS OF PLAGUE, TULAREMIA AND PSEUDOTUBERCULOSIS

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[Article by L. V. Samoylova, A. S. Vasenin, S. A. Piontkovskiy and Ye. A. Plotnikova, All-Union Scientific Research "Mikrob" Plague-Control Institute, Saratov]

[Text] Clinical and epidemiological observations have established the aerosol route of plague and tularemia infection. It was confirmed experimentally that aerosol infection of animals with pathogens of plague, tularemia and pseudotuberculosis is possible [6, 8, 10, 13, 15, 16, 19, 20, 24, 26].

Experimental investigation of the distribution and reproduction of pathogens of plague, tularemia and pseudotuberculosis in the case of infection via the respiratory tract is important to understanding of the pathogenesis of the pulmonary forms of the diseases. There are few works dealing with the dynamics of the infectious process with aerosol infection of animals with the above-mentioned pathogens [5, 12, 21]. Moreover, in the case of tularemia infection, no studies had been made in the first few postinfection hours, and there are only data concerning the increase in number of bacteria after 24 h or later. Fukui et al. [21] observed death of bacteria within 6 h after aerosol infection of guinea pigs with the virulent Alexander strain of *Y. pestis*, which was grown at 26°, but when bacteria grown at 37° and in vivo were given to guinea pigs, no reduction in number of bacteria was demonstrable within the early post-infection hours.

Our objective was to make a bacteriological study of the dynamics of the infectious process and determine the patterns of reproduction in guinea pigs of virulent pathogens of plague, tularemia and pseudotuberculosis after aerosol infection. Quantitative evaluation was made of the degree of infection of the lungs, regional lymph nodes, blood and viscera by pathogens of these infections. We used virulent strains of *Y. pestis*--No 231, *F. tularensis*--No 503--and *Y. pseudotuberculosis* No 1421 (serotype I) to infect animals. The pathogens were cultivated at optimum temperatures for growth on nutrient media: 28° for *Y. pestis*, 37° for *F. tularensis* and 22° for *Y. pseudotuberculosis*.

Prior to the main experiments, we determined LD₅₀ for guinea pigs with aerosol infection and, for comparison, subcutaneous infection. LD₅₀ for the pathogens of plague, tularemia and pseudotuberculosis constituted 480 (380-603), 38 (30-56)

and 7×10^4 (35×10^3 – 14×10^4) bacterial cells with aerosol infection. The figures were 8.9 (6.7–11.5) for the pathogen of plague and 13×10^4 (1×10^5 – 175×10^3) for the pathogen of pseudotuberculosis in the case of hypodermic infection. We could not determine LD₅₀ for *F. tularensis* in the case of subcutaneous infection, since 1 DCL [clinically lethal?] constitutes only 1 bacterial cell. It is apparent from these data that LD₅₀ with aerosol infection was many times higher than for subcutaneous infection, as determined for plague and tularemia. LD₅₀ for the pathogen of pseudotuberculosis was almost twice as high with hypodermic infection as aerosol. The obtained data confirm the opinion advanced by Ogarkov and Gapochko [9], who believe that animal susceptibility to different methods of infection varies, and that different nosological forms have their own specific distinctions. For this reason, in the main experiments we used doses calculated only for aerosol infection.

In the bacteriological study of dynamics of size of bacterial populations, we conducted 2–3 tests with each pathogen. Guinea pigs weighing 250–300 g were submitted to aerosol infection in doses of 50 to 90 LD₅₀. The size of aerosol particles constituted 0.5 to 5 μ m in 91.5%. The animals were dissected after 30 min, 1, 3, 6, 12, 24 h, 2, 3, 4, 5, 6, 7, 8 and 10 days. We suspended pieces of organs under sterile conditions, ground them in a mortar with sterile sand, the suspension in saline (1:5) was titrated and plated on appropriate solid nutrient media, and it was also used for biotests. *Y. pestis* and pseudotuberculosis were grown on Hottinger's agar with sodium sulfite, *F. tularensis* on fish-yeast agar with glucose, cysteine and defibrinated rabbit blood. In addition, to determine the quantity of *F. tularensis*, we used the biological method of "titration" of organ suspension in white mice [10]. After incubation of cultures for 2–3 days, we counted the colonies, with scaling to 1 g organ weight and 1 ml blood. According to the results (see Table), a specific pattern was observed with all three infections. In the first few hours after aerosol infection with the pathogens (3, 6 and 12 h), the number of bacteria in the lungs diminished, as compared to the initial level. With regard to pseudotuberculosis, this pattern was also demonstrated in a study of regional lymph nodes. When animals were infected with plague and pseudotuberculosis, the number of bacteria began to exceed the initial level between the 12th and 24th hour. After infection with tularemia, an increase in number of bacteria occurred between the 24th and 48th postinfection hour. Consequently, the number of *Y. pestis*, *F. tularensis* and *Y. pseudotuberculosis* not only failed to increase for 12 h, but even diminished significantly.

Elimination of bacteria could be attributed to the mechanisms of spontaneous immunity. Bacteria are eliminated from the lungs through the blood and lymphatic systems. In part, they are submitted to phagocytosis by blood macrophages and microphages. Phagocytosis of bacteria by alveolar macrophages, which have hydrolytic lysosome enzymes and perform the function of a fine aerosol filter [1, 22, 25], is another factor of natural resistance. Surfactant (surface-active film), which inherently has bactericidal properties, maintains surface tension of pulmonary alveoli and prevents transudation of fluid into the alveolar lumen, is considered to be significant as a factor of natural protection of the lungs [3, 17, 18, 23]. The outcome of the phagocytic reaction depends not only on functional state of phagocytes of blood, alveolar macrophages and pulmonary surfactant, but largely to extent of aggressive properties in the pathogen, its dosage and virulence. The highly virulent pathogens of plague and tularemia,

even in low doses, overcome rather rapidly the natural resistance. In the case of a mildly virulent pathogen, there is more marked resistance. Thus, with use of large doses of *Y. pseudotuberculosis* for infection, there was slower increase in number of bacteria in the lungs after 24 h and slower subsequent accumulation of the pathogen than when the animals were infected with the pathogens of plague and tularemia.

Results of bacteriological examination of guinea pigs submitted to aerosol infection with the pathogens of plague, tularemia and pseudotuberculosis

Time of testing	Quantity of bacteria per gram organ tissue (log)														
	plague					tularemia			pseudotuberculosis						
	1	2	3	4	5	1	2	4	1	2	3	4	5		
1/2						3,0									
1	3,1		2,3						4,8						
3	2,7					2,0			4,6		2,9				
6	2,6					2,0			3,9		2,0				
12	2,2					2,0	0,3	1,0	4,3		2,0				
24	4,4		3,2	3,1	2,1	2,0	0,3	1,0	5,4		2,8				
2	7,3	2,3	6,3	4,1	3,6	5,0	1,3	5,6	6,3		4,2				
3	11,4	3,4	6,4	5,0	4,3	6,0	2,3	6,6	6,5		5,07				
4	10,8	2,0	9,2	5,0	3,6	7,3	2,3	8,87	8,2	2,0	5,8	3,1	3,04		
5						10,3	2,3	9,6	8,1	2,1	6,9	5,6	3,5		
6									8,5	2,9	7,7	5,5	4,7		
7									9,04	2,3	7,4	6,3	5,3		
8									9,5	3,2	7,4	6,3	5,7		
10									10,2	4,2	10,1	9,04	8,04		

Key: 1) lungs 2) blood 3) regional node 4) spleen 5) liver

An important pathogenetic factor is generalization of the process and septicemia related to it, which occur on the 1st-2d day with the pulmonary form of plague and tularemia, and on the 4th-5th day with the pulmonary form of pseudotuberculosis. As a result, intoxication of the organism develops, which leads to impairment of vital functions and development of endotoxic shock. The toxic factor emerges very early [2, 7, 11], due to the large absorbing surface of the lungs. When septicemia develops rapidly, fewer septic changes occur in the spleen and liver that could be demonstrated by morphological examination. Such changes do not have time to develop. But our quantitative estimation of bacteria in organs enabled us to demonstrate already developed generalization. Animal death was observed on the 3d-5th day due to pneumonic plague and pneumonic tularemia and on the 7th-10th day due to pneumonic pseudotuberculosis. Death occurred in the presence of tens of billions of bacteria per gram lung and tens of millions and thousands of bacteria per gram of other internal organs. At the stage of generalization and septicemia in the case of pneumonic plague and pneumonic tularemia, no more than several thousand bacteria per milliliter accumulated in blood. Consistent demonstration of bacteria in blood in the presence of generalized infection and the quantity of bacteria in blood and organs warranted the belief that there are much fewer bacteria in blood than in organs. Bacteremia is the consequence of accumulation of bacteria in the primary focus (lungs) and other organs (spleen, liver). An enormous bacterial reservoir is created in organs as a result of their exponential accumulation.

It should be noted that utterly different correlations with regard to localization of bacteria in organs exist in the case of infection through the respiratory tract than with transcutaneous infection. In the latter case, bacteria are concentrated mainly in the spleen, liver and lymph nodes, i.e., in organs with a profusion of reticuloendothelial cells [4, 14]. With aerosol infection, the bacteria first accumulate in the lungs, then penetrate into blood and are localized in the spleen, liver, regional lymph nodes and other organs, then again return into blood. More bacteria accumulate in the lungs, sometimes twice as much as in the spleen and liver.

These studies enabled us to demonstrate a consistent decline in number of bacteria in the lungs, as compared to their initial number, for the first 12 h after aerosol infection with plague, tularemia and pseudotuberculosis. The range of accumulation of the pathogens were determined quantitatively in organs and blood at different stages of the infectious process. The demonstrated dynamics of size of the bacterial population could be used in preparing programs of specific and pathogenetic treatment.

Conclusions

1. A consistent decrease in quantity of bacteria in the lungs was demonstrated for the first 12 experimental hours after aerosol infection of guinea pigs with *Y. pestis*, *F. tularensis* and *Y. pseudotuberculosis*, after which elimination of bacteria stopped and they began to accumulate in lungs and other organs until the animals died.
2. Generalization of the infectious process and related septicemia occurred on the 1st-2d day in the case of pneumonic plague and pneumonic tularemia, on the 4th-5th day in the case of pneumonic pseudotuberculosis.

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STUDY OF PHAGOCYTIC ACTIVITY FOR *F. TULARENSIS* IN HIGHLY SENSITIVE ANIMALS
SUBMITTED TO MIXED INFECTION

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[Article by T. N. Dunayeva and K. N. Shlygina, Institute of Epidemiology and
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[Text] It is known that deviations from typical development of disease are observed in the case of mixed infections. We established that in animal species with little sensitivity to tularemia preinfection with other pathogens activated factors of protection and caused faster development of specific immunity reactions. In such cases, the animals withstood infection with massive lethal doses of *F. tularensis*.

The intensity of septicemia diminishes and duration of diseases increases in species that are highly sensitive to tularemia after being subjected to mixed infection [5, 6]. The mechanism of these deviations in the course of tularemia have not been identified.

Phagocytosis is one of the important factors of protection of organisms. We studied ingestive and digestive function of blood neutrophils in common voles and white mice infected with *Y. pseudotuberculosis* and guinea pigs infected with *Salmonella*. We used the opsonophagocytic reaction (OPR) with homologous bacteria and *F. tularensis* by a previously described method [7]. We ran the reaction simultaneously on animals with different duration of infection and control (intact) specimens. We examined 4-5 animals in each group. Blood was taken from the heart of voles and mice after sacrificing them and in vivo from guinea pigs.

On the day of running the OPR, animals with different duration of primary infection, as well as control animals, were infected subcutaneously, homolaterally with 0.3 ml dilution of *F. tularensis* culture (strain No 503) containing 10 bacterial cells per milliliter, which corresponded to 1 D_{clm} [median clinically lethal dose?].

Digestive and ingestive neutrophil function increased not only with regard to homologous bacteria, but *F. tularensis* in voles infected with *Y. pseudotuberculosis* in a dosage of 100,000 bacterial cells (Table 1). This intensification

was very distinct on the 4th-10th postinfection and less noticeable on the 14th day of pseudotuberculosis infection. Functional integrity of leukocytes persisted for the duration of the experiment in the reaction with *Y. pseudotuberculosis*, which was related to formation of specific immunity in the infected voles. In intact voles, the index of ingestion of *F. tularensis* showed a chronographic 2.66-3.54-fold increase, and in the reaction with *Y. pseudotuberculosis* a 1.33-1.55-fold increase, which reflected the difference in sensitivity of voles to infection with these pathogens.

Table 1. Phagocytic activity of blood neutrophils of voles infected with pseudotuberculosis

Postinfection day	OPR with <i>F. tularensis</i>						OPR with <i>Y. pseudotuberculosis</i>					
	ingestion index		index ratio, 60 min:30 min	phago- cytic neutro- phils,%		ingest. index		index ratio, 60 min:30 min	phago- cytic neutro- phils,%			
	30 min	60 min		30 min	60 min	30 min	60 min		30 min	60 min		
4th	11,5	5,7	0,49	46	23	10	4,2	0,42	40	17		
7th	12,2	9,2	0,75	49	37	10	6,0	0,60	40	24		
10th	12,7	6,0	0,48	51	24	8,7	7,2	0,82	35	29		
14th	9,2	16,5	1,78	37	66	6,2	4,2	0,65	25	17		
Intact: control for 4th, 10th and 14th experimental days	4,2	14,3	3,54	17	57	4,5	6,0	1,33	18	24		
control for 7th day of experiment	6,0	16,0	2,66	24	64	7,7	12,0	1,55	31	48		

OPR was done on white mice on the 7th and 11th days after infection with the pathogen of pseudotuberculosis in a dosage of 10,000 bacterial cells. The ingestion index for *F. tularensis* after 30 min constituted 15.5 and 20.5, and in intact mice 13.2. The reaction indexes rose after 60 min by 1.07-1.5 times in mice infected with *Y. pseudotuberculosis* and by 2.17 times in intact ones. In the reaction of *Y. pseudotuberculosis*, the index ratio constituted 1.37 on the 7th day of development of infection and 0.59 on the 11th day. In intact mice, the ingestion index increased by 1.98 times.

All 78 voles infected with *F. tularensis* on the 4th, 7th, 10th and 14th day after administration of the pathogen of pseudotuberculosis died, and most at the same time as control animals--on the 6th-7th day; 13 voles died on the 8th-10th day. However, in the case of mixed infection, there was a decrease in intensity of invasion of organs and particularly blood, and this was distinctly manifested when the interval between infection was 4-7 days (Table 2). In 11 voles, the results of microscopy of organ smears were negative, and presence of *F. tularensis* was established only by cultivation on blood agar of Yemel'yanova with penicillin.

Maximum invasion, scored at 3-4 points [9], which is typical for species that are highly sensitive to tularemia, was demonstrated in the spleen of 70.5%

of the voles, in the liver in 52.2% and in blood in only 39.7%. In all cases, invasion of organs and blood of 20 control voles was scored at 3-4. With mixed infection, organ smears showed vacuolized monocytes and in blood neutrophils with phagocytized *F. tularensis*. Phagocytic cells were found in 26% of the 78 voles that died, in the liver of 36.3% and blood of 32.4%. With intervals of 4, 7, 10 and 14 days between infections, phagocytic cells were demonstrated in 63.6, 57.6, 38.8 and 36.7%, respectively, of the voles that died. In the presence of tularemia infection alone, phagocytic cells were encountered very rarely, due to total lysis thereof and the fact that *F. tularensis* filled the entire field of vision.

Table 2. Intensity of invasion by *F. tularensis* of organs of dead voles submitted to mixed infection

Days between infections,	Number of voles	Demonstration of <i>F. tularensis</i> in smears					
		spleen		liver		blood	
		+	-	+	-	+	-
4	22	17	5	12	10	5	17
7	19	15	4	13	6	7	12
10	18	16	2	15	3	13	5
14	19	19	—	17	2	15	4
Totals	78		11(14.1%)		21(26.7%)		38 (48.7%)
Control	20	20	—	20	—	20	—

There were 74 deaths out of the 80 mice infected with *F. tularensis* 3, 5, 7 and 14 days after giving them *Y. pseudotuberculosis*: 38 died at the same times as control animals, on the 6th-7th day, 22 on the 8th-9th day and 14 mice on the 10th-12th day. There was very intensive invasion by *F. tularensis*, and bacteremia was scored at 1-2 in only 7 mice. Phagocytosis of *F. tularensis* was observed in smears of spleen in 6.8% of the 74 animals that died, in the liver in 17.7% and blood in 19.1%. When the intervals between infections constituted 3, 5, 7 and 14 days, phagocytosis was observed in 35.3, 40.0, 27.7 and 1.3% of the mice, respectively. The surviving 6 mice were infected after 20 days with a minimum dose of *F. tularensis*, and they died on the 7th-10th day.

The decline of septicemia and the incidence of phagocytosis coincided with the period of increase in nonspecific digestive activity of leukocytes during the first 7 days of development of pseudotuberculosis infection. Later on, phagocytosis, which acquired specificity for *Y. pseudotuberculosis*, had no appreciable effect on reproduction of *F. tularensis* in vivo and in the animals.

As can be seen in Table 3, we tracked an increase in phagocytic activity in against *F. tularensis* for a long time in guinea pigs infected with *S. typhimurium* in a dosage of 500 million bacterial cells: even 54 days after infection, we still observed some bacteriostatic effect of phagocytosis, as compared to intact animals.

Table 3. Phagocytic activity of neutrophils of guinea pigs infected with *S. typhimurium*

Postinfection day	OPR with <i>F. tularensis</i>					OPR with <i>Salmonella</i>				
	ingestion index		Ind. ratio 60 min:30 min	phago-cytic neutrophils, %		ingest. index		Ind. ratio 60 min:30 min	phago-cytic neutrophils, %	
	30 min	60 min		30 min	60 min	30 min	60 min		30 min	60 min
3	12,0	8,5	0,72	49	34	29,0	27,0	0,94	80	80
7	15,8	5,7	0,35	63	23	29,5	24,0	0,80	74	58
9	17,0	13,5	0,96	68	54	39	35,2	0,80	90	95
14	12,0	7,7	0,66	48	31	38,8	32,6	0,84	90	90
22	12,6	5,5	0,43	34	26	24,4	22,2	0,95	71	70
33	10,5	18,5	1,76	42	60	48	31,5	0,60	98	80
54	7,3	12,3	1,67	30	48	48,6	47,0	0,90	100	100
Intact	6,5	16,2	2,67	25	65	10,6	21,8	2,38	42	76

A total of 24 guinea pigs were infected with *F. tularensis* 3, 7, 9, 14 and 22 days after *S. typhimurium*. In 12 of them we used the OPR on the 5th day after infection with the pathogen of tularemia. Intensification of digestive activity of neutrophils was found in 8 guinea pigs: the ratio between ingestions indexes after 60 and 30 min of the reaction ranged from 0.47 to 0.93. In 4 guinea pigs, the decrease in bacteria contained in neutrophils was mild: after 60 min the ingestion index remained unchanged or increased by 1.6 times. In control guinea pigs infected only with *F. tularensis*, the ingestion index showed a 1.8-2.4-fold chronographic increase.

Table 4. Comparative time of death of guinea pigs demonstrating digestive activity of neutrophils

Day of death	Numb. of guinea pigs	Ingestion index ratio, 60 min:30 min		
		>1	1	<1
4-11	22	21	1	0
12-16	26	8	4	14
17-25	15	2	1	12

Nine control guinea pigs died of tularemia on the 8th-13th day, on the average on the 11.6th day. Of the 24 guinea pigs submitted to mixed infection, 19 died: 7 on the 10th-13th day, 4 on the 14th-15th day and 8 guinea pigs died on the 17th-25th day. Mean time of death ranged from the 13.6th to 16.3d day in different groups. Blood serum of some of the animals that died on the 17th-25th day showed antibodies to *F. tularensis* in titers of 1:10, 1:40 and 1:160. No antibodies were found in guinea pigs that died on the 13th-14th day.

No doubt, the protracted course of tularemia after mixed infection was related to intensification of phagocytosis. This can be clearly seen by comparing time of death of the 63 guinea pigs infected with *F. tularensis* at different intervals after salmonellosis and listeriosis, as well as controls, to the OPR parameters obtained on the day of tularemia infection (Table 4).

The data about surviving guinea pigs are of independent interest. In previous experiments, cases were observed where guinea pigs survived tularemia combined with other infections--salmonellosis, pseudotuberculosis and listeriosis. They demonstrated antibodies to *F. tularemia* and protracted (up to 40-118 days) *F. tularensis* carrier state [6].

The five guinea pigs that survived in this experiment were examined on the 32d day after administration of *F. tularensis*, on the 35th-46th day after infection with *Salmonella*. It was determined that all of the animals had had salmonellosis: positive agglutination test with serum dilution of 1:160-1:2560. When the agglutination test was done with tularemia diagnosticum and separate examination was made of lymph nodes, spleen, liver and lungs by means of biotests on white mice, no tularemia was demonstrable. We ran the OPR on three guinea pigs prior to infection, and it showed intensive digestion of *F. tularensis*. The ingestion index constituted 16, 19 and 12 with 30-min exposure and it dropped to 7, 8 and 8 after 60 min. The ratio between indexes constituted 0.43, 0.42 and 0.66. The number of bacteria ingested by 25 neutrophils constituted 86, 115 and 51 in the first smears and dropped to 21, 28 and 31 in the second ones. Intensification of digestive capacity of leukocytes could have caused destruction of a small number of inoculated *F. tularensis* at the adaptation phase, which is what prevented development of infection. Such instances had been occasionally observed in prior experiments with mixed infection [5, 6]. There were considerably more frequent descriptions of cases of survival of guinea pigs and white mice infected with *Y. pestis* combined with other pathogens [1, 2, 4, 8, 10]. Bacteriological and serological examination of surviving specimens failed to demonstrate evidence of plague. Probably, in these cases too, "cross phagocytosis" [3, 8] had a devastating effect on *Y. pestis* during the period of its adaptation in the animal. Not infrequently, such animals are considered "insusceptible," "resistant" or insensitive to infection. In reality, in such cases there is a negligible elevation of threshold of susceptibility due to temporary activation of the nonspecific factor of phagocytotic protection. Reinfection of these animals elicited development of fatal infection.

Conclusions

1. Infection of animals that are highly sensitive to tularemia with sublethal doses of pathogens of pseudotuberculosis and salmonellosis elicits an increase in ingestive and digestive activity of leukocytes with regard to homologous bacteria and *F. tularensis*.
2. Activation of the nonspecific protection factor, phagocytosis, causes deviations from the typical course of tularemia, prolonging the disease, reducing intensity of septicemia and, occasionally, raising the susceptibility threshold, which is related to death of small doses of the pathogen at the adaptation phase.

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A CASE OF TULAREMIA IN AMUR OBLAST

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 6, Jun 77 (manuscript received 21 Sep 76) pp 101-103

[Article by N. M. Busoyedova, O. A. Antip'yeva, A. I. Gulyayev, P. T. Moroz and V. F. Dzyubak, Khabarovsk Plague-Control Station, Department of Particularly Dangerous Infections of the Amur Oblast Sanitary and Epidemiological Station]

[Text] Endemic tularemia sites have not been found in Amur Oblast, although individuals who reacted positively in the allergic (average of 0.3-0.4%) and serological (1.4%) tests were encountered among the indigenous population [1]. In 1974, specific antibodies to *F. tularensis* were demonstrated in a titer of 1:80 in the passive hemagglutination reaction (PHAR) for the first time in field mice and 1:1280 in brown [or Norway] rats which were caught in Arkharinskiy Rayon. These data, as well as the closeness to Amur Oblast of the demonstrated endemic sites of tularemia served as grounds for assuming that they existed there. This assumption was confirmed when a case of tularemia was recorded in September 1975 in the village of Dzhalinda in Skovorodinskiy Rayon.

We submit an excerpt from the case history.

Patient A., 21 years old, became sick on the morning of 3 September 1975. He experienced headache, general weakness and detected a tender swelling in the left inguinal region. He went to the hospital and was admitted on 17 September.

Upon admission, 2 lymph nodes, 3×2 and 1.5×1.5 cm in size, were palpable in the left inguinal region, they were not tender, of solid elastic consistency and not adherent to the skin. Similar lymph nodes (the size of a bean) were found on the left side of the neck over the posterior surface of the nutatory [sternocleidomastoid?] muscle and on the right, under the scapula. No traces of portals of entry of infection were found. Upon admission and during the patient's 2-month stay in the hospital his temperature was normal. There were no complaints of weakness, perspiration, poor appetite or impaired sleep. The disease was in a mild form.

Blood tests on 18 Sep and several times later (24 Sep, 8 Oct and 12 Nov) failed to reveal deviations from normal.

Between the time he was admitted and 23 Oct, the patient received only multiple vitamins and ascorbic acid. After the diagnosis of tularemia was established, antibiotics were ordered: tetracycline in a dosage of 200,000 AU 4 times a day from 24 to 31 Oct, and streptomycin in a dosage of 500,000 AU [active units] twice a day from 31 Oct to 10 Nov. While the patient was hospitalized, there was a reduction in size of lymph nodes on the neck and under the scapula, and they were barely palpable at the time of discharge (19 Nov).

At first, the multiple enlarged lymph nodes caused suspicion of a blood disease; for this reason, the enlarged inguinal nodes were completely removed on 2 October, and one of them was submitted to histological examination, while the other was sent for bacteriological testing.

Macroscopic examination revealed the following: the nodes consisted of bean-like elements 2.5×2 cm in size, of flexible consistency, watery and yellowish on section. Microscopic examination revealed that the capsule of the lymph node was fibrous, structural pattern was intact, fine and rather large foci were found mainly under the capsule, some of them in the form of nodules consisting of epithelioid and clear reticular cells. Tiny necrotic foci filled with disintegrating leukocytes were visible in the center of many nodules. In one section, an element was found in the nodular region that resembled a giant multinuclear cell.

The origin of the above changes in the lymph node was not quite clear; they resembled pseudotubercular nodules and in some places presented elements of tuberculous eruptions, so that it was necessary to bacteriologically rule out pseudotuberculosis, as well as to give antituberculosis medication.

After this diagnosis was made, the Khabarovsk Plague-Control Station was informed about the patient and on 23 October (49th day of illness) blood was taken for serological testing for tularemia and pseudotuberculosis; the cutaneous tularemia test was made and one of the resected lymph nodes was taken for bacteriological examination. The allergy test was positive (hyperemia, infiltrate 1.0×1.0 cm in size) after 24 h. Blood serum revealed antibodies to *F. tularensis*: titer of 1:160 in the agglutination reaction (AR) and 1:2560 in the PHAR. When samples were taken again on the 69th and 97th days, antibody titer was 1:1280 in the PHAR, 1:160 and 1:40, respectively in the AR. No specific antibodies to the pathogen of pseudotuberculosis were demonstrated.

Suspension of lymph node tissue was used for hypodermic infection of 4 white mice, which died on the 9th-10th day. Dissection revealed hyperemia of vessels of subcutaneous cellular tissue, enlargement and hyperemia of regional (inguinal) lymph nodes; 2 mice presented enlargement and consolidation of the spleen and the other 2 failed to demonstrate any visible changes in the spleen.

A culture was isolated from the animals that died, which was typical in tinctorial and cultural-morphological properties for *F. tularensis*: the pathogen did

not grow on the usual nutrient media, it was agglutinated by antitularemia agglutinating serum (from the Irkutsk Plague-Control Institute) in a dilution of 1:800 (serum titer 1:3200), did not contain citrulline ureidase and did not ferment glycerin. The minimum lethal dose of culture for white mice and guinea pigs constituted 1 bacterial cell with subcutaneous infection. The species of the strain was confirmed by the Tularemia Laboratory of Irkutsk Plague-Control Institute.

Thus, on the basis of the positive tularin test, presence in the patient's blood serum of specific antibodies to *F. tularensis* and isolation of the pathogen from a lymph node, the diagnosis of tularemia was made on patient A. This case is unquestionably local, since the patient had not traveled anywhere for 1 year. During the last 10 days of August he often walked barefoot, in a T-shirt and shorts, and had noticed numerous mosquito bites.

Results of retrospective screening of the inhabitants of Dzhalinda
for tularemia

Group examined	Number of people tested	Positive results of			
		tularin test		PHAR	
		absolute	%	absolute	%
Timber management workers	123	8	6.4	8	8
School children	303	1	0.3	1	-
Totals	829*	71	8.5	64	61

*Translator's note: Numbers are copied as they were in source, but they do not seem to jibe.

Among the tested inhabitants of Dzhalinda, 8.5% showed a positive tularin test and their blood serum presented specific hemagglutinating antibodies in titers of 1:20 to 1:320 (see Table).

The results of this screening warrant the assumption that the subjects with positive reactions were infected somewhere in the vicinity of Dzhalinda. This is indicated by the fact that of the 8 timber management workers who had positive reactions 4 were indigenous inhabitants and the others had lived in the village for 10-15 years. It should be noted that positive reactions were demonstrated in a large percentage of the groups that were the most exposed to the risk of infection because of their occupation (see Table). The history of subjects with positive test results showed no mention of prior illness that clinically resembled tularemia.

Thus, in the vicinity of the village of Dzhalinda, near the national frontier, there is an endemic tularemia site, which can apparently be classified as the eastern, floodplain-swamp type, since this locality consists to a significant extent of the swampy region along the banks of the Amur River.

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DYNAMICS OF IMMUNOGLOBULIN M AND G SYNTHESIS IN THE PRESENCE OF EXPERIMENTAL TULAREMIA

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 7, Jul 77 (manuscript received 27 Sep 76) pp 123-126

[Article by I. S. Meshcheryakova, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The established heterogeneity of antibodies and possibility of differentiating them according to immunoglobulin classes make it possible to assess more completely and deeply the immunological changes that occur with various infections or vaccinal processes, determine involvement of IgM and IgG in serological reactions, as well as to determine the predominant link between different classes of immunoglobulins and specific diseases.

At the present time, the patterns of synthesis of different classes of immunoglobulins have been studied in relation to many viral and bacterial infections. These studies made it possible, in particular, to demonstrate that the presence and levels of IgG in serum could serve as an indicator of activity and severity of an infectious process, permitting more accurate determination of the etiological factor of disease and differentiate with sufficient reliability between postinfection and postvaccinal antibodies [1-3, 5-8].

Such differentiation of antibodies and identification of the role of different classes of immunoglobulins to the immunological process are quite important in tularemia infection. The serological and allergic diagnostic methods that exist at the present time for this disease do not permit detection of differences in immunological reactions of individuals who sustained infection and those who were immunized, which makes it difficult to assess the epidemiological situation in endemic tularemia sites and detect previously unknown sites of infection.

We conducted a study on rabbits weighing 2.5-3.0 kg in order to make a comparative evaluation of the nature and dynamics of antibodies synthesized in the presence of infectious and vaccinal processes referable to tularemia. The animals were infected with virulent *F. tularensis* strains No 55 (*Francisella tularensis mediaasiatica*) and 503/840 (*F. tularensis holarctica*), while vaccine strain 15/10 NIIEG [Scientific Research Institute of Epidemiology and Hygiene] was used for immunization (5 animals per group). The virulent strains were injected subcutaneously in a dosage of 10 million bacterial cells and

the vaccine subcutaneously in a dosage of 1 billion bacterial cells. Dynamic tests were made on the animals' serum: after 7, 14, 21, 30 days and then at 1-month intervals for up to 6 and 11 months in different groups of animals. In all we tested more than 100 samples of serum by two serological methods: agglutination reaction (AR) with bacterial diagnosticum and passive hemagglutination reaction (PHAR) with erythrocytic diagnosticum.

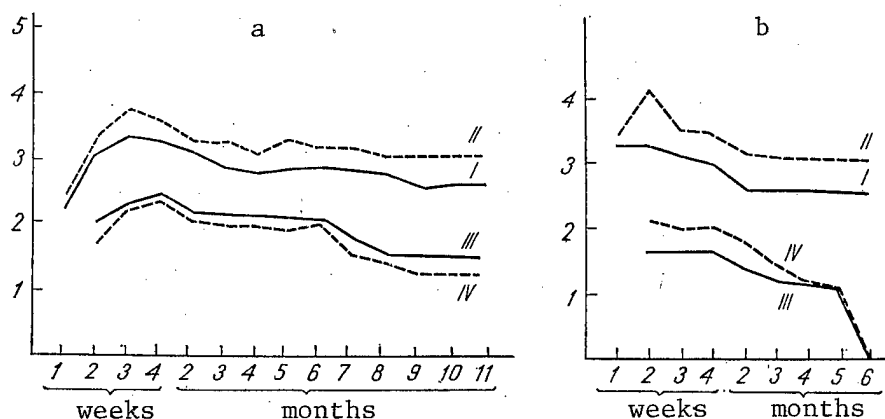
We used the simplest and most accessible method of treating antibody sulfhydryl bonds with reducing agents in order to differentiate between IgM and IgG. This led to selective breakdown of macroglobulins without having an appreciable effect on activity of microglobulin antibodies. We used cysteine hydrochloride (by the method of Chernokhvostova [4]) and 2-mercaptoethanol (by the method of Deutsch and Morton [9]) as reducing agents. Before treatment, we assayed overall or total antibody titer and after treatment, the IgG titer. We determined the presence of IgM on the basis of total absence of immune antibodies in the treated specimens of 4-fold or greater decline of titers in the sera, as compared to the control.

We failed to demonstrate any basic difference in indicators of the immunological reactions between treatment with the two reducing agents; however, it is preferable to use 2-mercaptoethanol which, unlike cysteine, does not form a sediment that makes it difficult to evaluate results. We submit below the results we obtained with use of 2-mercaptoethanol.

In animals infected with virulent strain No 55, only IgM was demonstrable 1 week later in average titers of 1:240 and 1:300 in the AR and PHAR, respectively. At this time IgG was not yet demonstrable; 2 weeks after infection they were synthesized and consistently demonstrable in infected animals, mean titers constituting 1:104 in the AR and 1:72 in the PHAR. We also found an appreciable elevation of overall antibody level in serum, which reached a maximum 3 weeks after infection: 1:2936 with the AR and 1:7040 with the PHAR. Highest IgG titer was recorded after 1 month, averaging 1:384 (AR) and 1:288 (PHAR). Subsequent long-term observation revealed gradual decline of overall antibody titers, which constituted 1:640-1:1280 after 11 months (duration of observation). It should be noted that throughout the study, IgG were demonstrable in blood; their titers also gradually decreased constituting 1:40-1:60 after 11 months, there being no appreciable difference between the AR and PHAR; in a number of cases the titer was lower in the PHAR than AR (see Figure).

Analogous studies of antibody structure and dynamics in rabbits infected with virulent strain 503/840 revealed the same basic patterns, some differences being noted only in mean antibody titers.

Thus, at the early stage of the infectious process after tularemia infection there is intensive production of IgM. IgG appear against the background of maximum macroglobulin synthesis (2d-3d week), reach a maximum by the 4th week and are demonstrable in the animals' blood for a long time. It was found that the PHAR is more effective for demonstration of IgM and AR for IgG. Since IgM are the original type of antibodies and first to appear in response to antigenic stimulation, expressly the PHAR is recommended for early detection of tularemia.



Dynamics of antibodies (mean titers) in infected (a) and immunized (b) rabbits; x-axis--testing time; y-axis, mean titer log

I, II) AR parameters before and after treatment of serum with 2-mercaptoethanol

III, IV) PHAR parameters

We demonstrated appreciable differences in nature and dynamics of antibodies when we examined immunized animals. IgM were consistently demonstrable in rabbit serum 1 week after immunization in both serological reactions, with mean antibody titers of 1:2280 (AR) and 1:3804 (PHAR), which reached a maximum after 2-3 weeks--1:2680 (AR) and 1:12,160 (PHAR)--after which the concentration gradually diminished, constituting 1:120 (AR) and 1:1600 (PHAR) by the 6th month. As in infected animals, the immunized ones showed IgG only after 2 weeks; however, the titers thereof remained low throughout the observation period (they did not exceed 1:40-1:160 in both reactions), declined rapidly and were not demonstrable after 6 months. In a number of cases, no IgG were demonstrable in immunized animals over the entire observation period.

Thus, we succeeded in establishing differences in structure and levels of immunoglobulins, as well as in time of their circulation in blood of infected and immunized animals. IgG production is attributable mainly to the infectious process. This warrants the assumption that IgG are an indicator of intensity of the immunological response in the presence of tularemia. The vaccinal process elicited chiefly production of IgM; IgG were demonstrable inconsistently, in low titers and disappeared faster from blood of experimental animals.

The different proportions of IgM and IgG in serum of infected and immunized animals can apparently serve as a criterion for differentiation between infectious and vaccinal processes by serological tests, and they permit more accurate determination of the immunological status of the organism in the presence of tularemia.

Conclusions

1. Appreciable differences were found in IgG level and time of persistence thereof in blood of animals infected with *F. tularensis* and immunized against tularemia.

2. The passive hemagglutination reaction was found to be more effective for studying IgM, whereas clearer results were obtained with the agglutination reaction for determining IgG.

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CYTOPATHIC EFFECT OF FRANCISELLA TULARENSIS ON CULTURE OF PERITONEAL MACROPHAGES

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 10, Oct 77 (manuscript received 15 Mar 77) pp 104-107

[Article by T. N. Maslova and R. A. Savel'yeva, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences]

[Text] It has been demonstrated that virulent *Brucella* strains, as well as *Shigella flexneri*, are capable of eliciting death of macrophages cultivated in vitro [1, 3, 7], and for dysentery bacteria this capacity is related to the presence of cytotoxin [3]. Studies with a culture of macrophages and *F. tularensis* revealed differences between the behavior of virulent and avirulent cultures. These data are of interest in connection with investigation of the virulence of *F. tularensis* [8-12]; however, the information in the literature on this matter is contradictory.

[page 105 missing]

... At different postinfection times (3 to 48 h), sample test tubes were removed from the incubator, pH of the medium, cloudiness were recorded and preparations made. The latter were fixed in methyl alcohol and stained according to Romanovsky-Giemsa. We observed simultaneously washed and unwashed preparations, both experimental and control ones.

In the first series of experiments we determined the behavior of the above-mentioned strains of *F. tularensis* in a culture of peritoneal macrophages from intact guinea pigs. The experiments enabled us to determine that the degree of ingestion by macrophages of all *F. tularensis* strains was relatively the same. After incubation for 5 h, there were marked qualitative and quantitative changes in the cell population of macrophage cultures infected with vaccinal and virulent strains. However, morphological cell changes were more marked. Regardless of the quantity of *F. tularensis* present in the cytoplasm of macrophages, the latter were damaged so severely that there was no question of irreversibility of the dystrophic changes.

F. tularensis ingested by macrophages elicited primary degeneration of karyoplasm with subsequent breakdown of cytoplasmic structures of cells. The most typical and frequent form of dystrophy was karyorrhexis and vacuolization of nuclear substance (Figure 1, a, b [photo not reproduced]). The cytotoxic effect of bacteria on cells was unrelated to intensive intracellular

reproduction of the pathogen. A small amount of bacterial cells ingested by a phagocyte, some of which were at the stage of digestion, was sufficient for manifestation of the cytotoxic effect of *F. tularensis* on macrophages. This was indicated by the eluated preparations, which made it possible to rule out repeated phagocytosis. By 24 h, there was complete degeneration of cellular elements. In their place we usually encountered accumulations of bacteria, the shape of which repeated the outline of the former cell (Figure 2 [photo not reproduced]). The fibroblastoid or stromal elements, which appeared in the macrophage culture at this time, were quite resistant to *F. tularensis*: completely destroyed macrophages and completely intact fibroblastoid cells could be seen (Figure 3 [photo not reproduced]).

Having become convinced that all of the strains had a markedly destructive effect on macrophages, we took a culture of strain No 503 killed by heat. When macrophages were infected with killed bacteria there was active phagocytosis, followed by complete digestion of ingested bacteria, without death or damage to macrophages.

The results of these experiments were indicative of very great lability of macrophages from animals sensitive to *F. tularensis*. The enzymatic systems of phagocytes were inadequate, even in relation to the vaccine strain, which had residual virulence and an antigen complex similar to that of virulent strains.

Negative results were obtained from experiments conducted to demonstrate the effect of bacterial metabolic products on macrophages through the nutrient medium: the cellular elements of macrophage culture did not undergo any visible morphological changes, and they were in the same condition as control elements.

A second series of experiments was conducted on albino rat macrophages for a comparative study of the toxic effect of *F. tularensis* on cells from an animal that is not very sensitive to this infection. It was shown that cells from rats did not undergo the marked pathological changes inherent in cultures of guinea pig macrophages. The total number of cells virtually failed to decline after cultivation for 24-48 h. We observed intensive phagocytosis of all strains: after 24 h there were signs of active digestion of captured bacteria, and the macrophages presented no appreciable signs of degeneration. We were impressed by the condition of the nuclear system, which presented no visible changes in this case (Figure 4 [photo not reproduced]). Thus, cells taken from insensitive animals were not subject to appreciable cytopathic effects, even of virulent strains of *F. tularensis*.

The results of our studies warrant the assumption that *F. tularensis* has a specific factor responsible for its cytopathic effect on macrophages, and this is consistent with previously obtained data concerning the existence of a toxic component in *F. tularensis*. The nature of this factor and mechanism of resistance of macrophages from insensitive animals are presently being studied.

Conclusions

1. It was shown that *F. tularensis* strains differing in virulence had a marked cytotoxic effect on macrophages from animals sensitive to this infection.

2. Macrophages from animals who are not sensitive to tularemia were more resistant to *F. tularensis*.
3. A culture of *F. tularensis* killed by heat was submitted to active phagocytosis by macrophages, without causing death of the latter.
4. The cytotoxic effect of *F. tularensis* is attributable to a factor in the nature of a toxin.

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MECHANISM OF CHANGES IN REACTIVITY OF ANIMALS TO TULAREMIA AFTER MIXED INFECTION

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[Article by T. N. Dunayeva, Ye. V. Ananova and K. N. Shlygina, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] Preinfection of animals with sublethal doses of certain pathogens has an inhibitory effect on development of tularemia [1]. The mechanism of this effect has not been determined.

Material and Methods

We made a comparative study of the pathogenesis and immunogenesis of listeriosis and salmonellosis in albino rats, as well as the effect of these infections on rat sensitivity to tularemia. In the experiments, we used *Listeria monocytogenes* strain No 681 serotype 1/2a, *Salmonella enteritidis* No 165 and standard *Francisella tularensis* strain No 503. Animals were infected subcutaneously with a sublethal dose of *Listeria* or *Salmonella* (500 million bacterial cells). We made a dynamic study of distribution of the pathogen in organs, intensity of plasma reaction in a regional lymph node and accumulation of specific antibodies in blood serum. We used cortisone-treated white mice for isolation of *Listeria*.

Results and Discussion

We demonstrated differences in the course of listeriosis and salmonellosis in rats, and in intensity of immunological reactions. Listeriosis had a brief course. Already on the 10th-15th day, the rats were free of the pathogen, and only a few specimens presented regional lymph nodes, from which *Listeria* were isolated. *Salmonella* was demonstrable in the spleen and liver up to the 10th day and in some cases up to the 18th experimental day. *Salmonella* persisted in lymph nodes of some rats for 38-58 days.

Appreciable differences were observed in intensity of plasma cell production. In rats with listeriosis, the increase in number of plasmoblasts did not lead to significant increase in number of plasmocytes. Maximum quantity thereof, which did not exceed 30-54 cells per 50 fields of vision, was recorded on the 6th-8th day. By the 10th experimental day, there was a decrease in quantity of plasmocytes (Table 1). *Salmonella* infection elicited intensive development of

the plasmocyte reaction, and immunomorphological changes lasted for a longer time. Maximum quantity of plasmocytes was noted on the 4th-10th day, reaching 112-360 cells per 50 fields. On the 28th day, the quantity of plasmoblasts and plasmocytes was greater in rats with salmonellosis than those with listeriosis.

Table 1. Dynamics of plasmocyte reaction and accumulation of antibodies in blood serum of albino rats infected with *Listeria* and *Salmonella* (dosage 500 million bacterial cells)

Post-infection day	Listeriosis			Salmonellosis		
	plasmoblasts*	plasmocytes*	agglutin. react.**	plasmoblast*	plasmocyte*	agglutin. react.**
1	11	2	—***	—	—	—
2	95	15	—	74	20	—
3	131	15	12,6	—	—	—
4	97	20	25,2	135	53	80
7	71	24	40	97	157	640
10	38	13	0-40	86	65	845
15-20	37	10	0-40	38	23	124
28	29	10	0	38	24	0-160
Intact	38	2,7				

*Arithmetic mean per 50 fields of vision.

**Geometric mean (reciprocals of antibody titers).

***No tests done (-).

Accumulation of circulating antibodies was consistent with differences in the immunomorphological reaction: brief and minimal with listeriosis, prolonged and sizable with salmonellosis. Already on the 4th experimental day, reaction titers reached 1:320 in some animals, and maximum titers were 1:2560 on the 7th-10th day. Antibodies in serum dilution of 1:40-1:320 were demonstrated in some rats 58 days after infection.

Concurrently with examination of some parameters of pathogenesis and immunogenesis of listeriosis and salmonellosis, we determined rat sensitivity to tularemia. The rats were infected with lethal doses of *F. tularensis* homolaterally. Concurrently, control animals were infected with the same doses. The inhibitory effect of listeriosis on development of tularemia infection was brief, and it was manifested when the interval between infections was 2 to 10 days (Table 2).

We observed heightened sensitivity to tularemia in rats with salmonellosis even when both were inoculated simultaneously, and it persisted for 1 month (Table 3). It should be stressed that the survival rate in experiments where intervals were 2-10 days was the same when tularemia was combined with listeriosis and salmonellosis. With a dosage of 100 million bacteria, 69.3% of the rats with listeriosis survived and 60.5% of those with salmonellosis. After infection with 1 billion *F. tularensis*, 16.2 and 20.5%, respectively, survived. When the interval between infections was 18 or more days, the death rate among rats with listeriosis was the same as in the control, whereas among those with salmonellosis 63 and 20% of those infected with 100 million and

1 billion *F. tularensis* survived. Consequently, the main difference was in duration of heterologous immunity due to development of the background infections.

Table 2. Inhibitory effect of listeriosis on tularemia in albino rats

Interval between infections, days	F. tularensis infective dose, quantity of bacterial cells	Infected (numera- tor) and deaths (denomi- nator)	Time of death, day	Surviving rats	Control	
					infected (numer.) & deaths (denom.)	time of death, day
0	100 million	6/4	2-3	2	6/4	2-3
	1 billion	6/6	2-3	0	6/6	2
2	100 million	10/1	19	9	12/12	2-3
	1 billion	10/10	2-9	0	12/12	2-3
4	100 million	23/4	2	19	13/13	2-3
	1 billion	23/17	2-3	6	13/13	2-3
7	100 million	5/2	4-7	3	5/4	2-3
	1 billion	5/5	2-3	0	5/5	2
10	100 million	5/2	3-11	3	*	
	1 billion	5/4	2-3	1		
18	100 million	5/4	2-3	1	5/4	2-3
	1 billion	5/5	2	0	5/5	2
32	100 million	5/4	2-3	1	*	
	1 billion	5/5	2	0		
Totals:						
100 million				38 (64,4%)	41/37 (90,2%)	
1 billion				7 (11,8%)	41/41 (100%)	

*See control for preceding time.

We can relate the prolonged change in reactivity of rats infected with *Salmonella* to the longer activation of their cellular reaction. It is known that the presence of antigen during development of immunocompetent cells is significant to determination of immunological specificity of these cells [3]. Administration of new antigen at intermediate stages of histogenesis could cause rapid appearance of immunocompetent cells that produce antibodies to this antigen--in our experiments tularemia antigen.

This phenomenon is well-demonstrable when rats are infected with a moderate dose of *Salmonella*--1000 bacterial cells--then *F. tularensis*. In this experiment, the immunomorphological reactions of rats developed more slowly than after infection with 500 million bacteria. On the 4th day, the mean quantity of plasmoblasts in a regional lymph node increased to only 34 per 50 fields, while plasmocytes remained at a low level, only 2 cells. Accordingly, there were no antibodies to *Salmonella* antigen in blood serum. On the 18th day, the number of plasmoblasts increased to 43 and plasmocytes to 27.5. Antibody titer was in the range of 1:10 to 1:320 (geometric mean 1:56.7). In this experiment, when rats were infected with lethal doses of *F. tularensis* they presented the same increase in resistance to tularemia as rats infected with a massive dose of *Salmonella*. The surviving rats were examined on the 20th day after tularemia infection. No antibodies to *Salmonella* were demonstrable in rats infected at a

4-day interval, in spite of the presence of bacteria in lymph nodes, spleen and, in one case, the liver. Titers of antibodies to *F. tularensis* ranged from 1:80 to 1:640.

Table 3. Inhibitory effect of salmonellosis on tularemia in albino rats

Interval between infections, days	F. tularensis infective dose, quantity of bacterial cells	Infected (numerator) and deaths (denominator)	Time of death, day	Surviving rats	Control	
					infected (numerator) and deaths (denominator)	Time of death
0	100 million	10/4	3	6	10/10	3
	1 billion	5/4	3	1	5/5	3-4
2	100 million	21/8	2-5	15	10/10	3-4
	1 billion	10/4	3-11	6	5/5	2-4
4	100 million	10/2	3-9	8	*	
	1 billion	5/4	2-3	1		
7	100 million	20/11	3	9	10/10	2-4
	1 billion	11/11	2-3	0	6/6	2
10	100 million	10/4	3	6	10/10	2-5
	1 billion	5/5	2-3	0	5/5	2-3
18	100 million	20/8	3-11	14	10/10	3-6
	1 billion	10/5	2-6	4	5/5	2
28	100 million	10/5	2-3	5	*	
	1 billion	10/5	2-3	0		
58	100 million	10/10	2-3	0	10/10	2-4
	1 billion	7/7	2-3	0	6/6	2
Totals:						
	100 million	111/48 (43,2%)		63 (56,8%)	60/60 (100%)	
	1 billion	63/51 (80,9%)		12 (19,1%)	32/32 (100%)	

*See control for preceding time.

Antibodies to both pathogens were present in rats infected with tularemia at an interval of 18 days: to *F. tularensis* in titers of 1:160-1:320 and to *Salmonella* in titers of 1:10-1:160. Consequently, administration of a massive dose of *F. tularensis* during the period of proliferation of plasmoblast cells (4th day) caused production of antibodies to the new antigen and inhibited production of antibodies to the first pathogen.

When *F. tularensis* was given to animals who already developed an immunological reaction to salmonellosis, we observed the opposite phenomenon: stimulation of production of antibodies to the first antigen. In rats infected with a massive dose and examined on the 22d day (20th day after tularemia infection) titers of antibodies to *Salmonella* reached 1:1280 (geometric mean 1:440). In the case of monoinfection, maximum antibody titer was 1:640, while the geometric mean constituted 1:124 (see Table 1). On the 31st day after administration of *Salmonella* (13th day after tularemia infection), antibody titers ranged from 1:40 to 1:1280 (mean 1:276), whereas in the case of monoinfection antibodies were not demonstrable in all of the rats and their titers did not exceed 1:160.

Reactivation of production of antibodies to listeriosis antigen was even more graphic. After a massive dose of *Listeria*, all 12 surviving rats, which were tested on the 20th day after tularemia infection (24th, 27th and 30th days after *Listeria* infection), presented antibodies to *Listeria* in serum dilutions of 1:40-1:1280 (geometric mean 1:300). Antibody production was much higher than after *Listeria* infection (see Table 1). Intensification of anamnestic serological reactions to heterogeneous pathogens has been described in great gerbils infected with plague [2] and rabbits immunized with *Staphylococcus* [4]. Activation of development of immunocompetent cells makes it possible to express within a short time the genetically inherent capacity of white rats to produce antibodies to *F. tularensis*. As we know, presence of antibodies enhances phagocytosis, which is important in the system of protective forces of the organism.

Conclusions

1. Experiments on albino rats infected with *Listeria* or *Salmonella* and then *F. tularensis* demonstrated differences in duration of nonspecific resistance to tularemia, which are attributable to the distinctions of pathogenesis and immunogenesis of the background infections.
2. Activation of immunomorphological reaction, which causes faster development of specific reactions of immunity to tularemia, is one of the important factors that enhances rat resistance to this infection after being submitted to mixed infection.

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EFFICACY OF TULAREMIA ANTIBODY ERYTHROCYTIC DIAGNOSTICUM FOR DEMONSTRATION
OF SPECIFIC ANTIGEN AND ANTIBODIES

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[Article by M. F. Shmuter, M. A. Aykimbayev, A. M. Yelyubayeva, D. I. Brikman
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[Text] A method was developed for commercial production of tularemia antibody erythrocytic diagnosticum in liquid and dry form at the Central Asian Scientific Research Plague-Control Institute in 1969-1972; TU-42 [technical specifications] and laboratory instructions for production of this product were compiled [1]. The results of experimental investigation were indicative of high sensitivity and specificity of this product for demonstration of both antigen in the passive hemagglutination reaction (PHAR) and antibodies in the antigen neutralization reaction (ANR) [1-3].

Following a test program coordinated with the State Institute of Standardization and Control of Medical Biologicals imeni Tarasevich, which was approved by the Committee for Vaccines and Sera of the USSR Ministry of Health, this diagnosticum was tested at the Irkutsk Scientific Research Plague-Control Institute of Siberia and the Far East and at the Omsk Scientific Research Institute of Endemic Infections. The results of this testing are submitted here.

Experimental series of the product, in dry and liquid form, were prepared for the trials. Specific Vi antigen content was assayed with this diagnosticum in 97 cultures of *F. tularensis* killed by boiling, as well as 114 cultures of other microorganisms (*Y. pestis*--40, *Listeria*--12, *Pasteurella*--12, *Erysipelothrix*--8, *Brucella*--17, *Salmonella* and *E. coli*--25). In addition, 313 suspensions of liver, spleen and extracts of femoral bones from 209 white mice that died of tularemia and 104 control mice were tested for specific antigen. For these tests, we used fresh, decayed and dry animal carcasses.

In order to check the expediency of using tularemia antibody erythrocytic diagnosticum for demonstration of specific antibodies with the ANR, we analyzed serum from 92 people who had been immunized or infected, rabbits and guinea pigs, as well as 102 control sera (from human donors--40, rabbits--20, guinea pigs--24, *brucella*--12, *cholera*--4 and tularemia agglutinating--2).

All of the material was coded by a special commission and analyzed in coded form.

The results of testing 211 cultures in the PHAR for presence of specific antigen confirmed the high sensitivity and strict specificity of tularemia antibody erythrocytic diagnosticum. PHAR with the tested diagnosticum was positive with suspensions of 97 tularemia cultures. The suspensions of the other bacteria listed above did not react with this diagnosticum.

Table 1. Sensitivity of *F. tularensis* strains in PHAR

Diagnosticum	Number of cultures	Results of PHAR with different concentr. of cultures (thous.bacterial cells/0.2 ml)								geom. mean titer
		31	50	62	100	125	200	250	500	
Liquid	97	2	1	11	13	18	31	19	2	147,6
Dry	97	—	2	6	4	18	39	25	3	175,4

Table 2. Results of testing suspensions of organs from white mice infected with tularemia

Dilution of material (thousand-fold)	PHAR with suspensions				Dilu- tion (thous)	PHAR with femur extract (70)	
	liver (69)		spleen(70)			diagnosticum	
	diagnosticum						
	liq.	dry	liq.	dry		liquid	dry
1:8	—	—	1	2	1:0,8	—	—
1:16	—	—	2	3	1:1,6	1	2
1:32	1	—	5	3	1:3,2	4	2
1:64	2	3	21	23	1:6,4	10	11
1:128	11	12	23	22	1:12,8	15	15
1:256	26	38	17	16	1:25,6	15	12
1:512	29	16	1	1	1:51,2	25	28
Titer geometric mean (thousand- fold)	1:283	1:246,8	1:103	1:97,5		1:19,8	1:20,4

In the PHAR with the tested product, *F. tularensis* strains were found to vary in sensitivity (Table 1). Shmuter et al. [4] had previously advanced a thesis to the effect that sensitivity of diagnosticum is related to virulence of cultures (presence of Vi antigen). In order to confirm this, killed suspensions of 2 tularemia cultures--virulent Schu of the Nearctic variety and the avirulent reference strain 21/400 in concentrations of 1 million and 1 billion bacterial cells per milliliter were studied at the Irkutsk Institute. A positive PHAR was obtained with Schu strain in a dosage of 100,000 cells, whereas with strain 21/400 it was negative even with a dosage of 200 millions cells/0.2 ml. This indicated that one could indirectly assess the virulence of *F. tularensis* according to sensitivity of the diagnosticum.

The results of testing 219 suspensions of organs from tularemia-infected white mice were indicative of the high specificity and sensitivity of tularemia antibody erythrocyte diagnosticum (Table 2). Positive results with the hemagglutination test, with suspensions of spleen and liver of 1:2000 and suspensions of femurs of 1:200, were not obtained with any of the 104 such suspensions from control animals.

The results of examining suspensions of white mouse organs were indicative of a higher antigen concentration in samples from decomposed and dry organs and lower in samples taken from fresh material, from infected dead mice. Such distribution of antigen is apparently related to the fact that there is less antigen per unit weight of fresh material than in dry and decomposed material. We were impressed by the higher concentration of antigen in the liver than the spleen, although usually more bacteria are isolated in bacteriological tests of the spleen than the liver. This distribution of antigen could apparently be attributed to the fact that, concurrently with reproduction of *F. tularensis* in the liver, there was destruction thereof and accumulation of antigen, not only of *F. tularensis* that multiplied in the liver, but antigen that had migrated from other parts of the body with blood.

Table 3. Results of testing serum of infected and immunized people and animals (ANR)

Titer	Serum from infected rabbits (20)		Titer	Serum from immunized guinea pigs (57) sick and immunized people (12)			
	diagnosticum			diagnosticum			
	liquid	dry		liquid	dry	liq.	dry
Negative	—	—	Negative	6	6	3	3
1:400	1	1	1:40	4	6	2	2
1:800	7	6	1:80	17	18	3	3
1:1600	9	10	1:160	17	16	2	1
1:3200	3	3	1:320	12	10	—	1
1:6400	—	—	1:640	1	1	2	2
Titer geometric mean	1:1299	1:1345		1:126	1:124	1:127	1:127
Total positive reactions, %	100,0	100,0		89,5	89,5	75,0	75,0

These results were indicative of the strict specificity and high sensitivity of tularemia antibody erythrocytic diagnosticum and its suitability for demonstration of specific Vi antigen, in both cultures of *F. tularensis* and suspensions of organs from rodents that died of tularemia, regardless of condition of the carcasses. There was virtually no difference between liquid and dry diagnosticum with respect to sensitivity.

We also explored the possibility of using the diagnosticum in the ANR in order to demonstrate specific antibodies in blood serum from healthy, sick and immunized people and animals. Antibodies in a titer of 1:64,000 were found in

tularemic agglutinating serum, in reactions with both dry and liquid diagnosticum. Specific antibodies were also demonstrable in serum from infected and immunized people and animals (Table 3). In control serum--Brucella, cholera agglutinating and serum taken from healthy rabbits, there were no specific antibodies. Antibodies in titers of 1:40-1:80 were found in only 2 out of 24 serum specimens from healthy guinea pigs and serum from 3 healthy people. Testing of the serum from these people in the PHAR with tularemia antigen diagnosticum revealed antibodies in the same titers. These data indicated that, in the cases mentioned, the people had had an anamnestic reaction due to prior illness or immunization.

These data were indicative of considerable specificity and relatively high sensitivity of the ANR with tularemia antibody erythrocytic diagnosticum and the possibility of using it in order to detect antibodies in people and animals who had been sick and immunized.

However, considering that it is more difficult to run the three-element reaction (ANR) than the two-element one (PHAR), use of tularemia antibody erythrocytic diagnosticum for demonstration of specific antibodies should be recommended only in the absence of antigenic diagnosticum, as well as for running two mutually checked reactions--PHAR with antigenic diagnosticum and ANR with antibody diagnosticum.

Conclusions

1. The PHAR with tularemia antibody erythrocytic diagnosticum is a specific and highly sensitive test, and it is suitable for demonstration of specific antigen in both bacterial suspensions and suspensions of organs and tissues from animals that died of tularemia, regardless of condition (fresh, decomposed or dry carcasses).
2. The PHAR with tularemia antibody erythrocytic diagnosticum makes it possible to indirectly assess the virulence of tested cultures of *F. tularensis*.
3. Tularemia erythrocytic antibody diagnosticum can be used in some cases for demonstration of specific antibodies in serum from people and animals who were previously sick and immunized by means of running the antigen neutralization reaction.

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ROLE OF LEMMINGS IN ENDEMIC TULAREMIA SITES IN THE ARCTIC REGION

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[Article by I. P. Algazin and I. I. Bogdanov, Scientific Research Institute of Endemic Infections, Omsk]

[Text] Introduction

Among the endemic mammalian species of the subarctic region that inhabit exclusively the tundra or forest-tundra, there is wide distribution of rodents represented by three vole species of the genus *Microtus* and four lemming species (3 of the genus *Lemmus* and 1 of the genus *Dicrostonyx*). The enormous territory of the Eurasian tundra, from Arkhangelsk to Chukotka is populated primarily by two lemming species: Siberian (*Lemmus sibiricus* Kerr.), which is the most numerous and inhabits the main tundra biotopes, and Arctic (*Dicrostonyx torquatus* Pall.), of which there are much fewer than the Siberian lemming, inhabiting the relative dry and elevated tundra regions [12].

Observations of Soviet and foreign scientists established that episodes of mass-scale reproduction, recurring every 3-4 years, which are associated with sizable migrations and death of most animals, are inherent in lemmings, and as a result their number occasionally decreases to 1/300-1/400th [10, 14].

When analyzing factors that affect the dynamics of rodent population size, in recent years much importance has been attributed to intrapopulation regulatory mechanisms [1, 4, 15], among which a place is reserved to deaths due to epizootic diseases, including tularemia [5]. It was possible to confirm, for the first time, the tularemic etiology of lemming death during a period of mass reproduction in one of the provinces of Lapland, in Sweden [13]. The author assumed that the cases of tularemia among humans in that locality were due to a tularemia epizootic in the lemming population.

Only indirect data were obtained for the territory of the USSR, which indicated a possibility of tularemia among these rodent species: intensive deaths during years of a "peak" in their number, coincidence of cases of the disease among the inhabitants with periods of mass reproduction and death of the animals, results of retrospective epidemiological analysis [9, 11]. Maksimov [7] proposed that a distinction be made of the tundra type of endemic tularemia site, in the belief that the lemming is the principal carrier of infection in this type of site.

In 1972, one of the present authors participated [2] in a study of lemmings trapped in the western part of Taymyr Peninsula, in the region of the Pyasina River, between the village of Valek and the estuary of Pura River, and in the lower reaches of the Pura, which yielded distinct seropositive data indicative of contact of these animals with *F. tularensis*. This served as grounds for a 4-year (1973-1976) study at a permanent base of an endemic tularemia site in East Taymyr, in the vicinity of the Novorybnoye trading post, in a typical moss and lichen tundra (73° north latitude). The area of the territory included in the study constituted about 27 km². The Siberian lemming settlements were in the humid lowlands, whereas settlements of the Arctic lemming were situated on elevations.

Material and Methods

Over the above-mentioned period, a total of 1013 lemmings were tested for tularemia, including 949 Siberian and 64 Arctic lemmings; in addition we examined 210 bird pellets, 29 mummified rodent carcasses, 126 samples of water from open reservoirs in the tundra and 128 samples of wet substrate collected near rodent nests. Finally, we examined 8300 bloodsucking mosquitoes and 4941 gamasid ticks.

The lemmings were caught using Gero press-type traps (equipped with a trip cord [tripping device]), which were placed in lines of 100, 150 or 200 traps at an average distance of 5 m from one another. Gamasid ticks were collected by brushing lemmings, as well as from nests using "thermoelectors." We used a biological test on white mice by the conventional method to isolate *F. tularensis* from rodents, environmental objects and arthropods. Biotests with gamasid ticks taken from animals were made immediately after brushing them out. Ticks of the nest-burrow complex were kept alive before the tests in nests, maintaining an optimum microclimate. Cultures of internal organs and blood of biotested animals that died were made by the impression method on coagulated yolk medium, blood and (for a control) beef-extract agar. Serological testing of pellets and mummified carcasses for tularemia antigen was performed using the antibody neutralization reaction (ANR). Specific antigens were demonstrated in rodents by means of the usual agglutination reaction and passive hemagglutination reaction with tularemia erythrocytic diagnosticum.

Results and Discussion

During the period of our observations, the number of lemmings changed in the following manner: In 1973, there was an average of 29 Siberian lemmings per 100 trap-days and 2 Arctic lemmings per 100 trap-days. In 1974, there was a complete depression of both species: we failed to trap a single animal in the vicinity of the station, nor did we find any fresh traces (droppings, evidence of gnawing, fresh nests and tunnels). In 1975, a new rise in number of animals began. There was an average of 3 Siberian lemmings and 0.6 Arctic ones per 100-trap-days, and the latter were trapped not only on elevations, but humid lowlands, i.e., the typical habitats of the Siberian lemming. The number of animals continued to grow in 1976: average of 9.9 Siberian lemmings and 7.2 Arctic ones per 100 trap-days, and we no longer observed penetration of the Arctic lemming into the habitats of the Siberian one.

Results of analysis of bird pellets and mummified lemming carcasses in
North Taymyr

Year	Where material was collected	Pellets			Mummified carcasses		
		total	positive results		total tested	positive results	
			absolute	%±t		abs.	%±t
1974	Syndassko	116	61	52.56±4.63	13	4	30.76±12.8
1974	Novorybnoye	9	2	22.22±13.85	3	-	-
1975	"	21	6	28.56±9.85	-	-	-
1976	"	64	25	39.06±6.09	13	7	53.84±13.9

A total of 17 taxons of gamasid and acariform ticks were associated with lemmings and their nests, 4 of which were the most numerous: *Laelaps lemni* Urube, *Haemogamasus ambulans* Thorel, *Hirstionyssus isabellinus* Oudms, *Parasitus netskyi* Pav. et Bogd. The first was encountered only on animals, the second and third on both animals and in nests, and the fourth usually in the nests.

During the first increase in number of animals (1973), 6 strains of *F. tularensis* were isolated, for the first time in the Soviet Union, from Siberian lemmings (5 cultures from live animals and 1 from a carcass). In 1974, when there were no live animals at the station, bacteriological tests were made of water samples from the moist substrate near nests referable to summer settlements of lemmings. In two cases, we succeeded in isolating a strain of *F. tularensis*. In 1974, during a period of gradual increase in animals, a culture of *F. tularensis* was isolated from water samples from a swampy region near settlements of Siberian lemmings. In 1976, 2 strains of *F. tularensis* were isolated from Siberian lemmings (1 from a live animal and 1 from a carcass), and again a culture of the microorganisms was isolated from a water sample from the substrate near a nest.

Examination of biological properties of all of the strains showed that they were referable to the Holarctic race of the pathogen (*Francisella tularensis holarctica* Ols.).

Data from a serological analysis for tularemia of bird pellets and mummified lemming carcasses (see Table) can serve as a graphic illustration of the dynamics of the epizootic process. In 1974, very few pellets and carcasses were collected in the vicinity of Novorybnoye. At the same time, according to statements of local residents, there was a migration of lemmings in the fall and spring, mainly to the north. Near the Syndassko trading post, which is north of Novorybnoye, on the coast of Khatanga Bay of the Laptev Sea, a large number of mummified carcasses and pellets was collected.* There was a high percentage of pellets and carcasses containing specific antigen, and ANR titers reached 1:1280. This warranted our assumption that there had been an intensive tularemia epizootic among lemmings in East Taymyr, in 1973. According to data in the literature [6, 8], epizootics had also been observed in the same year on Yugorsk Peninsula, Yamala and West Yakutia, i.e., they involved an enormous territory. In 1975, the percentage of pellets with antigen was

*V. A. Il'in collected the material near Syndassko.

considerably lower, ANR titers ranged from 1:20 to 1:640, while no cultures were isolated from animals. Considering the small number of lemmings, it is difficult to assume that there had been any significant epizootic at that time. In 1976, the quantity of pellets and carcasses containing antigen increased again, and antigen was demonstrable in suspension dilutions of up to 1:1280; cultures of *F. tularensis* were isolated from the lemmings. Considering the growth in number of animals, there is reason for us to talk about the start of a significant epizootic, which could be the cause of lemming depression in 1977.

The pathogen is transmitted via different routes in the lemming population. For example, we think the water factor is important to prolonged preservation of *F. tularensis* in the environment, which is added by the stable, low-temperature condition of tundra reservoirs and moist substrate near nests. The settlements of Siberian lemmings are in the humid lowlands, for this reason, many of their tunnels, burrows and spaces near nests are sometimes filled with seeping ground water. The feed tables are often near the very edge of small bodies of water. With a large number of rodents, these substrates, which are infected by the excreta of sick animals, could serve as a source of infection. In our opinion, isolation of *F. tularensis* cultures from water in a year of depression [of lemming population] serves as proof of the significance of water as the medium where the pathogen is preserved during periods of absence of lemmings.

Consumption by the lemmings of other lemmings and carcasses thereof may be another route of transmission of infection. Cannibalism is practiced by lemmings, particularly in years where their number is high [3]. When lemmings take over old nests (for permanent residence or as a temporary shelter), there can be contact with previously infected substrate and further spread of the pathogen. Finally, we should not rule out the role of the transmissive factor in transmission of the pathogen. We have data referable to bacterioscopic detection of *Francisella tularensis* in a suspension of *Haemogamasus ambulans* and *Hirstionyssus isabellinus* ticks caught in lemming nests. Transmissive infection of young animals by old ones in the nest is particularly likely. For the time being, it is difficult to assess the role of bloodsucking mosquitoes in circulation of the pathogen, since bacteriological examination thereof did not yield positive results.

Conclusions

1. Isolation of *F. tularensis* from live lemmings, fresh carcasses thereof and water, as well as demonstration of specific antigen in bird pellets and mummified lemming carcasses, are indicative of the existence of independent endemic sites of tularemia of the tundra type.
2. The coincidence of extensive tularemia epizootics with a peak in lemming population size and drastic reduction in population the following year are indicative of the role of epizootics of this disease in reducing the number of animals.
3. Water is the most probable medium where *F. tularensis* is preserved in periods between epizootics.

4. Apparently, lemmings are infected with *F. tularensis* through water; infection is possible as a result of cannibalism, as well as through nest-burrow gamasid ticks and the nest substrate.

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EXPLORATORY INSPECTION OF ENDEMIC SITES FOR TULAREMIA AND PLAGUE IN ARMENIA
BY SEROLOGICAL TESTING OF BIRD PELLETS AND EXCRETA OF PREDATORY MAMMALS

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[Article by B. P. Dobrokhotoy, A. G. Mnatsakanyan, I. S. Meshcheryakova and
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[Text] The geographic location, complex mountainous topography and diversity of the environment of Armenia are instrumental in the existence on its territory of endemic sites of various infections, which include widespread sites of tularemia and plague. Tularemia, which was first detected in 1949, is now known to exist in 33 out of the 36 rayons of this republic [6, 7, 9]. It has been established that there are meadow-field, steppe, forest, backwater-swamp and foothill-brook sites. According to the results of many years of bacteriological studies, the pathogen circulates the most often among common voles, less among Transcaucasian hamsters and water rats with the involvement of Ixodes ticks [9]. As for plague, most of Armenia is part of the Transcaucasian high-altitude site of this infection, within which three persistent epizootic districts were singled out: Leninakan, near Lake Sevan and Zangezur-Karabakh [1, 11]. Plague epizootics have been recorded almost annually since 1958. Onset and development of these epizootics are related to the common vole and its specific fleas, and the vole variety of *Y. pestis* is the pathogen.

Epizootiological observations of tularemia and plague sites have been pursued by the Armenian Plague-Control Station and its departments, as well as the sanitary and epidemiological station, for many years, and this enabled us to use this territory to test the exploratory ["reconnoitering"] method, which was developed in recent years, for detection of epizootics by means of demonstrating antigens of *F. tularensis* and *Y. pestis* in bird pellets and droppings of predatory mammals [2, 3, 5].

Between 12 September and 2 October 1974, we inspected northwestern and central regions of Armenia. Our itinerary crossed the Amasiyskiy, Gukasyanskiy, Kalininskiy, Stepavanskiy, Spitakskiy, Akhuryanskiy, Artikskiy, Aparanskiy, Talinskiy, Ashtarakskiy, Razdanskiy, Abovyanskiy, im Kamo, Martuninskiy and Vardenisskiy Rayons. Traveling by motor vehicle, in 14 days 2 people collected

in these areas 1973 bird pellets, 34 samples of excrements of wild predatory mammals and 52 rodent carcasses brought in by birds. Along the valleys between mountains, which have been developed by man and are typical of this locality, pellets were collected under communication and power line poles, and in the mountains, near rocky cliffs, exposed rocks at the peaks and triangulation towers. As a rule, dried rodent carcasses abandoned by birds near landing [alighting] places and excrements of predatory mammals were also found in the same places.

The material was tested concurrently for tularemia and plague. For demonstration of antigens of *F. tularensis* and fraction I of *Y. pestis*, we used only the antibody neutralization reaction (ANR) with standard tularemia antigenic and plague erythrocytic diagnosticums prepared at the Institute of Epidemiology and Microbiology imeni Gamaleya and the Central Asian Plague-Control Institute. We became convinced previously that the passive hemagglutination reaction with erythrocytes loaded with γ -globulins to *Y. pestis* and *F. tularensis* was not suitable for demonstration of antigens in bird pellets, in spite of its simplicity. We used a Takacs microtiterator and conventional methods for preparing specimens and running the ANR [4].

We performed our work independently of the annual scheduled inspection of endemic sites, which is made by the plague-control and sanitary-epidemiological stations, and thus we were able to compare the results (Tables 1 and 2).

Table 1. Results of bacteriological examination of small mammals and their ectoparasites for tularemia and plague in Armenia in 1974

Object tested	Tularemia			Plague		
	number of specimens examined	specimens with antigen		number of specimens examined	specimens with antigen	
		abs.	%		abs.	%
Small mammals	55 710	32	0,06	73 572	135	0,18
Fleas	71 061	2	0,003	287 576	478	0,17
Ixodes ticks	3 814	7	0,18	6 264	—	—
Gamasid ticks	86 890	—	—	168 752	6	0,004

Table 2. Results of demonstration of antigens of *F. tularensis* and *Y. pestis* by the ANR in field specimens collected in an exploratory inspection of sites in Armenia in 1974

Tested object	Tularemia			Plague		
	total specimens examined	specimens with antigen		total specimens examined	specimens with antigen	
		abs.	%		abs.	%
Bird pellets	1973	73	3,7	1973	42	2,1
Predatory mammal excrements	34	3	8,8	34	1	2,9
Mummified rodent carcasses	52	8	15,4	52	5	9,6

Of the *Y. pestis* strains isolated from mammals in 1974, 122 (90.5%) were obtained from common voles, 7 from Persian jirds, 4 from an Asia Minor suslik and 2 from weasels; *F. tularensis* was also isolated the most often from common voles--31 (97%) and only 1 strain from a shrew carcass. These data, as well as the sites where pathogen cultures were isolated from fleas, Ixodes and gamasid ticks were entered on map-diagrams (Figures 1 and 2) and served as a sort of check map that we were to confirm by means of the exploratory inspection.

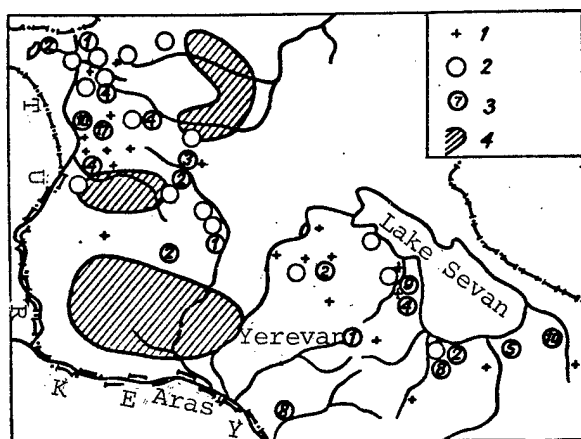


Figure 1.

Tularemia epizootics in Armenia in 1974

- 1) sites of isolation of *F. tularensis* strains from mammals, Ixodes ticks and fleas
- 2) sites of collection of pellets and excrements of predatory mammals
- 3) points, in which *F. tularensis* antigen was demonstrated (the numeral shows percentage of positive tests)
- 4) area inspected, but no pellets were found

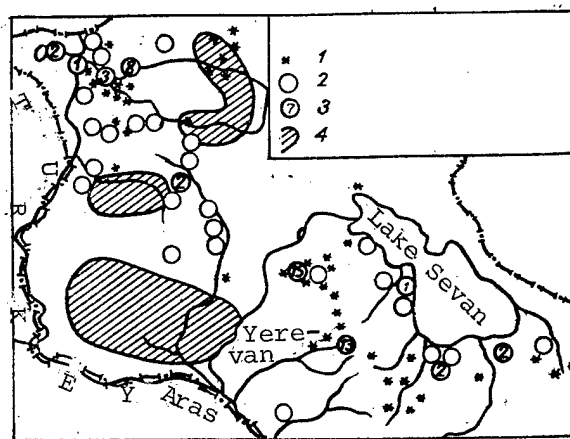


Figure 2.

Plague epizootics in Armenia in 1974

- 1) sites of isolation of *Y. pestis* from mammals, fleas and gamasid ticks
- 2) sites of collection of pellets and excrements of predatory mammals
- 3) points at which *Y. pestis* antigen was demonstrated (the numeral shows percentage of positive tests)
- 4) area inspected, but no pellets found

Material was collected at 37 points, and in 20 of them positive results were obtained in tests for tularemia, 10 in tests for plague. The sites of these pellets coincided rather well with the boundaries of the epizootic territory, and they enabled us to assess its dimensions in 1974. We could not collect any bird pellets only in places where there were very few rodents. There were few specimens with antigen in each inspected point: *F. tularensis* antigen was presented in 10-17% of only three of these points, and in two points 13-15% of samples revealed fraction I of *Y. pestis*. At other points, the incidence of positive tests on pellets did not exceed 1-8%. This warranted the assumption that there was a low intensity of tularemia and plague epizootics in 1974 over most of the territory of Armenia. At five points, we found both *F. tularensis* and *Y. pestis* antigens. The existence of simultaneous epizootics of these infections in the same places had already been reported in Armenia [8, 10]. We succeeded in demonstrating simultaneous infection with both pathogens in

the same animals. Antigen of both *F. tularensis* and *Y. pestis* was contained in the remains of two common voles (in 2 pellets) and two mummified common vole carcasses. In the neutralization reaction, the titers of tularemia antibodies of these 4 specimens constituted 1:20 and 1:12, and plague antibody titers were 1:40, 1:128, 1:80 and 1:24.

The amount of antigen in bird pellets can be estimated from the ANR titer. Presence of pellets in the environment leads to gradual flushing out of the antigen with atmospheric precipitations, so that one can tentatively estimate the time of the epizootic. In this case, the mean ANR titer ranged in different points from 1:12 to 1:128 with tularemia antigen and from 1:12 to 1:1428 with plague antigen (maximum titers were 1:512 and 1:2560, respectively). Thus, we detected both epizootics occurring at the time of our inspection and those occurring several months previously.

Analysis of the contents of pellets, in which pathogen antigen was found, revealed (Table 3) that *Y. pestis* circulates almost exclusively among common voles, whereas *F. tularensis* circulates among common voles (73.4%) and golden hamsters (19%). We found fraction I of *Y. pestis* in 5 out of 52 common vole carcasses, whereas *F. tularensis* antigen was found in 7 common voles and 1 golden hamster. This is consistent with the findings of many years of observation of local sites--common voles are the main hosts of *Y. pestis* in the Transcaucasian high-altitude site [11]; of the 410 strains of *F. tularensis* isolated from mammals in 1968-1972, 374 (91.2%) were obtained from common voles, 21 (5.1%) from golden hamsters and only 11 (2.7%) from water rats [6]. It can be assumed that in Armenia there is prevalence of a distinctive variant of a steppe site for tularemia, with very limited distribution of other types of sites.

Table 3. Results of analysis of contents of bird pellets, in which *F. tularensis* and *Y. pestis* antigens were found

Food constituents	Demonstration in pellets of			
	tularemia antigen (69 pellets)*		plague antigen (42 pellets)	
	absolute	%	absolute	%
Mammals:				
total**	105	100	58	100
common vole	77	73.4	55	94.8
golden hamster	20	19.0	1	1.7
gray hamster	2	1.9	—	—
water rat	1	1.0	—	—
unidentified species***	5	4.7	2	3.5

*Contents of 4 pellets were not analyzed.

**Together with the remains of mammals, 3 pellets contained remains of small birds, 7 contained remains of insects and 1 those of a lizard.

***This includes 1 specimen of *Apodemus sylvaticus* and 1 specimen of *Mustela erminea*.

The question of advantage of examining bird pellets and droppings of predatory mammals, as compared to ordinary bacteriological methods, was of interest. The probability of detecting antigens of the pathogens of tularemia and plague in

this material increases substantially, due to the fact that birds and predators select sick and weakened animals out of the wild animal population, or else pick up their carcasses. The data we obtained enabled us to tentatively assess the efficacy of such "selection." In the 111 pellets analyzed, there were remains of 163 animals, i.e., 1.5 per pellet. Accordingly, in all 1973 collected pellets, there were at least 2959 animals, of which 73 had had tularemia and 42 plague. Accordingly, there was 1 positive pellet per 41 and 70 animals captured by the birds. Bacteriological examination revealed 1 *F. tularensis* culture in 1741 trapped animals and 1 *Y. pestis* culture in 545 trapped animals (see Table 1). Thus, the effectiveness of serological testing of animal residue in bird pellets was 42 times greater with respect to tularemia and 7.7 times greater with respect to plague. The difference is apparently attributable to the distinctions of the infectious process in common voles, in whom tularemia is always acute and causes death of these animals, whereas in the case of plague chronic forms are also observed, with which the sick animals are less accessible to birds. Similar findings were made when the ANR was used on carcasses of animals that were not completely consumed by birds and abandoned on their resting sites: 1 finding of tularemia antigen was referable to 6.5 such mummified carcasses and 1 plague antigen per 10.4 carcasses.

Conclusions

1. Use, for the purpose of an exploratory inspection of tularemia and plague sites in Armenia, of the method of demonstrating pathogen antigen in bird pellets and excrements of predatory mammals made it possible to define rather accurately the size of the territory involved in epizootics, intensity of the latter and animal species involved in the epizootic process.
2. A significant part of the Armenian highlands has mixed and conjugated sites of plague and tularemia, epizootics of which in 1974 often occurred on the same territory and occasionally in the same common voles.
3. Serological testing with the antibody neutralization reaction of remains of small mammals from fish pellets was 42 times more effective for tularemia and 7.7 times more effective for plague than bacteriological examination of animals caught in traps during a zoological inspection of the territory.

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SOME PATTERNS OF CHANGE IN NUMBER OF CARRIERS AND EPIZOOTIC ACTIVITY IN A
LOCAL BACKWATER ENDEMIC SITE FOR TULAREMIA IN VORONEZH OBLAST

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[Article by V. R. Krasil'nikov and V. S. Sil'chenko, Oblast Sanitary and
Epidemiological Station, Voronezh]

[Text] Dedicated to the discoverers of tularemia in the USSR:
S. V. Suvorov, A. A. Vol'ferts and M. M. Voronkova.

The Povorinsk endemic tularemia site has long drawn the attention of researchers [7, 8, 12-14] as a classic example of a local backwater site. It is located in the northeastern part of this oblast, in the floodplain of the Khoper and Svintsovka Rivers. It covers an area of more than 6000 ha. The location of this site on the boundary between forest-steppe and steppe regions provides for a significant abundance and diversity of fauna [11, 12]. It is inhabited by more than 35 species of rodents, insectivores and carnivores [or predatory mammals] [1]. The most frequently encountered species are the water and common redbacked [or bank] voles, common and striped field mice, and the house mouse. The yellow-necked field mouse, beaver, common shrew, water-shrew and muskrat [or desman] are common to these areas. Bloodsucking insects are widely represented by horseflies, mosquitoes, biting midges and gnats. Ixodes ticks are represented by an insignificant number of Ixodes ricinus and Dermacentor marginatus.

Epizootic and epidemiological observations have been pursued of this site since 1930,* but they started to be systematic and scheduled in 1958. In the period from 1958 to 1974, 19,326 mammals (Table 1), 98,700 bloodsucking Diptera, 408 Ixodes ticks and 1672 water samples were examined. As a result, 370 cultures of *F. tularensis* were isolated, of which 352 were from the water vole, 4 from the striped field mouse, 1 from water-shrew and 13 from water. No cultures were isolated from ticks.

As a result of studies of the dynamics in rodent population size and epizootic activity between 1958 and 1974, it was possible to divide this time into two

*Ye. G. Reznikov, S. A. Rastorguyeva, A. S. Sokol'skaya, A. V. Rayskaya, V. G. Klenova, T. D. Markova, B. G. Chebotarev, Ye. N. Yezhova, L. M. Grishayev, M. P. Lysenko and others.

periods. The first period (1958-1968) was characterized by a large population of water voles (35-88% filled traps along the shoreline during the spring flood tide), while the number of other rodents held at a constant low level (maximum trapped 16.5%). The second period (1969-1973) began with an unusually rigorous winter without snow, which caused, along with drying of swamps, a severe depression of the water vole population lasting to the end of this period; the number of small rodents was rapidly restored and reached a high level by the summer of 1970 (50-70% of traps had animals).

Table 1. Species composition and quantity of animals examined

Species	Quantity of animals		Cultures isolated	
	absol.	%	abs.	%
Water vole	17 654	91,35	352	98,6
Common redbacked vole	574	2,97	—	—
Common vole	67	0,35	—	—
Striped field mouse	707	3,66	4	1,1
Common field mouse	132	0,68	—	—
Yellow-necked field-mouse	44	0,23	—	—
House mouse	52	0,26	—	—
Common shrew	73	0,38	—	—
Water-shrew	7	0,04	1	0,3
Other mammalian species	16	0,08	—	—
Totals	19 326	100,0	357	100,0

It was established that all of the epizootics in this site were referable to a period of large number of water voles, i.e., up to the winter of 1968-1969. There was very negligible involvement of other rodents in the tularemia epizootic, 1.4% of total cultures isolated. We failed to demonstrate any tularemia epizootics from 1969 on, when there was a large number of small rodents (Figure 1).

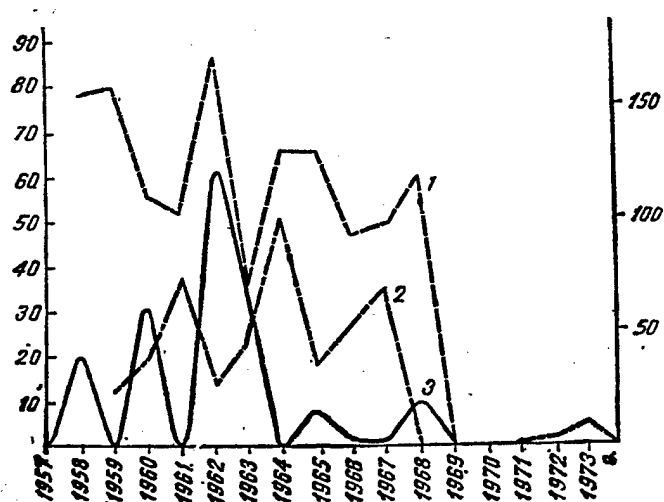


Figure 1.
Dynamics of water vole population size and intensity of tularemia epizootics in the Povorinsk endemic site

- 1) number of water voles in the spring (flood tide)
- 2) fall
- 3) intensity of epizootics

Y-axis: left, % caught in traps; right, number of cultures isolated per 1000 tested animals

The results of further investigation of epizootic activity at the Povorinsk backwater endemic site for tularemia warranted consideration thereof as being of the single-host type [10]. Epizootic outbreaks were observed mainly among water voles, and they were the cause of the epizootic problem in this site [4].

Apparently, there must be a minimum (threshold) number of water voles, which are the main carrier of *F. tularensis*, for epizootics to occur. In the Povorinsk enzootic site, this level corresponded to about 30% trapped water voles. It should be thought that this was close to the threshold level.

Table 2. Quantity of animals tested annually

Year	Water vole			Other species			Total ani- mals tested	Cultures isolated from water	Number of cultures	Cultures/ 1000 tested animals
	number of tests	% trapped	number of cultures	number of tests	% trapped	number of cultures				
1958	1 270	80,0	4	23	—	—	1 293	—	4	3,1
1959	1 997	80,5	—	110	16,5	—	2 107	—	—	—
1960	1 030	56,7	67	71	10,4	—	1 101	—	67	61
1961	1 262	51,7	—	34	10,3	—	1 296	—	—	—
1962	1 184	88,5	167	185	15,7	2	1 369	2	171	123
1963	1 011	35,3	52	68	18,5	—	1 079	4	56	48
1964	1 005	66,6	—	16	5,5	—	1 021	—	—	—
1965	2 172	65,5	31	47	9,3	—	2 219	4	35	14
1966	2 358	46,3	4	229	10,3	1	2 587	—	5	1,9
1967	2 868	49,1	1	156	11,7	2	3 024	—	3	0,9
1968	1 478	60,1	26	51	9,7	—	1 529	3	29	17
1969	—	—	—	42	7,8	—	42	—	—	—
1970	1	0,5	—	298	49,2	—	299	—	—	—
1971	—	—	—	146	50	—	146	—	—	—
1972	—	—	—	14	16	—	14	—	—	—
1973	16	2,0	—	83	21,6	—	99	—	—	—
1974	2	2,0	—	99	22,5	—	101	—	—	—
Totals	17 654	—	352	1672	—	5	19 326	13	370	—

From 1958 to 1968, when the number of water voles was consistently above the threshold level, tularemia epizootics were recorded almost annually. However, their intensity varied, and it was unrelated to fluctuations in number of carriers. Thus, the 1958 epizootic occurred with an 80% level of trapped animals, whereas in the spring of 1959, when there were somewhat more water voles, no epizootic was found, although more animals had been examined than in 1958 (Table 2). By the spring of 1960, the incidence of trapping animals dropped to 56% and, in spite of this, there was a tularemia epizootic (61 cultures of *F. tularensis* per 1000 tested animals). Water voles multiplied intensively in 1961 (52% trapped), but there was no epizootic.

In 1962, the maximum number of water voles for the entire observation period was recorded, and the most intensive epizootic was demonstrated among them (123 cultures per 1000 examined animals). The epizootic continued in 1963, but it did not affect the number of water voles, which reproduced intensively,

and by the spring of 1964 the incidence of trapping them reached 66%, but no epizootic occurred that year. In 1965-1967, the dynamics of intensity of epizootics were identical to 1962-1964, but the epizootics were not as acute.

Table 3.
Incidence of tularemia among people and isolation of *F. tularensis* at the Povorinsk site

Year	Number of sick cases	Number of cultures isolated	Year	Number of sick cases	Cultures isolated
1930	2	—	1951	4	1
1934	103	—	1952	3	2
1936	32	—	1953	1	—
1938	183	37	1958	1	3
1939	6	2	1959	—	4
1941	33	—	1960	1	67
1943	377	—	1962	2	171
1945	1481	3	1963	1	56
1946	347	3	1965	—	35
1947	1	—	1966	—	5
1948	10	6	1967	—	3
1949	62	—	1968	—	29
1950	5	—	1969—1974	—	—

Notes:

1. There were no cases of tularemia among people and no cultures isolated in years not listed here.
2. In 1943 and 1945-1946, cases of tularemia among people were related to a tularemia epizootic among mouse-like rodents--common voles and house mice.

Thus, from 1958 to 1968 the intensity of epizootics changed drastically. It increased from 1958 to 1962 and decreased by 1968. The rise and decline were not uniform, presenting a wavy pattern with 2-3-year period. The phase of growth in intensity consisted of 2 waves with a 2-year period (1958-1959, 1960-1961). The phase of decline consisted of 2-3-year waves (1962-1964, 1965-1967). The entire cycle covered 10 years.

Long-term observation of water vole population size enabled us to conclude that the tularemia epizootics at the Povorinsk site definitely had an influence on it, but it was not always the same and depended on the phase (rise, decline) of epizootic activity. At the phase of rise, when the epizootics had a 2-year period, the number of water voles dropped significantly (1958, 1960); at the phase of decline in intensity of epizootics, which occurred in 3-year waves, the number of water voles dropped drastically (1962, 1965), whereas the second year, in spite of the continuing epizootic, the number of animals was restored (1963, 1966).

The tularemia epizootics, which caused considerable fluctuation in number of water voles in different years [14], could not elicit a lasting decline thereof. A lasting decline was caused by abiotic factors and anthropogenic factors [5, 6, 9]. When the number of water voles dropped below the threshold level, this site moved to an inactive epizootic phase. Kalabukhov [2], Karpov [3] and Kondrashkin [4] arrived at the same conclusion with regard to backwater sites.

We analyzed data on incidence of tularemia among people who had been in the range of the Povorinsk site (hunting for water voles, water use, agriculture, etc.) in order to characterize more fully the changes in epizootic activity of this site. Our findings revealed (Table 3) that there was significant variation of morbidity in different years (Figure 2): years with no tularemia problem were followed by years with marked incidence of the disease among people. We were impressed by the coincidence of sequence of periods of epizootic activity and epidemic well-being. Here too, we observed rises and extinctions

of infections, the same cycles and waves as with the epizootics. From 1933 to 1939, one 3-year and two 2-year waves of morbidity were recorded, and the entire cycle covered 7 years. From 1940 to 1949, the 10-year cycle included two 2-year waves. Significant changes have been occurring since 1946: drastic decrease in outbreaks of tularemia with further change to isolated sporadic cases of disease. That year marked the beginning of systematically implemented scheduled preventive immunization against tularemia.

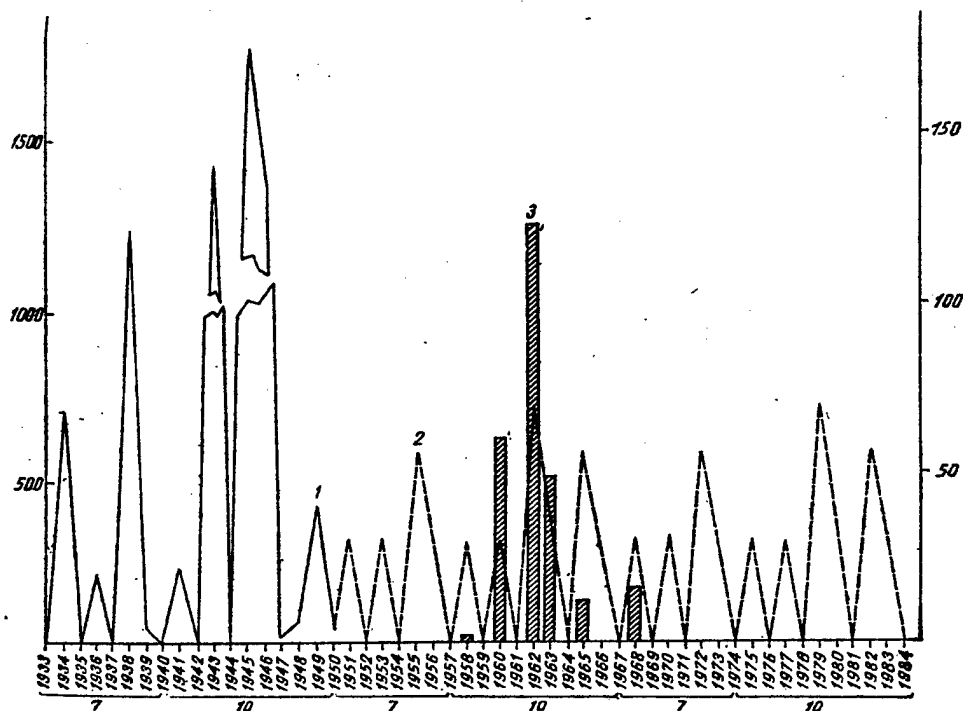


Figure 2. Incidence of tularemia in Povorinskiy Rayon and intensity of epizootics. X-axis, years; y-axis: left, number of sick cases per 100,000 population; right, number of cultures isolated per 1000 tested animals

- 1) number of sick cases per 100,000 population
- 2) estimated morbidity curve
- 3) number of cultures isolated per 1000 tested animals

If we assume that there is alternation of 7- and 10-year cycles and, on this basis, plot a hypothetical morbidity curve (it is also the epizootic curve), we would find that since 1958 it coincides entirely with the actual curve of epizootic activity. An increase in number of water voles started in 1974; tularemia epizootics were demonstrated among them in 1975 and 1977, which corresponded with our projected curve of epizootic activity. Investigation and demonstration of the distinctions referable to dynamics of tularemia epizootics, their influence on the number of voles, which are carriers of this infection, make it possible to produce a long-term forecast and plan epidemic control measures for endemic tularemia sites in backwater-swamp regions.

Conclusions

1. In a backwater endemic site in Povorinsk, tularemia epizootics occurred when there was a large number of water voles; other rodents and Ixodes ticks did not play an appreciable role in maintaining endemicity of the infection.
2. The threshold number of water voles, which presented a threat of a tularemia epizootic constituted a 30% incidence of trapping the animals; if the number was above the threshold level, the fluctuations in epizootic activity at the site did not depend on changes in number of carriers.
3. Epizootic activity at this site was characterized by alternation of 7- and 10-year cycles, which consisted of a certain number of waves occurring with 2-3-year periods; at the phase of rise in intensity (2-year waves) of epizootics, there was significant decrease in number of water voles, at the phase of decline (3-year waves), the number of animals dropped drastically the first year and rose the second year, in spite of the epizootic.
4. Onset of tularemia among people at the site usually coincided with periods of high epizootic activity in water voles.
5. The demonstrated distinctions of dynamics of epizootic activity and its influence on number of the main carriers of tularemia infection made it possible to formulate a long-term forecast and plan epidemic-control measures.

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SENSITIVITY OF SOME *F. TULARENSIS* STRAINS OF THE HOLARCTIC RACE ISOLATED IN KAZAKH SSR TO DIFFERENTIAL DOSE OF ERYTHROMYCIN AND OLEANDOMYCIN

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[Article by O. B. Chimirov, Central Asian Scientific Research Plague-Control Institute, Alma-Ata]

[Text] Determination of sensitivity to erythromycin and oleandomycin of different strains of *F. tularensis* is an additional test for differentiation of its geographic races [3, 5]. This test is based on the high resistance of the Holarctic race of the pathogen of tularemia to erythromycin (100 µg/ml or more) and oleandomycin (400 µg/ml or more), whereas strains of the Central Asian and Nearctic race of *F. tularensis* are sensitive to the above-mentioned doses of these antibiotics [1, 3, 5].

Previously [3], strains of the Holarctic race of this microorganism, which were isolated in the USSR, were characterized only by resistance to erythromycin and oleandomycin, and for this reason this trait served as an additional differential test. However, recent works [2, 4] established that some strains of the Holarctic race isolated in Taymyr, Buryat ASSR, Yakutsk ASSR, Chita and Khabarovsk Oblasts, Maritime Kray and Sakhalin, as well as some strains isolated in Italy, France, Sweden, China and Japan, were found to be sensitive to erythromycin and oleandomycin.

Our study of a number of strains of the Holarctic race of *F. tularensis* isolated in 1973-1975 in Kazakh SSR revealed that not all of the strains were resistant to differential doses of erythromycin and oleandomycin. For this reason, we decided to determine the degree of sensitivity of *F. tularensis* of the Holarctic race to these macrolides.

We took 33 strains of *F. tularensis* isolated in Kazakh SSR in 1973-1975 for our experiment; 6 of them were isolated from Turgay Oblast, 18 from Taldy-Kurgan Oblast and 9 from Uralsk Oblast. All of them presented typical morphological, cultural and biochemical properties; they did not decompose glycerin on Downs' medium and had no citrulline ureidase activity [6], i.e., they were referable to the Holarctic race and caused death of white mice given hypodermic injections of isolated bacterial cells.

Experiments were conducted on Heddleson's liver-glucose-glycerin-cysteine agar with addition of 10% defibrinated rabbit blood at pH 7.2. The antibiotics were

diluted in distilled water and specific doses (100 µg/ml erythromycin or 400 µg/ml oleandomycin) were added to dissolved agar cooled to 45°C at the same time as defibrinated blood. After thorough mixing, the medium was decanted in Petri dishes and kept for 1 day at room temperature.

Table 1. Behavior of tested strains with erythromycin (E) and oleandomycin (O)

Where strains were isolated	Strains tested	Number of strains resistant to E & O	Number of strains sensitive to E & O
Turgay Oblast	6	5	1
Taldy-Kurgan Oblast	18	10	8
Uralsk Oblast	9	9	-
Totals	33	24	9

In the experiments, we used a suspension of 1 billion bacterial cells per ml saline (according to the enteric standard of GKI [State Control Institute of Medical Biologicals]), prepared just prior to inoculation on media with antibiotics from a day-old culture of *F. tularensis* grown at 37°C in Heddleson's agar. We plated 4 strains in each Petri dish containing medium with antibiotics, one of which was known to be resistant to the antibiotic in question, and was a typical representative of the Holarctic race (strain No 263), another was a typical representative of the Central Asian race of *F. tularensis* sensitive to these antibiotics (strain No 31).

Using a 1-ml pipette, we inoculated 0.05 ml, which corresponded to 50 million bacterial cells. The same suspension was used to inoculate a control Petri dish with medium containing no antibiotic. The cultures were incubated for 10 days at 37°C and examined daily. We assessed sensitivity or resistance of the strains to antibiotics on the basis of appearance or absence of growth of *F. tularensis* on this medium, as well as in comparison to bacterial growth in the control dish. Concurrently, we examined under a microscope smears of cultures that grew in medium with antibiotic, which were stained by the Gram method.

The results of this experiment revealed (Table 1) that there were strains that were both resistant and sensitive to the tested doses of antibiotics among the examined cultures. We failed to demonstrate a difference in virulence of resistant and sensitive strains of *F. tularensis* of the Holarctic race for white mice--all of the animals died after hypodermic infection with isolated bacterial cells.

In determining the degree of sensitivity to erythromycin and oleandomycin, we found (Table 2) that the minimum inhibitory concentration (MIC) of antibiotics for these strains was considerably lower than the differential dose of erythromycin (100 µg/ml) and oleandomycin (400 µg/ml). As to other antibiotics, all of the strains were highly resistant to penicillin (MIC = 1000 µg/ml) and polymyxin (MIC = 2000 µg/ml) and sensitive to tetracycline (MIC = 5 µg/ml) and streptomycin (MIC = 2.5 µg/ml).

We failed to demonstrate any correlation between reaction to erythromycin, oleandomycin and biochemical activity of the tested cultures, as well as

their source: we found strains that were both sensitive and resistant to what was previously considered differential doses of these macrolides among strains that did not decompose glycerin and did not have citrulline ureidase activity.

Table 2. Characteristics of strains of *F. tularensis holarctica* sensitive to differential doses of erythromycin and oleandomycin

Strain No	Source	Date of isolation	MIC, $\mu\text{g/ml}$	
			erythro- mycin	oleando- mycin
205	Ar. terrestris	27/VIII 1973	6	12
316	D. marginatus	22/VI 1975	12	100
332	D. marginatus	23/VI 1975	6	100
333	D. marginatus	23/VI 1975	6	100
334	D. marginatus	25/VI 1975	6	50
344	D. marginatus	20/VI 1975	6	50
355	D. marginatus	20/VI 1975	6	50
356	D. marginatus	20/VI 1975	6	50
358	D. marginatus	20/VI 1975	6	50

Thus, the demonstrated difference in response to differential doses of erythromycin and oleandomycin of strains of the Holarctic race of *F. tularensis*, which were isolated on the territory of Kazakh SSR, confirmed the data of Brikman et al. [2] to the effect that this test is not suitable for differentiating between geographic races of the pathogen of tularemia.

Conclusions

1. Among strains of the Holarctic race of *F. tularensis*, which were isolated in Kazakh SSR, some were sensitive and others resistant to erythromycin and oleandomycin.
2. Sensitivity of the bacteria to erythromycin and oleandomycin was unrelated to the source of the pathogen or to virulence and biochemical properties of strains.
3. The presence of strains of the Holarctic race of *F. tularensis*, which were both resistant and sensitive to differential doses of erythromycin and oleandomycin, on the territory of Kazakh SSR makes it impossible to differentiate between their geographic races according to this property.

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ENDEMIC INFECTIONS IN THE NORTHERN PART OF THE FAR EAST. REPORT 1: DETECTION OF ENDEMIC SITES IN CHUKOTKA

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 10, Oct 78 (manuscript received 8 Aug 77) pp 101-104

[Article by G. P. Somov, F. N. Shubin, I. M. Gopachenko, G. N. Leonova, T. P. Ivanenko, S. A. Sorochenko, A. N. Sotnikova, V. G. Kuznetsov and S. P. Kruglyak, Vladivostok Institute of Epidemiology and Microbiology, Siberian Branch of the USSR Academy of Medical Sciences, Novosibirsk; Maritime Kray Plague-Control Station, Ussuriysk]

[Text] The rapid industrial development of the northern part of the Far East, because of the exceptional wealth of natural resources in that region, has advanced several biomedical problems aimed at improving man's life under extreme conditions. The question of presence of endemic sites of infection in the vast territory of the northern Far East, about the existence of which virtually nothing was known until very recently, is of some importance among these problems.

The major outbreaks of Far Eastern scarlatinoid fever (human pseudo-tuberculosis in Chukotka [Chukotsk Peninsula] drew attention to the study of endemic diseases in the northern Far East [1, 3, 6]. The results of serological studies pursued in this regard revealed that there were antibodies to the pathogens of tularemia, leptospirosis, pseudotuberculosis, brucellosis, ornithosis, toxoplasmosis and Q fever in the blood of people and animals [4, 5].

A many-year complex program was elaborated for the study of endemic infections in the northern Far East in order to resolve the problem that emerged; implementation thereof was started in 1974 in the form of organizing annual expeditions. We submit here some of the results of 3-year studies, the purpose of which was to find endemic sites of some infections in Chukotka, investigation of their structure and mechanisms of circulation of pathogens under the rigorous climate and geographic conditions of the North.

We first made a serological study of 1027 reindeer with antigens of *Y. pseudo-tuberculosis* and *Yersinia enterocolitica* using the agglutination reaction, *F. tularemia* by the indirect hemagglutination reaction, *Rickettsia* by the CFT [complement fixation test] and arboviral infections by the hemagglutination inhibition reaction. As a result, it was established that antibodies to the

pathogen of pseudotuberculosis serotype I were present in $13.3 \pm 0.7\%$ of the animals, to serotype III pseudotuberculosis in $10.5 \pm 1.03\%$, to *Y. enterocolitica* (strain No 443) in $2.92 \pm 0.55\%$, tularemia in $10.58 \pm 1.4\%$, North Asian tick-borne [mite?] typhus in $1.05 \pm 0.4\%$, Q fever in $1.42 \pm 0.4\%$ and tick-borne encephalitis in $1.6 \pm 0.53\%$ of the tested reindeer. Analysis of 85 samples of blood from Arctic susliks also demonstrated antibodies to serotype I of *Y. pseudotuberculosis* in 5 animals and serotype III in 10, and when blood from 23 susliks was tested with antigen of tick-borne encephalitis virus antibodies were found in 2 animals.

Thus, the obtained data were indicative, in the first place, of the possibility of existence in Chukotka of endemic sites of these infections and, in the second place, of the possibility of circulation of the pathogens of zoonoses among small mammals and their nest-burrow parasites under the rigorous climate conditions of the Chukotsk tundra, as was demonstrated in Taymyr [2]. Migratory birds, which could be the link between endemic sites of diseases in remote climate and geographic zones, play a part in this process.

The microbiological and virological studies made it possible to confirm in part, the results of serological studies. In all, 268 specimens of mammals were caught and submitted to virological testing in different parts of Chukotka, including 182 Arctic susliks, 46 northern redbacked voles, 21 large-toothed redbacked voles, as well as a few root voles, brown rats and northern pikas, 95 migratory birds represented by 11 species and the contents of 45 eider nests.

Material from these animals (liver, spleen, segment of intestine) was delivered for tests in a frozen state in insulated containers with liquid nitrogen. For testing for pseudotuberculosis, macerated organs and segments of intestine were plated separately in buffer solution by the method of Paterson and Cook [7]. The cultures were kept at 4°C for 30 days, and periodically subcultured on differential nutrient media.

We isolated 9 strains of *Y. pseudotuberculosis* from Arctic susliks, thereby establishing, for the first time, spontaneous carriers in this species. In addition, isolation of *Y. pseudotuberculosis* strains from susliks showed that endemic sites for pseudotuberculosis can exist in the northern part of the Far East which, in turn, enables us to relate outbreaks of this infection to circulation of the pathogen in the environment.

Concurrently, the delivered material was tested by a similar method for *Y. enterocolitica*. We isolated a total of 48 *Y. enterocolitica* strains. Most of these strains were homogeneous in biochemical properties. They fermented to acid glucose, maltose, mannitol, saccharose, xylose, sorbitol, did not split lactose, dulcitol, raffinose, did not form hydrogen sulfide, whereas there were both positive and negative variants with regard to production of indole and fermentation of rhamnose. All of the strains were nonmotile at 37°C . At both temperatures, they reacted positively with methyl red and formed acetyl methylcarbinol at 22°C .

Spontaneous *Y. enterocolitica* carriers were found among the Arctic suslik, brown rat, northern redbacked vole, shrew and six bird species: long-tailed duck, tufted duck ["chernyat'"], Temminck's stint [sandpiper], glaucous gull and alpine ptarmigan.

The incidence of *Y. enterocolitica* infection is rather high among different animal species. For example, it reached $15.93 \pm 2.7\%$ in the Arctic suslik. However, no antibodies to *Y. enterocolitica* were demonstrated in a test of blood serum from 85 susliks with live cultures of isolated strains and autologous strains.

Material from 238 small mammals and birds caught in the valleys of the Anadyr and Tanyurer Rivers were submitted to bacteriological analysis for salmonellosis. Two strains of *Salmonella heidelberg* were isolated, which were typical in morphological, biochemical and antigenic properties. Spontaneous *Salmonella* carriers were found among the northern redbacked voles, thereby demonstrating for the first time the endemicity of this salmonellosis in the extreme northeastern part of Asia.

For demonstration of arboviruses, we examined 237 birds of 20 species, 30 rodents (northern pika, northern redbacked and large-toothed redbacked voles), 63 Arctic susliks and 1 hare. All of the material was analyzed in 97 biotests on suckling mice. We isolated four strains of viruses, which were identified as tick-borne encephalitis virus according to biological and antigenic properties. Spontaneous carriers were found in two bird species (ruff and long-tailed duck), the Arctic suslik and northern redbacked vole. The virus of tick-borne encephalitis was isolated from the ruff in the early spring, which indicates that the virus is carried by birds to the northern regions of the Far East. Isolation of the virus from the Arctic suslik and northern redbacked vole, on the contrary, indicated that there was an endemic site for tick-borne encephalitis in Chukotka. It can be assumed that nest-burrow parasites and, perhaps, mosquitoes are involved in the process of circulation of tick-borne encephalitis virus in the environment of the Extreme North, since the percentage of seropositive results with regard to tick-borne encephalitis virus was about the same when we tested reindeer grazing in different physico-geographic regions of Chukotka (see Table).

Results of serological testing of Chukotka reindeer with antigens of pathogens of some zoonanthroponotic diseases ($M \pm m$)

Physicogeographic region	F. tularensis		Tick-borne encephalitis virus		Pathogen of Asian tick-borne rickettsiosis		Pathogen of Q fever	
	sera	anti-bodies found, %	sera	anti-bodies found, %	sera	anti-bodies %	sera	anti-bodies %
Anyuysk-Chukotsk bald peak and tundra highlands	302	4.0 ± 1.13	322	2.2 ± 0.82	—	—	—	—
Anadyr-Penza bald peak and forest-tundra plateau	403	9.9 ± 1.48	403	2.0 ± 0.7	302	1.7 ± 0.74	302	2.0 ± 0.81
Koryaksk bald peak and forest-tundra highlands	139	9.4 ± 2.48	130	1.4 ± 0.99	246	0.8 ± 0.57	246	0.8 ± 0.57

Only serological tests were made (see Table) for detection of tularemia and endemic rickettsiosis. Antibodies to the pathogens were found in reindeer from all of the examined physicogeographic regions of Chukotka.

Thus, as a result of these studies in Chukotka, for the first time there was demonstration of endemic sites of pseudotuberculosis, enteric yersiniosis, heidelberg type salmonellosis and tick-borne encephalitis. Two animal species play a large part in circulation of the pathogens of zoonoses, and they are also the most numerous--Arctic suslik and northern redbacked vole--and this creates the conditions for formation of combined sites of infection.

Considering the data on isolation of tick-borne encephalitis virus on Taymyr Peninsula [2] and the results of our studies, it can be concluded that tick-borne encephalitis virus circulates in northern Siberia and Far East. The absence of Ixodes ticks at these latitudes warrants the assumption that there are special routes of circulation of the virus in the environment, and this requires further in-depth investigation.

Conclusions

1. Endemic sites of pseudotuberculosis, enteric yersiniosis, heidelberg type of salmonellosis and tick-borne encephalitis were found for the first time in the subarctic tundra and forest-tundra of Chukotka.
2. Isolation of tick-borne encephalitis virus from the ruff and long-tailed duck during the spring migration was indicative of the possibility that migratory birds brought the virus to Chukotka.
3. The results of serological studies are indicative of the expediency of further investigations for detection of endemic sites for tularemia and endemic rickettsiosis in Chukotka.

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CURRENT STATUS OF TULAREMIA PREVENTION IN THE USSR AND FUTURE TASKS

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[Article by G. P. Nikolayevskiy, V. I. Frolov and V. I. Morozov]

[Text] The achievements of Soviet health care in the area of preventing infectious diseases are common knowledge. Considerable advances have been made, not only in the area of controlling infections that were widespread in pre-revolutionary Russia, such as typhus and relapsing fever, smallpox, cholera, malaria, anthrax and others, but considerable work has also been done on the study and development of a system of measures for the prevention of many diseases that had not been given attention in tsarist Russia.

One of these diseases is tularemia. No doubt, it was encountered in our country since time immemorial; however, the illness in people most often was diagnosed as plague, plague-like disease, fever, etc.

In the years that have passed since the pathogen of tularemia was identified in the USSR (1926), Soviet researchers have accumulated basic material on the microbiology, laboratory diagnosis, pathology, symptomatology, therapy, immunology and prevention of this infection. Soviet researchers made greater advances than foreign authors in the study of many of these areas.*

From 1926 to 1927 (a 9-month period), 561,510 cases were recorded among people in the USSR. Since there was no mandatory registration, which was adopted in 1941-1942, the morbidity records of prewar years were, of course, incomplete.

One can arbitrarily distinguish five periods in the dynamics of tularemia among the people of the USSR. The first period (1926-1932) was referable to active detection tularemia in our country; in these years there were 11, 287 cases; the second period (1933-1940) was one of investigation of the infection and development of a system of epidemic-control and preventive measures, and already 40,002 cases of tularemia were found; considering the absence of mandatory registration of diseases in these years, it can be assumed that the true incidence of tularemia was somewhat higher; the third period (1941-1949) was a period of maximum incidence of the infection among people, and there were

*Olsuf'yev, N. G., MED. PARAZITOL., No 3, 1977, pp 273-282.

482,413 registered cases. It should be noted that in these years, mandatory registration of tularemia had already been adopted, and this guaranteed a full record of cases in the nation, with the exception of occupied territories.

The wide distribution of tularemia during the war and early postwar years was attributable to failure to adherence to principles of agriculture due to military operations, intensive reproduction of rodents, who were the sources of infection, migration of the public, long-time presence or exposure to endemic tularemia sites. In addition, tularemia vaccine was not yet in use for mass inoculation of the public, and this practice was adopted in public health in 1948-1949.

The fourth period (1950-1964) was characterized by a decisive attack on the infection by means of an effective system of measures, the principal one being mass scale vaccination of the public with live tularemia vaccine in enzootic regions. During these years, there were still 34,280 registered cases of this disease among people. In this period, there was intensive development and completion of the system of measures for tularemia prevention, and the minor outbreaks of tularemia (1954, 1957) were related to development of regions where there were previously unknown endemic tularemia sites.

Thus, the combined ameliorative measures in endemic sites of infection, based on a comprehensive study of tularemia, as well as regular mass-scale immunoprophylaxis of the disease made it possible to reduce the incidence of tularemia to isolated cases, in spite of the poor epizootic situation in the country.

It can be considered that tularemia in the USSR has been a controlled infection since 1965, the fifth period in the dynamics of human morbidity. Since that time, there have been isolated cases of the disease and cases among small groups of unvaccinated individuals (usually not immunized because of medical indications). As a result, 1703 cases of tularemia among humans were recorded in the USSR in 13 years (1965-1977), although the epidemic activity of many enzootic sites of infection was very high. This was indicated by the intensive epizootics among rodents, isolation of *F. tularensis* cultures annually from both rodents and ticks, as well as environmental objects, mainly water. The advances made in the USSR in the control of tularemia are even more impressive, if we consider that endemic sites of infection are still being found virtually all over the country.

At the same time, the intensive epizootic situation with regard to tularemia in the USSR makes it imperative to implement mandatory and proper tularemia-control measures. When such work is not done properly or sufficiently, group diseases occur. In these years, group diseases constituted more than 90% of all cases of tularemia among people. Isolated, sporadic cases are encountered rather seldom. Most active were endemic sites of the backwater-swamp and mountain (foothill)-brook types, in which more than 97% of the tularemia-stricken people were infected.

As a rule, group cases occurred in sites of the backwater-swamp type in a typical period of the year, summer and fall, particularly July and August. Unimmunized people became ill in endemic tularemia sites, among those engaged in mowing hay, gathering mushrooms and berries, vacationers, swimmers in

waters contaminated by excrements of rodents with tularemia. The transmissible and water routes were the principal ones in transmission of infection in these sites.

The cases of group disease in endemic sites of the mountain (foothill)-brook type in Transcaucasian republics were distinctive. There, the following causes were involved in infecting humans: water factor of transmission in Armenian SSR, and people became ill in March and September-October; alimentary route (March) in Azerbaijan SSR; in Georgian SSR through ticks when people came in contact with livestock grazed in endemic tularemia sites (October-November).

It should be noted that these group cases were due not only to the activity of some endemic tularemia sites, but flaws and serious infractions of the rules for preventive tularemia-control measures.

Most of the tularemia cases are recorded in the RSFSR (74.7% of all morbidity since 1972), Transcaucasian republics--14.6% and Kazakh SSR--8.5%, with 2.2% for the rest of the regions.

In assessing the measures for tularemia prevention, we must mention that they are combined, and this is reflected in the relevant work plans. At the same time, the principal preventive measures are implemented by public health agencies and institutions, unlike other zoonotic infections, for which measures implemented by the USSR Ministry of Agriculture, other ministries and agencies play a large part. With regard to tularemia, other ministries and agencies implement measures for the control of rodents and vectors.

In order to properly organize tularemia-control measures, in the USSR much attention is given to inspection of endemic tularemia sites, exploration of regions where there may be such sites. In our country, considerable territories of endemic sites are inspected annually, up to 400,000 rodents, up to 800,000 bloodsucking arthropods, many samples of water, grain and other material. The method of inspecting sites by means of demonstrating antibodies in bird pellets and droppings of carnivorous mammals is being used more and more.

Epizootics are also monitored and regulated by means of planned control of rodents and vectors, together with concerned ministries and agencies. A total of 30-60 million ha fields and 3-4.5 billion m² in populated areas are treated annually in our country for eradication of rodents (Table 1).

Table 1. Parameters of treatment of areas against rodents*

Regions	1974	1975	1976
Fields, thousands of hectares	37,188	31,045	62,567
Populated centers, thous. m ²	4,367,210	3,867,389	3,241,779

*Not counting the work done by plague-control institutions.

Substantial work is being done in the USSR with regard to inspection of regions in which no endemic sites are known, but they could be present. Such sites are being inspected with particular intensity in regions under development, in particular along the route of the Baykal-Amur Railroad.

Of course, with regard to tularemia prevention attention is focused mainly on vaccination. Work in this direction is being constantly refined. Considering the existing system of measures for the prevention of tularemia, the USSR Ministry of Health issued an order on 5 February 1976, "On Measures for the Prevention of Tularemia Among the Public." This order approved the "Methodological Instructions for Planned Preventive Inoculations Against Tularemia," and described distinct concepts of territories enzootic for tularemia, procedure for detecting them, reporting and eliminating enzootics. The methodological instructions call for a differentiated approach to scheduling inoculations on the basis of exact determination of groups that are really exposed to the risk of *F. tularensis* infection. At the present time, within the limits of an administrative region deemed to be enzootic for tularemia, scheduled inoculations cover only the public residing or working in the enzootic territory of the village soviet with sites of the meadow-field, steppe, backwater-swamp or foothill-brook type, as well as inhabitants of adjacent village soviets if the landform and economic indicators of their territory do not differ from the rural soviet with established enzootic.

A definite approach has also been formed to planning of inoculations in accordance with epidemic activity of enzootic tularemia sites. For example, in sites of the meadow-field type, one does not have to immunize children up to 14 years of age, retired persons, the disabled, and there are expanded contraindications for immunization. In areas with inactive endemic sites, where only isolated cases of the disease had been recorded in the past, inoculations have become strictly occupational.

This order augmented appreciably the role of health ministries of Union republics, as well as local health agencies, in planning immunization of the public, since the question of declaring and eliminating enzootic nature of regions is within their competence.

Monitoring of immunization in the country and immunological structure of the population revealed that, in general, this work is being done correctly, and the immune stratum of public exposed to the risk of infection by *F. tularensis* constitutes about 85%, i.e., it meets the established requirements (Table 2).

It should be noted that the number of people immunized against tularemia in the USSR annually is quite sizable (Table 3), from 5,812,000 to 13,814,000 (in the period following adoption of scheduled mass scale vaccination, i.e., after 1949).

Prompt forecasts of the epizootic and epidemic situation in different regions is important to the prevention of group morbidity and proper organization of preventive measures. Sufficient attention is given to making them in all of the republics. Thus, epizootic complications pertaining to tularemia in West Siberia and the Ukraine in 1972 were forecast in good time. However, in cases where little zooparasitological work is being done, which is used

to make forecasts, the latter are not justified. For example, in the same year (1972), the favorable forecast was not confirmed in Kazakh SSR. As a result, the measures were not implemented to a sufficient extent and there were cases of the disease among people in North Kazakhstan Oblast.

Table 2. Results of checking the immune stratum

Parameter	1972	1973	1974	1975	1976
Number of populated centers inspected	2 799	3 430	4 114	4 644	4 307
People tested	419 983	466 181	450 287	386 504	371 162
Incidence of positive results, absolute	354 698	397 220	386 448	334 206	312 748
Immune stratum, %	84,5	85,2	84,5	86,5	84,3

Table 3. Preventive vaccination and incidence of tularemia

Year	Number of sick cases	Immunized public, thous.	Year	Number of sick cases	Immunized public, thous.
1941	18 498	—	1959	982	12 780
1942	103 581	1,3	1960	1338	9 905
1943	27 085	4,0	1961	926	10 767
1944	3 848		1962	700	10 783
1945	136 616		1963	1591	10 232
1946	76 231	468	1964	569	13 121
1947	5 446	768	1965	131	11 387
1948	71 554	4 399	1966	271	10 194
1949	35 534	13 814	1967	272	10 200
1950	6 544	7 563	1968	182	9 999
1951	2 948	6 589	1969	50	8 957
1952	2 503	9 871	1970	22	9 066
1953	2 038	7 052	1971	59	8 100
1954	3 210	7 718	1972	205	9 370
1955	2 921	6 800	1973	115	9 086
1956	1 126	7 300	1974	114	7 252
1957	5 623	9 200	1975	178	7 800
1958	1 261	11 260	1976	55	5 812

The success of work for tularemia prevention among the public depends largely on how well-informed the public is about this infection and preventive measures. According to data at our disposal, the scope of work on health education with regard to tularemia can be deemed quite sufficient.

Plague-control institutions and some scientific research institutes concerned with epidemiology, first of all, the Scientific Research Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, render methodological assistance in questions of tularemia to public health agencies and institutions.

The low incidence of tularemia among people in the USSR is indicative of the high efficacy of tularemia-control measures implemented by public health agencies and institutions. Nevertheless, it is still important to systematically improve the efficacy and quality of work for the prevention of tularemia.

This is attributable, first of all, to the further expansion and intensification of man's contact with the environment, animals of different species in the course of farming, as well as during recreation. Under the 10th Five-Year Plan, work must be continued to develop new regions and considerable reclamation. As shown by experience, such development of territories requires thorough investigation and implementation of appropriate measures for the prevention of endemic infections, including tularemia.

At the same time, a law is being worked out in the USSR for the protection of the animal kingdom, which is the first legislation in the world whose objectives are to regulate social relations in the area of protection and use of the animal kingdom. Of course, this has its mark on the system of measures dealing with prevention of endemic infections, and it requires relevant research.

Finally, one should not overlook the fact that the importance of tularemia to public health is attributable to the wide distribution of endemic sites of infection in our country, their high epidemic activity and exceptional persistence. Moreover, tularemia is widespread all over the world. Even the fragmentary data submitted to WHO by some nations indicate that tularemia is distributed in Europe and Asia, America and Africa. This data do not reflect the true distribution of tularemia in foreign countries, since they have no government system of keeping records and, moreover, little attention in general is given to tularemia, which is an infection with a low mortality rate.

All this is indicative of the need for some revision of the system of tularemia-control measures, in order to improve their efficacy and reduce their cost. The question arises of further differentiation of measures in accordance with local agricultural conditions, presence of enzootic and anthropurgic sites of infection, absence of epizootics in the wild and among domestic (including farm) animals.

At the same time, one should avoid unwarranted reduction in scope of measures for tularemia prevention. One should proceed quite cautiously and after comprehensive investigation in altering the system of measures in any particular region.

As we learn from many years of experience, proper and thorough implementation of the existing system of measures for tularemia prevention is a reliable guarantee of protecting the public against this infection.

The prospects for improving tularemia prevention are closely linked with improvement of the quality of work done by therapeutic and preventive institutions in the area of detection, keeping records, registration and treatment of patients suffering from this infection, as well as implementation of strict monitoring by sanitary and epidemiological institutions along the lines of State health inspection to see that the established standards and rules are followed for protection of sources of water, potable water and foods against contamination by *F. tularemia*, as well as other preventive measures.

Conclusions

1. Combined health-improving measures, based on a comprehensive study of tularemia in the USSR, in endemic sites and planned vaccination against this disease have made it possible to reduce to isolated cases the incidence of tularemia, which showed itself to be epidemically quite active, in spite of the epizootiological activity of enzootic infection sites.
2. The intensive epizootic situation in our country with respect to tularemia makes it imperative to implement on a mandatory basis appropriate tularemia-control measures.
3. Prospects for improving the efficacy of tularemia prevention are related to further differentiation of measures in accordance with activity of infection sites and local agricultural conditions.

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MAIN RESULTS OF EXPLORING ENDEMIC SITES OF HUMAN DISEASES IN THE AMUR-BUREYA SECTION OF THE BAYKAL-AMUR RAILROAD

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[Text] In accordance with a plan approved by the USSR Ministry of Health, in 1975-1977 the Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, together with the Khabarovsk Kray Sanitary and Epidemiological Station, Khabarovsk Plague-Control Station and some other institutions, explored the distribution of endemic sites of human diseases in the Amur-Bureya section of BAM [Baykal-Amur Railroad]. This is the most easterly part of the Mainline Railroad extending over about 500 km (between Urgal and Komsomolsk-na-Amure), which is to be among the first to start operation. Construction and rapid development of this territory are leading to a drastic growth of population. There is immeasurably more intensive contact between construction workers and inhabitants of previously existing and newly built settlements with the natural biotopes surrounding them. The need for such exploration was attributable to the virtually complete absence of information that could serve as the basis for speedy organization of prevention of endemic diseases.

Material and Methods

The Amur-Bureya segment of BAM traverses a territory with a diversified landform: along the Upper Bureya intermontane trough, over the Bureya ridge, along the gradually widening and changing Amgun River valley over a considerable distance, intersecting the relatively even areas of the Chukchagir-Evoron depression and, finally, out into the Amur valley. Aerovisual inspection of the route and adjacent strip revealed that biocenotic conditions for existence of the pathogens of endemic infections were present only in the area whose absolute altitude did not exceed 650-750 m. Within this territory, five key districts were selected by means of analysis of cartographic data. They are situated along the route at about 100 km from one another (near the settlements of Ust-Urgal, Suluk, Badzhal, Sonakh, Kondon); they contained all of the main

elements of the mesotopography and reflected the most typical landform features of the locality. In each such district, using the same program and standard methods [5, 6], we pursued zooparasitological studies and gathered material for virological and bacteriological studies to detect the following infections: tick-borne encephalitis, tularemia, pseudotuberculosis and enteric yersiniosis, leptospirosis and rickettsiosis. The local inhabitants were screened by means of a set of serological and epidemiological methods. Isolation of pathogens of endemic infections and serological tests were performed in specialized permanent laboratories in Moscow and Khabarovsk using conventional techniques.

In all, about 11,500 adult *Ixodes* ticks were caught and tested for pathogens of endemic infections. In all of the key districts, enlarged photos were taken of their location and a record kept of their number with a load factor of 3-4.5 rating units per km², for a total of 536 flag-hours. To determine the number of small mammals, we processed about 23,00 trap-nights. A total of 1244 animals referable to 18 species were trapped and examined bacteriologically. For demonstration of leptospirosis, we examined about 600 head of cattle and pigs. The local inhabitants of 14 settlements situated along the railroad construction route were submitted to epidemiological examination; almost 3000 blood serum samples from local residents were submitted to serological tests with antigens of various pathogens of endemic infections. We submit here only the principal results of this work.

Results and Discussion

It was established that small mammals are represented in all areas essentially by *Cl. rutilus* (northern redbacked) and *Cl. rufocanus* (large-toothed redbacked) voles, the number of which is relatively small on the whole, but constitutes up to 14 per 100 trap-days in some of the most easterly parts of the route in forests where there is a significant admixture of wide-leaved trees. The biotopes near water are inhabited by the muskrat (*O. zibethica*) and Ungur vole (*M. maximoviczii*). The average number of the latter constitutes more than 3 per 100 trap-days in the floodplain habitats in the Chukchagir-Evoron plain. There are many house mice (*M. musculus*) and rats (*R. norvegicus*) in residential and farm buildings, which penetrate intensively into new settlements. The striped field mouse (*A. agrarius*), to which as we know are closely linked manifestations of hemorrhagic fever with the renal syndrome in the Far East, was not found anywhere. The density of hoofed and other wild mammals, which are hosts for adult *Ixodes* ticks, does not limit the abundance of these arthropods. *I. persulcatus* is the most widespread and *D. silvarum* is encountered less often. The detection of isolated *H. concinna* and *H. japonica douglasi* is of no appreciable significance to dissemination of pathogens. The tayga tick [or mite] was found everywhere, but different segments of the route differ basically in number and distribution thereof. The number of ticks varies, depending on absolute altitude, nature of mesotopography and other features of the locality, which ultimately determine the temperature and mesoclimate features of a given region. Under the most suitable conditions, the mean number of tayga ticks constitutes 13.5 and the maximum is 54 per flag-hour, and they are usually encountered in some quantity or other over virtually the entire territory. In the region of the Bureya ridge, the average number of ticks is about one-tenth and constitutes only about 1.6, whereas maximum does not exceed 14 per flag-hour. *I. persulcatus* is situated here in the river plains and other lower mesotopographic regions. There is an average of less

than 0.1/flag-hour of *D. silvarum* in the regions of the Upper Bureya depression and Bureya ridge. The average number of this tick species reaches 4 and the maximum is 30/flag-hour only in some parts of the Amgun River valley and Chukchagir-Evoron plain.

A total of 852 standard (10 adult ticks in each) pools of *I. persulcatus* were used in biotests on white mice, and 110 strains of tick-borne encephalitis virus were isolated; this virus was found in all areas. But infectivity of the samples varied in different segments of the route, ranging from 1.3 to 29%. This is consistent with the incidence of virus carriers among these vectors, which ranges from 0.1 to 3.4% [2]. In general, the endemic sites in the eastern part of BAM do not differ from many epidemiologically intensive sites situated in other parts of the range of tick-borne encephalitis, according to this parameter [7]. On the basis of the number and degree of infection of ticks, it should be considered that the most dangerous sites of tick-borne encephalitis are distributed mainly in the most easterly part of the surveyed territory (from Berezovyy to Komsomolsk-na-Amure), as well as in some spots of the valley in the middle part of Amgun River. An average of up to 10 tayga ticks and, in addition, up to 6 *D. silvarum* attack a man spending an entire day continuously in these sites in the tayga. A considerable part of the railroad route near the Amgun and Bureya regions, which are covered mainly by grass and wild rosemary "listvenichniki" [foliage, or could refer to larch groves with ground cover of grass and wild rosemary], present less hazard with regard to tick-borne encephalitis. There is even less danger in the region of the Bureya ridge, where there is prevalence of wild rosemary "listvenichniki" and where man is attacked by an average of less than one tick per day during the period of peak seasonal activity of *I. persulcatus*.

According to a survey made in 1975, about 23% of the local residents reported having contact with ticks during the spring and summer. Anti-hemagglutinins were found in the serum of about 11% of the individuals who had lived in that locality for at least 5 years. According to the results of passive hemagglutination and complement fixation tests, up to 10% of the inhabitants are infected in the spring-summer period. Still, the intensity of contact of people with endemic sites was and, for a number of reasons, still is relatively low. It will increase drastically in the next few years, after construction is completed on a number of planned settlements, as well as railroads and highways. This will increase significantly the danger of epidemic manifestation of endemic sites for tick-borne encephalitis.

Tularemia, which was found in the southern part of Khabarovsk Kray in a strip of mixed coniferous and broad-leaved tree forests [1, 3], had not been recorded before our studies in the Amur-Bureya interfluvial region. Bacteriological examination of gathered *Ixodes* ticks failed to yield positive results. However, the tularin test was positive in all settlements, in 6-10% of the people who had lived there continuously and were never immunized against tularemia, whereas serological agglutination and passive hemagglutination tests were indicative of the probability of infection of up to 17% of this group. The results of examining small mammals in 1975 and 1976 were negative. In 1977, we used 86 spleens from rodents trapped in early July near Lake Evoron for 22 biotests. We isolated two cultures of *F. tularensis* from a northern redbacked and Ungur vole. Thus, for the first time it was proven that

there are tularemia sites in the northern regions of Khabarovsk Kray, and retrospective diagnostic methods showed that it is possible for people to contract this infection.

The endemic tularemia sites along the Amur-Bureya part of BAM are located near backwater and lowland reed-grass meadows. They are apparently epizootically closely linked with the Ungur vole and muskrat, and they must be classified as the backwater-swamp type. Epidemically, they remained inactive due to the relatively low risk of infection, which can apparently occur mainly by the water route. With the current scope and rate of development of this territory, special attention must be given to proper water supply to prevent people from being infected with tularemia.

As for pseudotuberculosis and enteric yersiniosis, in 1975 4 cultures of *Y. pseudotuberculosis* were isolated from northern redbacked and large-toothed redbacked voles trapped in the tayga at a considerable distance from the village of Kondon [4, 8]. Since pseudotuberculosis is a widespread and ubiquitous infection, these findings were indicative of the existence of environmental conditions for its epidemic manifestation over the entire territory surveyed. As we know, people are infected the most often right in settlements and, as a rule, this is related to synanthropic rodents. In July 1976, there were several cases of the disease among BAM construction workers living in the same building in one of the settlements, and this was the first time they were recorded in Verkhnebureinskiy Rayon of Khabarovsk Kray. *Y. pseudotuberculosis* was isolated from house mice caught in this building [or room]. Potential further epidemic complications were averted by prompt preventive measures. An inspection of residential and farm buildings in 9 other settlements, which was made during this period, resulted in isolation of 7 more cultures of *Y. pseudotuberculosis* in 4 of them, from brown rats and house mice. Thus, the real threat of future epidemic outbreaks of pseudotuberculosis in settlements along the BAM route is rather great, and this requires regular rat extermination work and other preventive measures.

Concurrently with cultures of *Y. pseudotuberculosis* in the same Kondon station, in 1975 10 cultures of *Y. enterocolitica* were isolated from northern redbacked and large-toothed redbacked voles, large Japanese field mouse (*A. speciosus*) and shrews (*S. caecutiens*) [4, 8]. Demonstration of this pathogen, which is close to *Y. pseudotuberculosis* and apparently widespread, is thus far one of the few finds in USSR. We know of outbreaks of enteric yersiniosis (enterocolitis) among people of some West European countries. The significance of this infection to human pathology in the USSR remains unclear, due to the fact that it has been little-studied. The intensive and extensive epizootic that we found among wild mammals is indicative of the existence of endemic yersiniosis sites. Its epidemic manifestation, in particular along the BAM route, as well as manifestation of pseudotuberculosis, should apparently be linked to carriers among synanthropic rodents.

As shown by the serological tests (agglutination reaction--lysis) on small mammals with 13 standard *Leptospira* strains [9], in the Amur-Bureya interfluvium the endemic sites for leptospirosis could be situated mainly in the lakeside and riverside floodplain meadows, as well as lowland meadows away from floodplains in the Chukchagir-Evoron plain. There, about 25% of the Ungur

voles had specific antibodies to *Leptospira*, most often referable to the grippotyphosa serogroup.

Antibodies were found in 66% of the pigs (438 serum specimens tested), and more than half the positive cases were referable to the icterohaemorrhagiae serogroup. In 1977, 5 strains of *L. pomona* were isolated from pig kidneys at a meat-packing plant in Komsomolsk-na-Amure. Antibodies to *Leptospira* antigenically related to this serogroup were encountered the most often in cattle also (157 sera tested, 56% of them were positive). Retrospective serological tests on about 1000 local residents showed that there was some probability of leptospirosis in about 4%. On the basis of the aggregate of the above-mentioned facts, the possibility of fresh leptospirosis cases is unquestionable, but in our opinion mass scale epidemic manifestations are unlikely. However, one must devote attention to prevention of leptospirosis among workers at livestock complexes, which are to be created in the near future along the route of BAM.

The Amur-Bureya interfluvial area is north of the presently known range of tick-borne rickettsiosis in North Asia. However, the hemocyte test revealed that 7% of the *I. persulcatus* (2400 ticks were tested) were infected with *Rickettsia* referable to the tick-borne spotted fever group. Negative results were obtained from analogous studies of 1500 *D. silvarum* ticks. We checked 374 standard pools (10 specimens each) of taya tick imagoes and 57 pools of *D. silvarum* using the biotest on guinea pigs. No rickettsial strain was isolated. But, when the biotest guinea pigs were submitted to serological analysis, we obtained 12 positive results (about 3%) with antigens of the tick group of *Rickettsia* in the former case and 2 (also about 3%) in the latter. Two out of 628 blood serum samples from local residents reacted with antigen of tick-borne rickettsiosis and 6 with antigen of Q fever in the complement fixation test. These facts warrant the assumption that there could be sites of these forms of rickettsiosis in the territory that passes through the eastern section of BAM.

Conclusions

1. As a result of a comprehensive exploration of endemic sites of human diseases in the Amur-Bureya section of the BAM, which constitutes one-sixth the total length of the route, endemic sites were found of tick-borne encephalitis, tularemia, pseudotuberculosis enteric yersiniosis, and evaluation was made of the probability of distribution of leptospirosis and endemic rickettsiosis. The obtained data serve as the basis for organizing preventive measures, as well as more detailed cartographic and prognostic interpretation.
2. Of the infections found in the eastern part of BAM, tick-borne encephalitis and pseudotuberculosis may be of greatest epidemic significance; the hazard of different sections of the route with regard to tick-borne encephalitis is not the same, and it depends on a number of environmental factors that determine the number taya ticks and incidence of virus carriers among them.

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EXPERIMENTAL VALIDATION OF AEROSOL IMMUNIZATION WITH LIVE TULAREMIA VACCINE

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[Article by V. I. Agafonov, K. G. Gapochko, I. Libikh, O. P. Misnikov, M. Erikhleb, I. Votruba, F. Faltus and A. Matsela, Military Medical Academy imeni Kirov, Leningrad]

[Text] Olsuf'yev et al. [5] showed that Soviet-made dry live tularemia vaccine from Gayskiy strain No 15 (reconstituted), when given epicutaneously, subcutaneously and intracutaneously in doses of 0.1-10 million live bacteria produces in experimental animals, in particular guinea pigs, intensive immunity to tularemia, not only with subcutaneous but aerosol infection with a virulent pathogen. At the same time, we know of results obtained from experiments on monkeys, as well as human volunteers, by American researchers [7-10], according to whom live tularemia vaccine produced on the basis of the Soviet strain, although it protects against death after aerosol infection with the virulent American Schu strain, does not prevent development of the pulmonary form of the disease which, it is true, ended with a spontaneous cure.

We made an experimental assessment of the intensity of immunity resulting from subcutaneous and epicutaneous administration of the Soviet commercial dry live tularemia vaccine derived from Gayskiy strain No 15 (reconstituted) to aerosol infection of immunized animals to virulent *F. tularensis*.

Material and Methods

In our work, we used series No 3 (control No 290) of dry live tularemia vaccine produced by the Odessa Institute of Virology and Epidemiology imeni Mechnikov, which was used for hypodermic and intracutaneous immunization of guinea pigs weighing 250-300 g, using a dosage of 1 million live bacteria. We used two strains of *F. tularensis* for aerosol infection of immunized animals: European No 130 and American Schu.

Strain No 130 was isolated in CSSR in 1956 by Dr Krivinka from a hare. According to the data of Libikh, isolated cells (<9) of this strain, when used for hypodermic infection, elicited 100% death of white mice and guinea pigs within 5-6 days. Inhalation by guinea pigs of 600 live bacteria caused death of all animals within 6-10 days, whereas a dosage of 6000 cells elicited the same

effect 1 day earlier. LD₅₀ of this strain for guinea pigs was ~2 bacteria for hypodermic and aerosol infection.

The American Schu strain, isolated in the United States from a patient, was obtained from the laboratory of Dr Lukash (CSSR). LD₅₀ thereof for guinea pigs also constituted ~2 bacteria for aerosol and hypodermic infection; however, death occurred 1 day earlier than after infection with strain No 130.

Guinea pigs were submitted to aerosol infection 1 month after vaccination in a dynamic aerosol chamber. We used a fine-particle aerosol, with mean-mass diameter of $3.6 \pm 1.2 \mu\text{m}$, obtained upon dispersion of a suspension of virulent strain. No 130 strains were used for infection in ascending doses, from 15 to 60,000 LD₅₀, and Schu was used in doses of 18 to 200 LD₅₀.

Results and Discussion

Hypodermic immunization provided protection for all guinea pigs against aerosol infection with 15 LD₅₀ of the European strain and only 44% of the animals with 180 LD₅₀. After inhalation of more massive doses of strain No 130 (3425-60,000 LD₅₀), degree of protection dropped to 20-60%. After intracutaneous immunization, intensive immunity was demonstrated only in 36-48% of the animals after aerosol infection with 15-180 LD₅₀ of European strain No 130 (Table 1).

Table 1. Efficacy of live tularemia vaccine against aerosol infection with European and American strains of *F. tularensis*

Immunization method	European strain (No 130)			American strain (Schu)		
	LD ₅₀	number of exper. animals	protection, %	LD ₅₀	number of exper. animals	protection, %
Subcutaneous	15-180	59	44-100	18-200	32	0-
Intracutaneous	3425-60000	10	20-60			
Aerosol	15-180	50	36-48	18	20	5
	18-180	75	72-96	18-200	80	0-14
	3425-60000	24	20-64			
Control (no vaccination)	15-180	20	0	18-200	20	0

At the same time, aerosol infection with the American Schu strain in doses of 18 to 200 LD₅₀ consistently caused death of animals vaccinated either sub- or intracutaneously.

Studies of duration of immunity after hypodermic immunization of guinea pigs using aerosol infection with European strain No 130 revealed that vaccination with 1 million live bacteria produced insusceptibility in 60-80% of the animals to infection with 25-50 LD₅₀ for 6 months after immunization (Table 2), but did not protect them against higher doses (6000 LD₅₀).

Thus, the Soviet commercial tularemia vaccine caused formation of immunity to the European strain, but was entirely ineffective against the American strain of *F. tularensis*.

Table 2. Results of aerosol infection fo guinea pigs with European strain of *F. tularensis* at different intervals after immunization

Immunization method	Infection dose, LD ₅₀	Time of infection, day		
		32	95	180
Subcutaneous	25-50	4/5 (80%)	6/10 (60%)	7/10 (70%)
	6000	4/10 (40%)	1/10 (10%)	0/10 (0%)
Aerosol	25-50	5/5 (100%)	9/10 (90%)	10/10 (100%)
	6000	4/10 (40%)	2/10 (20%)	1/10 (10%)

Note: Number of surviving animals is given in numerator and number of infected animals in denominator.

In view of these data, as well as positive results previously obtained by Soviet and American researchers using aerosol immunization against tularemia [1-4, 6], we tried to enhance the efficacy of the commercial, dry live tularemia vaccine for use in aerosol form.

Considering the rapid inactivation of the vaccine strain when the dry vaccine is reconstituted, dispersion thereof, as well as when the aerosol vaccine particles hover, we used a specially developed stabilizing medium for dilution of the vaccine; this medium contained amino acids, low molecular sugars and glycerin.

The vaccine was sprayed by means of an ejector type pneumatic, direct jet aerosol generator with output of 1 ml/min in a special aerosol chamber 0.5 m³ in size, at a temperature of 20°C and relative air humidity of 70%. We collected samples of aerosol 90 s after producing the aerosol cloud, and results of testing them revealed (Table 3) that an aerosol with biological concentration of $5.75 \pm 0.65 \cdot 10^5$ bacteria per liter, with weight concentration of $12.3 \pm 0.4 \cdot 10^2$ µg/l, was produced when spraying vaccine containing 1.13 ± 0.08 billion live bacteria per ml stabilizing medium. Restoration (survival) of microorganisms under these conditions was $40.9 \pm 2.46\%$. Mean-mass diameter of aerosol particles was in the range of 3.4 ± 0.1 µm, while the biological aspiration dosage* for guinea pigs constituted a mean of $1.15 \pm 0.13 \cdot 10^6$ live bacteria.

Spraying tularemia vaccine in a statistic chamber 15 m³ in size also demonstrated that it was possible to create a "working" concentration of aerosol vaccine ($4.6 \cdot 10^4 \div 6.2 \cdot 10^5$ bacteria per l), that would assure inhalation of an immunizing dosage by man and animals.**

Thus, these experiments demonstrated convincingly that a physically and biologically rather stable aerosol is produced by means of reconstitution followed by dispersion of live tularemia vaccine, and it can be used for immunization against tularemia by the inhalation method.

*The aspiration dosage (AD) was determined by the formula, $AD = C_b \cdot t \cdot v$, where C_b is biological concentration of aerosol (in microorganisms per liter), t is exposure time (in minutes) and v is pulmonary ventilation volume, taken to be 0.2 l/min.

**Translator's note: Chamber size is indicated in source by two different figures.

Table 3. Characteristics of main physical and biological parameters of aerosol vaccine obtained by reconstitution and dispersion of live tularemia vaccine

Exper. No	Initial concentration, bacter./1 ml	Weight concentration, µg/l	Mean-mass diam. of aer. partic. µm	Biolog. concentration, bacteria per l	Reconstitution, %	Aspiration dose for guinea pigs, 10-min exposure, number of live bact.
1	1.26×10^9	13.7×10^3	3.6	8.27×10^8	47.6	1.65×10^6
2	1.13×10^9	12.6×10^3	3.7	6.42×10^8	45.1	1.28×10^6
3	1.40×10^9	11.3×10^3	3.1	5.10×10^8	32.2	1.02×10^6
4	0.95×10^9	11.7×10^3	3.2	4.82×10^8	43.1	0.96×10^6
5	0.87×10^9	12.1×10^3	3.4	3.67×10^8	34.9	0.73×10^6
6	1.16×10^9	12.7×10^3	3.6	6.24×10^8	42.3	1.25×10^6
Means	$1.13 \pm 0.08 \cdot 10^9$	$12.3 \pm 0.4 \cdot 10^3$	3.4 ± 0.1	$5.75 \pm 0.65 \cdot 10^8$	40.9 ± 2.46	$1.15 \pm 0.13 \cdot 10^6$

We tested the efficacy of aerosol immunization with live tularemia vaccine on guinea pigs submitted to aerosol infection with the European and American strains of *F. tularensis* (see Tables 1 and 2).

After aerosol immunization with a dosage of $1 \cdot 10^5$ – $1 \cdot 10^6$ live bacteria and inhalation infection with 15–180 LD₅₀ European strain No 130, performed 1 month after immunization, 72–96% of the infected animals survived, versus 100% death in the control group. This was substantially higher than the results of subcutaneous and intracutaneous immunization, with which the level of protection constituted 44–100% and 36–48%, respectively.

We demonstrated a distinct advantage of the aerosol method over the hypodermic one in a follow-up test at the long postvaccinal term (3–6 months) when guinea pigs were infected with 25–50 LD₅₀ of the European strain No 130: aerosol immunization protected 90–100% of the animals and hypodermic only 60–70% (see Table 2).

At the same time, the aerosol method of immunization, like the hypodermic (intracutaneous) method, was found not to be effective enough against massive doses of the European virulent strain (>3000 LD₅₀) and did not protect against aerosol infection with the American strain Schu, even in moderate doses (see Tables 1 and 2).

Thus, one can develop aerosol vaccine for immunization by inhalation by means of reconstituting of commercial live tularemia vaccine in a special stabilizing medium followed by dispersion, and its efficacy exceeded that of hypodermic (intracutaneous) inoculations against tularemia. The basic possibility of using tularemia vaccine in aerosol form, along with cutaneous and intracutaneous jet injector methods, makes it possible to use it for creation of a universal product which, in certain epidemic situations, could be used with optimum results.

Conclusions

1. Commercial Soviet dry, live tularemia vaccine was found not to be sufficiently effective against aerosol infection with the virulent American Schu strain in experiments on guinea pigs.

2. Immunization with reconstituted dry live tularemia vaccine by the aerosol method was more effective in experiments on guinea pigs than hypodermic and intracutaneous immunization in the case of aerosol infection with the virulent European strain of *F. tularensis*.

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CURRENT PROBLEMS OF ENDEMICITY AND PREVENTION OF TULAREMIA

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[Article by N. G. Olsuf'yev, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences]

[Text] Comprehensive and in-depth work has been done in the USSR on the problem of endemicity of tularemia. This was aided by the teaching of Academician Ye. N. Pavlovskiy about endemicity of human diseases. The results of studies of tularemia formed an important foundation for the prevention of this infection.

To date, endemic tularemia sites have been found on the territory of 14 Union republics (Kirghiz SSR was an exception). It was shown that the sites are distributed from the western frontiers of the USSR, including Kola Peninsula, Kaliningrad Oblast and Moldavian SSR, to the east, to Chukotsk Peninsula, Kamchatka and Sakhalin, Khabarovsk and Maritime Krays. The northernmost point where tularemia was found in the USSR is on the Pyasina River on Taymyr Peninsula (73° north latitude) and the southernmost point is in Kulyab, Tadzhikistan (38° north latitude) and Zangilan in Azerbaijan (39°05' north latitude).

There is spotty distribution of sites over the country, they coincide with areas where conditions favor their existence. They are arranged relatively diffusely in the northwest, center, south and southeast of European USSR, in the forest-steppe part of West Siberia, as well as foothills and low elevations of Altay, Kuznetsk Alatau, Tarbagatay and Tyan-Shan. In other regions, the focal territories are more local, coinciding mainly with river valleys and, in Transcaucasia, with foothills and, in part, mountain regions. The geography of endemic tularemia sites in the USSR has not been sufficiently studied; new territories with sites are found virtually every year. Thus, relatively recently, tularemia was discovered in Lithuanian SSR, Tuva ASSR, Amur Oblast, Yamal and Taymyr peninsulas, etc. For this reason, it is imperative to further study the nosogeography of tularemia in a number of regions of our country, in particular, Kazakhstan, East Siberia, the Far East, Extreme North of RSFSR, Baltic region, some parts of the Ukraine, Belorussia, etc. One should also define the distribution of endemic tularemia sites in regions where the infection was discovered in the past, but then was not manifested for a long period of time.

Soviet researchers have devoted much attention to *F. tularensis* isolated from different geographic regions and different sources. A major achievement was development of an intraspecific taxonomy of *F. tularensis* and distinction of three geographic races differing in biochemical and pathogenetic properties: Holarctic, Central Asian and Nearctic (or American). In the USSR, there is distribution of the Holarctic race, which is inherent in most territories with endemic sites, and the Central Asia race, which was discovered only in river valleys in Central Asia. The virulence of these two races is similar, but differs from the Nearctic race, which is more pathogenic. Drastic differences were recently demonstrated in Holarctic strains, with regard to sensitivity to erythromycin and oleandomycin, as well as lincomycin. On this basis, it was suggested that the Holarctic race be divided into two biotypes (biovars): I--erythromycin-sensitive and II--erythromycin-resistant [2, 4, 14]. Some differences have been detected in their distribution. The erythromycin-sensitive strains are found primarily in East Siberia, the Far East and northern USSR, as well as Western Europe (France, Italy and Sweden) and East Asia (Japan and China); erythromycin-resistant strains prevail in the central zone and southern part of European USSR, the Caucasus, Kazakhstan and West Siberia, and abroad in Central Europe. In store is the important task of continuing to accumulate data on distribution of these biotypes of the Holarctic race.

The main sources of tularemia infection in the environment--water rats, common voles, house mice, muskrats, hares, etc.--have been identified and studied comprehensively. Determination was made of secondary carriers of infection. In all, tularemia has been recorded in 82 species of wild vertebrates. The infection has been modeled experimentally in a considerable number of species of wild mammals, and separation thereof into three groups was validated on the basis of susceptibility and infectious sensitivity, as well as their significance as sources of infection [10]. These data turned out to be quite relevant to defining their role in maintaining endemic tularemia sites.

The infection circulates in endemic sites chiefly because of the large-sized populations of animal species of the first group, which are highly sensitive to tularemia: water rats, common voles, house mice and others. For a long time, it was believed that, in these animals, tularemia is always lethal and that it is not inherent in them to contract the disease with development of chronic carriers. However, because of the rare demonstration of antibodies in blood to *F. tularensis* in animals of the first group, for example, hares, water rats, etc., the question arises as to their possible role as carriers. Our experiments with common voles (*Microtus subarvalis*) revealed that it is possible, in some cases, for them to experience a nonlethal infection [11]. When voles were fed carcasses of white mice and common voles that had died of tularemia, some of the animals were infected and contracted the disease. Surviving voles revealed bacteria in internal organs, antibodies in blood, immunity to re-infection with *F. tularensis* and elimination of the pathogen in urine. Such recovered animals can apparently be involved in maintaining the infection in an endemic site.

Determination was made of the great importance of Ixodes ticks in transmission and prolonged preservation of tularemia infection in the environment. It was also proven that bloodsucking Diptera (mosquitoes, horseflies) play an important role as mechanical vectors of this infection. In the USSR, spontaneous

F. tularensis infection was demonstrated in 74 species of bloodsucking arthropods. Determination was made of the important role of water in transmission of infection among amphibian species of rodents, for example, the water rat and muskrat, as well as spontaneous infection of hydrobionts, for example, mollusks, caddis flies and other invertebrates (total of 14 species) in aquatic tularemia sites. It was proven that *F. tularensis* can survive in water (or ice) at low temperature without a change in properties for up to 9-10 months. *F. tularensis* was repeatedly detected in the water of different reservoirs, including running water, which is of epidemiological importance.

As a result of the information accumulated in the USSR about endemic tularemia sites, a classification (typology) thereof was worked out on the basis of landform [5, 8]. It comprises seven main types: backwater-swamp, foothill (mountain)-stream, meadow-field, forest, steppe, tugay (valleys of desert rivers) and tundra. Subtypes and variants have been described for some types of sites. This list justifies classification of tularemia as a multizonal infection. Distribution of different types of sites was identified, as well as routes of circulation of pathogens in them, etc. But still, some sites have not yet been studied enough. Typing the sites is important, not only in the theoretical, but practical respect, since it permits making differentiated epizootiological forecasts and implementing preventive measures. In the USSR, the most widespread and epidemiologically dangerous endemic tularemia sites are of the backwater-swamp, foothill (mountain)-stream, meadow-field and steppe types.

The spatial structure (topology) of tularemia sites was worked out, which involved division of site territories into subordinate entities (ranks): range of endemic sites (pathogen), region of endemicity, province of endemicity, etc.

Studies were pursued of the patterns of the epizootic tularemia process, which are characterized by drastic fluctuations of intensity related to changes in number of rodents, which are the main carriers of infection. Thus, in the central belt of European USSR the rise in number of common voles and, accordingly, of tularemia epizootics in meadow-field sites are observed at 2-3-year intervals, and they last about 1 year; the higher these rises, the more intensive the epizootics. The current standards of agriculture do not, unfortunately, always have a retarding influence on the epizootic process. For example, in the fall and winter period of 1977-1978, there was a drastic increase in common vole population in the central belt of European USSR, in particular in Tula and adjacent regions of Moscow, Ryazan, Kaluga and Orel Oblasts, against the background of which there was an intensive tularemia epizootic and cases thereof among people. In West Siberia, a rise in water rat population of backwater-swamp sites, which occasionally reaches very high levels, is observed at intervals of 10-11 years, and each time it is associated with intensive tularemia epizootics. The fluctuations in number of water rats are related essentially to changes in water level in the lake-swamp depressions of the Siberian lowlands. When they are filled with water, the zone of aquatic and swamp vegetation suitable as a habitat for animals becomes larger.

Many years of observations have shown that endemic tularemia sites persist in natural biocenoses, and they can exist for an unlimited time, unless

submitted to radical changes under the influence of man's industrious endeavors or ameliorative measures are not thorough enough. Persistent backwater-swamp endemic tularemia sites have been active for 40-50 years in the Volga River delta (retrospectively for about 100 years), along the Don River and its tributaries, in the central reaches of the Ural River, several parts of West Siberia, Yakutia, etc. For about the same time, meadow-field sites have been active in Moscow, Tula, Ryazan, Orel Oblasts and other places. In the former Mikhnevskiy Rayon, in the south of Moscow Oblast, we have observed the existence of a meadow-field site since 1938 and to this day, i.e., 40 years, in the very same places. Infection is demonstrable almost annually. We know of instances of epidemic outbreaks of tularemia in regions where there had been no human cases for a long time and where preventive measures had been unjustifiably discontinued (for example, in Rostov Oblast in 1975).

Epidemiological observations of the last decade confirmed the previously obtained data concerning the predominant significance in the USSR of water rats, small mouse-like rodents, particularly common voles and house mice and, in some places muskrats, to human infection. Unlike the United States and West European countries, hares as a source of human infection play an insignificant part in the USSR (usually no more than 1% of all cases of the disease). Infection from water rats more often occurs in the summer, through blood-sucking Diptera (transmissive route), while hunting or through water, and from muskrats when hunting or also through water. Infection from mouse-like rodents occurs more often during fall and winter agricultural work (transportation of fodder, straw, etc., from the fields), when rodents invade houses (household infection) and through water (for example, from wells).

An epidemiological landform zoning was effected in a number of oblasts on the basis of typology of sites, their spatial structure and epidemiology of sick cases, which served as the foundation for planning and implementing preventive measures.

Laboratory diagnostic methods for detecting tularemia in man, as well as in epizootiological inspections of endemic sites of infection, have continued to be upgraded. Serological tests with the use of hemagglutination have been introduced to practice [6]. A high-speed microscopy method--immunofluorescence microscopy--was proposed [1]. A rather effective method has been developed for searching for tularemia epizootics in the field by means of detection of antigen in bird pellets, droppings of carnivorous mammals and other objects [3]. Under practical conditions, it was shown that this method, which is based on use of the antibody neutralization test in hemagglutination (ANT), is highly effective for inspection of virtually all types of endemic sites, particularly the meadow-field, steppe and tundra types. This method has been introduced to the practice of sanitary and epidemiological stations.

A set of measures to reduce the number of rodents, such as water rats, small mouse-like rodents, etc., was proposed in order to depress endemic tularemia sites. Methods have been developed for eradication of Ixodes ticks. However, these ameliorative measures are implemented only for limited sites because of their complexity. Development of large reservoirs, with total flooding of water rat habitats, filling swamps and other reservoirs, overall plowing of land over large areas, etc., are among the measures that lead to destruction

of the biocenosis to which the tularemia sites are related in one way or another. These are large-scale measures, and they are implemented in accordance with the general national economy plan. Several reservoirs were created on the Volga River and its tributaries, the Dnepr and other rivers, and this led to eradication of several rather active tularemia sites of the backwater-swamp type.

Immunization of the public with live tyularemia vaccine is the simplest and most reliable means of preventing tularemia available to medical workers. One inoculation given superficially (transcutaneously) provides for formation of immunity lasting an average of 5 years and, in some cases, up to 15 years. It was learned that a jet injector can be used for tularemia vaccination, which simplifies the immunization procedure considerably and reduces outlay of vaccine [12, 13]. The cutaneous (transcutaneous) allergy test with tularin is used extensively to determine presence (preservation) of immunity after vaccination. This skin test is simple and quite harmless. In recent years, it was learned that screening of immunized people by serological methods (agglutination, passive hemagglutination and other tests) makes it possible to pick up an additional number of immunopositive subjects whose skin test is negative. The number of such people increases as a function of postimmunization time, and could constitute 15% after 3-5 years. This question requires further comprehensive investigation.

In the last 30 years, mass scale immunization of the public residing in the zone of endemic tularemia sites has justified itself entirely as the main, quite safe and highly effective means of preventing tularemia. Let us recall that the annual incidence of tularemia (according to registration that was far from complete) constituted 6000 cases per year in the prewar years, when there was no vaccine, reaching 10,000 in some years, 40,000-60,000 cases during the Great Patriotic War and first few postwar years, with a maximum of 136,000 per year [9]. In subsequent years, morbidity dropped drastically under the influence of vaccination of the public and other measures, and at present it constitutes 100-200 registered cases per year. These are essentially sporadic cases and, less often, minor epidemic outbreaks. It can be considered that at least 10,000 cases of tularemia among the public are prevented each year in our country, chiefly as a result of vaccination.

The brilliant advances made in the USSR in the control of tularemia require further reinforcement by means of thorough use of vaccination and other measures. Tularemia is an endemic infection and reduction of human morbidity due mainly to vaccination does not mean that there has been depression of the endemic sites, which are widespread, quite persistent and, in a number of cases, highly active (several hundred virulent *F. tularensis* cultures are isolated annually in different parts of the country).

Previously, there was extensive and planned immunization (vaccination, revaccination) of the entire rural population (with the exception of children of certain ages and individuals for whom vaccinations were contraindicated for health reasons) living in an administrative region that was enzootic for tularemia. A region was considered enzootic when tularemia sites were found on its territory, even in limited sections and regardless of time of detection. In recent years, it was recommended [7] that scheduled immunization should

cover only people living or working in the enzootic territory of the rural soviet, as well as of neighboring rural soviets, if their territories do not differ from the territory with established enzootic with regard to landform and economic indicators (distribution of farms, management of agriculture, etc.), within the limits of an administrative region designated as being enzootic for tularemia.

In regions with inactive endemic tularemia sites, where isolated cases among people had occurred in the past, for example, in Baltic republics, Central Asia, the Far East, etc., one should vaccinate hunters, shepherds, foresters, etc., residing in the zone of endemic tularemia sites (or traveling to enzootic territories) and those whose occupation involves the risk of tularemia infection.

The corrections made in planning and implementing tularemia vaccination made it possible to reduce somewhat the scope of inoculations given in the nation. The procedure for removing a territory from the enzootic list was defined. In view of the considerable persistence of endemic tularemia sites, removal of formerly enzootic regions from the list should be done with great caution; it should be based either on radical biocenotic changes in the sites under the influence of anthropogenic factors or negative results of thorough many-year epizootiological surveys. In the latter case, the method of detecting tularemia antigen in bird pellets and droppings of predatory mammals, which permits more extensive inspection of territories, should be helpful. Use of this method does not preclude the traditional bacteriological inspection method, but it supplements and expands them appreciably. Long-term absence of tularemia cases among humans in a given region formerly stricken with tularemia should not by itself serve as grounds to deem it to have been self-cleared of endemic sites of infection if no radical changes in biocenoses have taken place.

The following main tasks pertaining to investigation of endemicity and prevention of tularemia can be advanced for the next few years: 1) further accumulation of data on nosogeography of the infection with more accurate delineation of the boundaries of territories with sites and determination of the properties of the pathogen distributed in them; 2) further determination of conditions of existence of different types of endemic sites, refinement of methods of epizootiological and epidemiological inspection thereof, as well as forecasting situations and means of improving problem territories; 3) definition of groups of population subject to vaccination, depending on the distinctions of endemic sites where they live, as well as risk of infection; 4) further comparative study of different methods of determining vaccinal immunity in order to establish its duration; 5) continuation of work on epidemiological zoning of territories in different oblasts, krays and republics for the purpose of more precise determination of the scope and optimization of preventive measures.

Conclusions

1. The main results have been summarized of studies of endemicity and prevention of tularemia in the USSR, with indication of advances made in this field, which excel in a number of cases the achievements of foreign specialists.

2. The main tasks referable to investigation of tularemia in the future were formulated.

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COMPARATIVE EVALUATION OF EFFICACY OF DIFFERENT METHODS OF DETERMINING
IMMUNITY OF INDIVIDUALS INOCULATED AGAINST TULAREMIA

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 10,
Oct 79 (manuscript received 7 Jun 78) p 113

[Author abstract of article by V. I. Timoshenko, Institute of Epidemiology and
Microbiology imeni Gamaleya, USSR Academy of Medical Sciences]

[Text] A study of samples of inhabitants of Tula, Ryazan, Smolensk, Kaliningrad
(vaccinated 1-5 years before the screening) and Rostov (vaccinated 2 weeks to
1.5 months before the screening) Oblasts in order to compare the efficacy of
seroallergic methods of determining immunity in those vaccinated against tula-
remia. We determined the immunological status of immunized people by means
of the usual methods of running the tularin test and serological reactions--
agglutination (AR) and passive hemagglutination (PHAR). In all, we screened
1145 people immunized against tularemia.

Analysis of the findings on 1056 people immunized 1-5 years before our study
revealed that 860 (81.4%) reacted positively with tularin (86.5% positive
in the AR and 88% in the PHAR). In the group of people immunized 3-5 years
before the tularin test and collecting blood samples, we tested 530, 76.8%
showed a positive tularin test [TT] result and 89.2% were seropositive (as
a whole with AR and PHAR). The geometric mean titers constituted 1:18 with
the AR, 1:30 with the PHAR, and maximum values were 1:80 and 1:320, respectively.
The frequency of differences between the results of the TT and serological
reactions constituted 15%, i.e., 15% of the seropositive people had no allergic
reaction to tularin. In the group immunized 1-2 years before the TT and taking
blood, we tested 526 people, 85.6% of whom had a positive TT and 91.9% were
seropositive (as a whole with AR and PHAR). Geometric mean titers were 22.6
for the AR and 1:50.5 for the PHAR, the maximums being 1:80 and 1:640, res-
pectively. Differences between the results of the tularin test and serological
reactions were observed in 6.3% of this group.

Examination of 89 people immunized against tularemia 2 weeks to 1.5 months
before the study, using the same three tests, revealed that 81 (91%) reacted
positively. Coinciding positive results were obtained with the AR, PHAR and
TT in 73 (82%) cases. Differences (negative TT with positive serological
reactions) were noted in 5 cases (5.6%). Geometric mean titers constituted
1:79 in the AR, 1:182 in the PHAR, the maximum values being 1:1280 and 1:10,240,

respectively. All of the tested reactions were quite effective: 91% according to TT results, 96.6% for AR and PHAR together.

Thus, it was established that in determining the immunological status of individuals immunized against tularemia the tularin test does not pick up 6-15% (average of about 10%) of the immunopositive individuals. This was established by comparing the results of the tularin test and serological reactions (AR and PHAR).

The number of immunopositive individuals that were not demonstrable by the tularin test increased with increase in time after vaccination. Consequently, one should use the tularin test combined with one of the above-mentioned serological tests in order to objectively assess the stratum of the population that is immune to tularemia.

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FURTHER INVESTIGATION OF AN ENDEMIC TULAREMIA SITE ON BIRYUCHIY ISLAND

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 11, Nov 79 (manuscript received 20 Feb 79) pp 96-97

[Author abstract of article by V. S. Bessalov and L. A. Teremenko, Kherson Oblast Sanitary and Epidemiological Station]

[Text] The tularemia site on Biryuchiy Island, Kherson Oblast, was discovered in 1961. It has been quite active for 18 years; many cultures of *F. tularensis* (5 to 68) are isolated annually there from *Ixodes* ticks, and during years of rise in rodent population, from them, as well as their nests, larvae and nymphs of *Ixodes* ticks, from gamasid ticks and from water. This site is under constant observation. The collected material is submitted to bacteriological examination, and bird pellets to serological testing. The scope and results of studies pursued in 1961-1969 have been published.

From 1970 to 1977, a total of 28,312 bacteriological tests were made: 2345 rodents, 25,655 *Ixodes* ticks, 129 samples of water, etc., have been examined. We isolated 5 cultures of *F. tularensis* from rodents and 339 from *Ixodes* ticks. Serological tests were done on 242 bird pellets, 21% of which contained tularemia antigen in titers of 1:20 to 1:640.

In this period, two drastic rises in rodent population were recorded on the island, in 1970 and 1977. In the summer of these years, the average number of rodents was in the range of 10.5-18.0% trapping incidence, the maximum being in the range of 25.0-35.0%. In other years, mean population of rodents did not exceed an incidence of 4.0% trapped and maximum of 13.0% trapped. In all the years of observation of this site, the largest rodent population size was referable to 1977, and in the fall of that year the percentage of trapped animals reached 70 in some places. The rise in rodent population was referable to house mice in 1970 and house mice, as well as common voles, in 1977. In 1973, there was also some increase in number of common voles.

Each rise in rodent population on the island was associated with onset of intensive epizootics among them. The epizootic of 1970 and early 1971 was confirmed by isolation of pathogen cultures from mummified carcasses of common voles; in 1973, tularemia cultures were isolated from fresh vole carcasses collected in the steppe. That same year, *F. tularensis* antigen was found in bird pellets, which were examined in the Tularemia Laboratory of the Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of

Medical Sciences. Positive results with relatively high titers (average 1:178) were obtained in testing 36% of the pellets, which was indicative of a recent epizootic. In the winter and spring of 1977-1978, we also observed an intensive epizootic, which was confirmed by isolation of *F. tularensis* cultures from the substrate of vole nests and their mummified carcasses. Aside from rodents, white-toothed shrews were involved in the epizootic, and a culture was obtained from them (in a group biotest on 4 animals).

Each rise in rodent population was followed by a 2-3-year period of severe depression. Thus, after the increase in number of common voles in 1973, there was none trapped for the next 3 years (1974-1976), traces of their vital functions were barely noticeable in the steppe, while the number of house mice in these years, even in the summer, constituted 0.1-0.9% trapped.

The drastic decrease in rodent population size in 1974-1976 led to a decrease in *Ixodes* ticks, particularly species for whom rodents are food providers at the preimago stages: *Dermacentor marginatus* Sulz., *Rhipicephalus rossicus* Jak et K.-Jak. The index of profusion of the most numerous species of *Ixodes* ticks, *D. marginatus*, constituted only 3.1 in 1977, which is 1/10th-1/15th the usual level. This was the smallest number of this tick species for the last 10 years.

In spite of the small rodent population in 1974-1976, tularemia epizootics did not stop among them, as confirmed by annual isolation of pathogen cultures from *Ixodes* ticks and positive results of antibody neutralization tests in the serological examination of bird pellets.

A set of preventive measures is being implemented in order to prevent tularemia among humans and carrying the infection out beyond the limits of this island.

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A METHOD OF DEMONSTRATING BACTERIOCINS IN FRANCISELLA TULARENSIS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 3, Mar 80 (manuscript received 1 Feb 79) pp 43-44

[Article by M. A. Aykimbayev, G. M. Kunitsa and O. B. Chimirov, Central Asian Scientific Research Plague-Control Institute, Alma-Ata]

[Text] Protein-like substances with a narrow range of antibacterial activity were discovered in the family of *E. coli* by Gratia, in 1925, and they were named colicins [3, 4]. Colicin-producing strains are called colicinogenic, whereas the actual biological phenomenon related to this is called colicinogeny. A phenomenon similar to colicinogeny was also discovered in a number of other bacteria, in particular, *Y. pestis*--pesticinogeny [1], vibrios--vibriocinogeny [2], etc. This phenomenon found in bacteria was named bacteriocinogeny [5]. Such a phenomenon was not described for *F. tularensis*, but by analogy we gave the name of tularecins to potential bacteriocins of *F. tularensis*, and tularecinogeny for the phenomenon.

Our objective here was to determine whether tularecinogeny exists in *F. tularensis* and to describe the techniques demonstrating this phenomenon.

Material and Methods

In our experiment, we used 60 strains of *F. tularensis*, including 32 of the Holarctic and 28 of the Central Asian race. They did not differ from typical strains of these races in cultural, morphological and biochemical properties, and they were highly virulent for white mice: the lethal dosage for them constituted 1 bacterial cell given subcutaneously. The museum strains had been submitted before the experiment to three passages in white mice in order to restore virulence. In the experiment, we used subcultures of strains that had grown on liver agar with blood from organs of third passage biotest animals that had died. As nutrient medium, we used Heddleson's liver agar, pH 7.2, containing 2% agar, 1% glucose, 2% glycerin, 0.1% cysteine hydrochloride, 20% hydrolysine and 5% defibrinated rabbit blood.

In preliminary experiments, we selected 5 out of the 60 strains as indicators, by means of cross testing of *F. tularensis* sensitivity to bacteriocin, and the others served as producers. Of the 5 selected indicator cultures, 3 (Nos 113, 31 and 178) were referable to the Central Asian geographic variety and 2 (Nos 255 and 264) to the Holarctic.

We determined bacteriocinogenicity and sensitivity to autologous bacteriocin and bacteriocin of other strains of *F. tularensis* in each of the five indicator strains, as well as producer strains. We inoculated by the plaque method, with plaques 0.5 cm in diameter, 4 strains from a 2-day agar culture in Petri dishes on the surface of dried liver agar with blood, and incubated them at 37°C for 48 h. The purity of the grown cultures was checked by microscopy of smears prepared from these plaques, as well as inoculation in beef-extract broth. The plaques were killed by decanting 0.3 ml chloroform in the lid of the dish with cultures, which was turned upside down, and leaving it there for 30 min. In order to remove chloroform fumes, the dishes were kept at room temperature for 3 h. Then we layered the indicator strains on the surface of the killed cultures using a round piece of sterile filter paper, 10 cm in diameter, profusely saturated with a suspension of 2-day culture in saline, containing 10 billion bacterial cells per ml. After 10 min, during which the suspension had time to spread in the agar, the filter paper was removed. The cultures were incubated at 37°C for 18-20 h, and we evaluated the results by examining and measuring the zones of depressed growth of the indicator culture around plaques of the tularecin-producing strain.

Results and Discussion

When the five indicator strains were layered on plaques of 60 producer strains, we found that most of the producer strains formed a zone of depressed growth around 1, 2 or all 5 indicator strains, 0.1 to 0.7 mm wide from the margin of the producer plaque. The most stable results were obtained with use of Central Asian strains Nos 178, 113 and 31 as indicators. It should be noted that when strains were used simultaneously as both indicators and producers (5 indicator strains), i.e., when we tested the effect of a strain on itself and on one another, the zones of depressed growth were the same as with other producers. Hence, the strains used as indicators were also tularecin producers, i.e., they were tularecinogenic and sensitive to tularecin.

Probably any other strain of *F. tularensis* in the S form could have the same property if cultivated on a layer of blood agar.

We were unable to demonstrate basic differences between strains referable to different geographic races, with respect to tularecinogenicity and sensitivity to tularecin, i.e., all of the strains, regardless of which race they belonged to, produced bacteriocins, which were demonstrable by means of indicator strains of the Holarctic and Central Asian races. Differences in size and dimensions of zones of depressed growth of indicator strains were apparently unrelated to the producer strain's geographic race.

The question of tularecinogeny, from the standpoint of specificity of effect on different strains of *F. tularensis*, requires further investigation.

Conclusion

The described method of demonstrating bacteriocinogeny made it possible to discover this phenomenon in *F. tularensis* for the first time.

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MILITARY EPIDEMIOLOGICAL DOCTRINE (BASED ON PROTECTION OF TROOPS AGAINST
EPIDEMICS DURING THE 1941-1945 GREAT PATRIOTIC WAR)

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5,
May 80 pp 11-17

[Article by I. I. Yelkin, First Moscow Medical Institute imeni Sechenov]

[Text] Apparently some epidemiologists, who are veterans of the Great Patriotic War, are correct in proposing that military field epidemiology be singled out as a special section of military epidemiology, by analogy to military field surgery and military field therapy [10]. This is correct because the living and working conditions of troops in the army in action differ radically from conditions in peacetime. There are also radical differences in forms of development of the epidemic process. In order to participate in the discussion of this question, we have tried to formulate here a military epidemiological doctrine, as it developed through the experience of the 1941-1945 Great Patriotic War.

During the period preceding the Great Patriotic War, great advances had been made in the USSR in the control of infectious diseases. By 1940, such diseases as smallpox, cholera, plague, relapsing fever and dracunculiasis had been eradicated. There was a drastic decline in morbidity referable to most infectious diseases. For example, the incidence of typhoid fever and typhus in 1940 was one-fifth the level in 1913. For expressly this reason, the period of defensive combats was characterized by epidemic uneventfulness, both among the public in the area of troop action and among reinforcements coming to the front, as well as in the troops in the field.

During the period of counterattack and gradual liberation of occupied regions of our country, the situation changed. Already during the period of the battles near Moscow, a large number of typhus cases was found among the inhabitants of liberated regions near Moscow: 125 cases in December 1941, 426 in January 1942, 623 in February and 972 in March [10].

As they established "new order" in occupied territory, the fascists subjected the local population to the most cruel exploitation and torture, implementing a genocide policy. In the villages, the people were evicted from buildings that had not been destroyed in order to billet the troops, and the residents of villages and cities found shelter in cold barracks, mud huts or remote

small villages. Thus, more than 1000 people lived in 60 peasant huts in a village in Vitebsk Oblast. Grain and cattle confiscation led to a semi-starvation subsistence.

No work was done to control epidemics. Moreover, we know of instances when the occupationists artificially created conditions for the spread of epidemics. As a result, epidemics of different infectious diseases, primarily typhus, raged among the local inhabitants of occupied territory. Thus, when the Velikolukskiy Rayon was liberated, 5205 cases of typhus were found among the inhabitants, and in the liberated territory of Nevel'skiy and Rosokhinskiy Rayons there were 5806 patients with typhus [8].

In March 1943, after liberation of the first regions in Belorussia, Soviet power agencies came face to face with the enormous number of people in these areas stricken with typhus. Half the inhabitants were stricken in some villages. One can realize the mass scale of typhus by the following examples: 97.7% of the inhabitants had suffered typhus in the years of occupation in the Vselitskiy Rural Soviet of Ushachskiy Rayon in Polotsk Oblast, 86.6% in the Molodetskiy Rural Soviet and 72.0% in some villages of Rechitskiy Rayon [2].

During the battle of Stalingrad, along with the difficult operational situation dangerous epidemic conditions developed. There was a real danger of cholera being brought to the Volga River region from fascist-occupied parts of the Ukraine, where there were mass scale cases of cholera in some cities at this time [5]. There was the threat of spread of other intestinal infections also.

The German prison and concentration camps, in which there were constant typhus and intestinal infection epidemics, were particularly dangerous sources for the spread of infectious diseases. In one camp, more than 4000 prisoners of war died of typhus and starvation between 13 September and 31 December 1942. In another camp, four barracks were burned, along with close to 300 patients in them, by order of the German command in order to eradicate an outbreak of typhus [2, 8].

During the offensive battles to liberate Hitlerite-occupied regions of our country, under the difficult epidemic conditions created by the occupationists, of course there were also cases of typhus in the Soviet troops. As a rule, this happened during heavy combat in units (chasti) leading the offensive. For example, in the troops of one front, in 1943 89.6% of the typhus cases had been infected in sites among the civilian population in a region liberated from occupation [2].

At the final stage of the battle of Stalingrad, the troops on the front liberated large prisoner of war and concentration camps for the civilian Soviet population, where typhus and dysentery raged. Moreover, our troops took as prisoners hundreds of thousands of enemy soldiers and officers, among whom morbidity was high, and there were sites of typhus and intestinal infections. The military medical service on the front was confronted at this time with an utterly new epidemiological task, that of protecting prisoners of war, as well as internees of fascist German concentration camps, against epidemics [5].

Furthermore, we know of instances when the Hitlerites took steps for artificially spreading typhus among our troops. Thus, during the combat operations in the

region of Staraya Russa (December 1941), during an attack by our troops the Hitlerites chased about 700 prisoners of war from a camp, in which there was a typhus epidemic, and under the threat of machineguns forced them to move toward our troops. The Army's military medical service did not take the necessary preventive steps, and outbreaks of typhus flared up among both the public and military units [7].

The data obtained from sanitary and epidemiological reconnaissance indicate that the fascist German troops were also severely stricken with typhus. Thus, in the enemy's 9th Army alone, there were 4971 registered cases of typhus, 328 of whom died, in the period from 1 January to 30 July 1942. It is not in vain that the commanding officer of the 9th Army wrote in his order: "Recently, the number of cases in the army of typhus almost reached the number of casualties." And then: "... there is a new enemy emerging before us--the typhus louse. This new enemy must be destroyed. Every soldier must be shown that a louse is more dangerous than enemy firearms" [7]. In the health service records of the 87th German Infantry Division for 25 July 1942, there is a reference to the report of regiment physicians to the effect that 50% of the regiment personnel suffered from diarrhea.

Our conceptions of tularemia broadened significantly during the Great Patriotic War. The etiology, epidemiology and symptomatology of tularemia had been studied quite intensively in prewar years. Endemic sites of this disease had been discovered in the USSR. Measures were developed to prevent it. Military operations disrupted the normal activities of the public in the zone near the front. Grain remained unharvested and grass unmown. A good feed base was created for rodents, and in the fall of 1942 a large number of house mice, striped field-mice, common redbacked voles, common field-mice, common voles, shrews, etc., appeared in dugouts and mud huts. A high density of these animals turned out to favor a tularemia epizootic in places where tularemia persisted in nature in water rats and, because of their small number, had not been detectable in peacetime. Against the background of intensive epizootics among mouse-like rodents, tularemia cases appeared in the military units in many sectors of the front. New endemic tularemia sites were discovered, in particular, in the northern part of RSFSR--Kalinin, Novgorod and Leningrad Oblasts [8].

One can judge the intensity of the tularemia epidemics even by the fact that in some parts of Stalingrad Oblast more than 75% of the inhabitants were stricken [5].

The nature of a number of outbreaks in the troops confirmed the fact that the aspiration route of tularemia infection holds a prominent place in the epidemiology of this infection. Before the war, so-called threshing outbreaks occurred, during which people were infected by inhaling infected dust when threshing grain. During the war, people were infected when they slept on straw contaminated by excrements of sick rodents, as well as when eating dry bread and bakery goods damaged by rodents, using water from primitive wells infected by the carcasses of rodents that had died of tularemia. Finally, there were outbreaks among the civilian population in the area near the front, as well as in the troops, which were related to transfer of the pathogen by mosquitoes [9].

Typhoid fever did not spread appreciably in the troops, although there were many cases among the public of liberated regions. Evidently, this was due to the effect of regularly instituted measures in the troops in action.

Dysentery was observed much more often. This was attributable to poor sanitary conditions, particularly on marches. Many cases were related to arrival of reinforcements and less so to contact with the public in dysentery sites. There was prevalence of *S. flexneri* (up to 93%) in the etiology of dysentery. From 15 to 25% of all cases of dysentery were of the chronic type [7].

The rapid offensive of German troops and related temporary occupation of Soviet territories caused the forced evacuation of an enormous number of residents of regions near the front, industrial enterprises, sovkhozes and kolkhozes to eastern regions. This led to overcrowded housing, overload on public transportation, communal, cultural and medical institutions and public eating facilities, as a result of which there was worsening of sanitary and hygienic working and living conditions instrumental in the spread of infectious diseases. Heroic efforts were required on the part of public health and Soviet power agencies to prevent the epidemic spread of typhus, relapsing fever, typhoid fever and other infectious diseases [11, 12].

Thus, war factors, which activated the epidemic process and caused the spread of infectious diseases in the troops and among the population, were also present during the Great Patriotic War, like in any other war. However, they were immeasurably more active, since the Great Patriotic War was the most devastating, the large in coverage and involvement in it of people, of all the wars known to the history of mankind. A total of 61 nations with a population of 1700 million people, i.e., three-quarters of all mankind, participated in it. The number of victims in this war was 50 million people [1]. However, in spite of this, the Great Patriotic War was not associated with epidemics either in the rear or army in action [6].

This can only be attributed to the fact that in the USSR, by virtue of the rapid development of the national economy, improved material welfare and culture of the people, creation of a solid system of national health care, the necessary conditions were provided for successful prevention of infectious diseases, even in wartime.

The army's military medical service entered in the war well-prepared, with regard to organization, to encounter the danger of an epidemic. The sanitary and epidemic-control service was singled out as part of this service, from the division to the Main Military Medical Directorate of the army, as well as special scientific and practical institutions in the form of SEO [sanitary and epidemiological departments] in the army and SEL [sanitary and epidemiological laboratories] on the front; there were infectious hospitals, bath and disinfection companies (ODR) and frontline disinfection instruction detachments (DIOF). The bath and laundry services for the troops in action were transferred to the military medical service.

From the very first days of the war, advancement of personnel qualifications became a first and foremost task. There were continuous classes at the frontline SEL in the form of courses, meetings of medical epidemiologists,

microbiologists and laboratory technicians of medical battalion platoons. Classes for advanced training of physicians specializing in infectious diseases were organized at the frontline infectious hospital, and disinfection specialists were trained at DIOF's. When preparing for offensive operations, there were mandatory educational gatherings of epidemiologists at frontline SEL's where problems were solved that dealt with protection against epidemics in offensive operations, maneuvering of epidemic-control equipment was discussed and scientific papers were delivered on current [or pressing] problems in accordance with the epidemic forecast.

Once a year, the Main Military Medical Directorate in Moscow held educational meetings (conferences), to which the chief frontline epidemiologists, army epidemiologists and certain other specialists were invited. At such gatherings, a survey was usually made of the epidemic status of the army in action broken down into fronts and districts, and there was detailed discussion of knowhow gained and future tasks. These educational meetings were of enormous importance, both to exchange of knowhow and advancement of administrative epidemiological cadres.

A military epidemiological doctrine gradually developed through this public [sic] experience in preventing infectious diseases among the troops in action, and by the end of the war it acquired orderly finalization.

Here are the main theses of this doctrine.

1. The epidemic process is a single entity, and is not divided into military and civilian. It is imperative to arrest outbreaks of epidemics among the civilian population in regions of troop formation and action, through the joint efforts of the military medical service and civilian public health in order to prevent penetration of infectious diseases into the troops. Sometimes the military medical service had to do this alone, since the civilian public health service was destroyed in occupied territory [11, 12].

2. Major changes were made in the medical evacuation of infectious patients. During World War I, it was the practice in the Russian army of evacuating all infectious patients to rear-area hospitals. In the first place, this worsened the patients' condition and, in the second place, it led to spread of infection. As a rule, multistage evacuation was not permissible during the Great Patriotic War. It was mandatory to directly evacuate infectious patients to their destination from a regimental or division medical post to an army infectious hospital, which moved to the division rear area on the level of first-line mobile field hospitals. The evacuation was effected by motor transport of the infectious hospital. Only when the hospital could not cope with this task was it permissible to transport infectious patients in vehicles traveling in that direction, with the mandatory disinfection of the vehicle at the hospital. This procedure for medical [therapeutic] evacuation assured the return to the ranks of about 75% of the infectious patients from hospitals in the army area. A small number of patients received additional treatment, due to combat conditions, in infectious hospitals in the frontline area.

3. Firm "epidemiological barriers" developed in the practice of epidemic-control back-up of the troops during the war, between the rear and the front,

which were compelled to prevent passage of any infectious patients from the rear to the front and from the front to the rear area. These barriers, which protected the troops in action from infectious diseases carried from the rear area, were referable to reserve regiments and medical check points at all stages of advancement or replacements. A system of epidemic-control measures at all stages of evacuation of casualties and the sick prevented infections from being carried from the front to the rear area, and its elements were: early detection of infectious cases among the wounded, isolation and treatment thereof, as well as sanitary processing of the wounded and sick to prevent pediculosis (see Figure) [13].

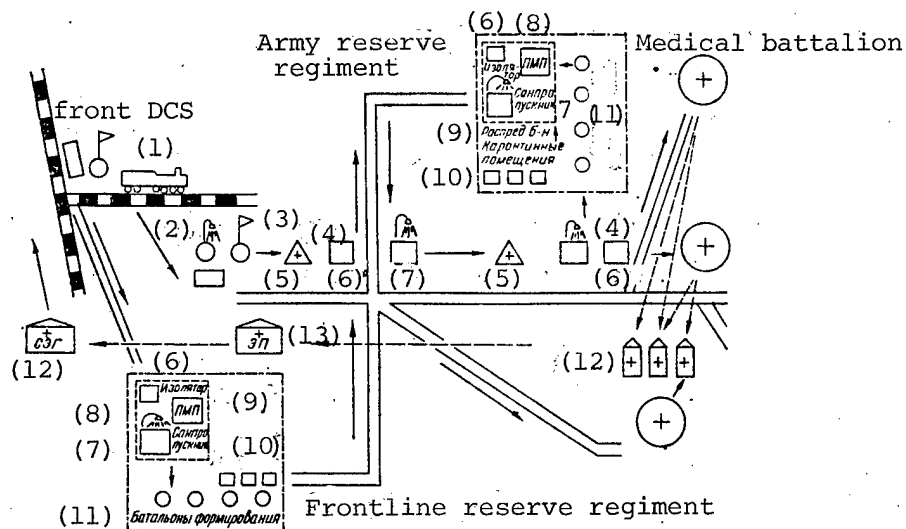


Diagram of "epidemic barrier"

Key:

- 1) bath and disinfection train
- 2) ODR department
- 3) army DCS [disease control station]
- 4) ODEB [separate road maintenance battalion]
- 5) medical post
- 6) isolation ward [facility]
- 7) disinfection center
- 8) regimental medical center ODEB
- 9) clearing battalion
- 10) quarantine buildings
- 11) formation battalions
- 12) clearing station

4. A very important element of the system of infectious disease prevention is early and accurate record-keeping about diseases. This is always difficult to do, particularly under conditions of an army in action. During the war, it was customary to keep a record of patients according to their surnames from the regimental medical post up to and including the military health administration of the front. It was mandatory to report an infectious patient by telegraph to

the chief of the division medical post, medical posts of different military units, mobile field hospitals, etc. The chiefs of infectious hospitals made reports of patients admitted to their hospitals.

5. Sanitary and epidemiological reconnaissance was the basis for assuring satisfactory conditions in the troops with regard to epidemics. The military medical service performed it systematically, constantly upgrading the forms and methods of work. Everyone is aware of the requirements for continuity, zoning and succession of reconnaissance. With the experience of war, the requirement of effectiveness was also added: it was necessary not only to detect a patient or some sanitary-epidemiological disturbance, but to take the necessary steps. Sanitary and epidemiological reconnaissance acquired special importance during periods of preparations and implementation of offensive operations. It was necessary to gather sanitary and epidemiological information about the region of forthcoming battles. This was done by interrogating prisoners and deserters, thorough examination of seized documents, orders, instructions, reports, outpatient and hospital logs. Interrogation of physicians taken prisoners were useful. Thus, from an interrogation of two physicians captured in the Velikiye Luki area, we learned that German medical officers were utterly uninformed about the symptomatology and epidemiology of tularemia. Not infrequently, the most valuable information was gained from secret agent and troop reconnaissance, as well as partisan detachments. Troop and army sanitary and epidemiological reconnaissance was conducted together with advancement of the troops; in the most important sectors, the work was intensified by means of forming operational epidemic-control groups manned by personnel from the frontline sanitary and epidemiological laboratory, bath and disinfection companies and frontline disinfection-instructor detachment.

6. On the basis of the data from sanitary and epidemiological reconnaissance, information about epidemiology and epidemiological geography, epidemiological forecasts were made, i.e., prediction of possible changes in the epidemic situation, in this case, on the territory of troop actions. These forecasts had to be based on systematic investigation and analysis of diverse factors affecting development or regression of the epidemic process. They were very important in planning epidemic security of offensive operations [6].

7. Epidemic security of offensive operations was the subject of constant attention on the part of the military medical service of the army in action. During the period of preparation for offensive operations, the health department of the army advanced its epidemic-control reconnaissance groups to the most important sectors, and they consisted of the following: SEO mobile laboratory, disinfection equipment of the ODR and horse-drawn bath detachments. The chief of the mobile laboratory served as group leader. The amount of disinfection equipment and horse-drawn bath detachments was determined by the tasks set forth and number of units (soyedineniya) serviced. Before battles, the sanitary and epidemiological status of the troops was checked, and any detected flaws were eliminated. The personnel was submitted to sanitary processing and provided with a change of underwear. During this period, there were educational gatherings of division epidemiologists, at which the plan of protection of troops against epidemics was studied in detail.

The medical specialists of the army's SEO base laboratory supervised epidemic control work in the rear area of the army. Special attention was given to epidemic-control measures in the reserve regiment, army roads, at centers where prisoners were assembled who were released from German prison and left the encirclement. Before an offensive, infectious hospitals were disposed on the level of the first-line clearing stations. Following the offensive units, the disposition of infectious hospitals was effected in accordance with the combat and epidemic situation or "leapfrogging," or else the infectious patients were transferred for completion of treatment to a clearing station moved for this purpose, while the infectious hospital moved forward. In some cases, a frontline infectious hospital was assigned as reinforcement. It was extremely important for the infectious hospital not to lag behind the troops. The field laundry detachments were also as close as possible to the troops. Before battles, the personnel was issued clean underwear, and it was mandatory for it to be treated with antiparasite agents.

There was mandatory separation of the flow of patients from the flow of wounded, starting at the regimental medical center in order to prevent infectious diseases at clearing stages. At the regimental medical center, all those suspected of having an infectious disease were put in an isolation ward, examined by a physician and sent to an infectious hospital. The second mandatory rule was sanitary processing of the wounded and sick at all stages of evacuation [clearing stages] starting at the first-line field hospitals. Finally, strictest adherence to sanitary and epidemiological regimens at hospitals and medical transport made it impossible for infectious diseases to spread and be carried to the rear area.

Thus, in the course of providing epidemic security of offensive operations, a new chapter of military epidemiology was formed. After the victory over fascist Germany and imperialistic Japan, the Main Military Medical Directorate of the Soviet Army organized an in-depth and comprehensive study of the experience gained in medical care. Already in 1945-1946, a competition was organized for scientific works dealing with Soviet medical experience in the Great Patriotic War. Hundreds of participants in the war described their observations and offered extremely valuable opinions. The USSR Council of Ministers issued a decree on 26 March 1946 concerning the publication of a multivolume work entitled "Experience of Soviet Medicine in the Great Patriotic War of 1941-1945." Publication of the 35 volumes of this work was completed in 1955; the 31st volume deals with infectious diseases [3] and the 32d with epidemiology [2]. These volumes, which were written by many authors who participated in the war, were a priceless contribution to Soviet military medicine. One cannot teach students to understand military epidemiology without making extensive use of the most valuable material contained in these books.

There is another major work that serves the same purpose, the "Military Medical Encyclopedia," in five volumes [4]. It also contains many valuable articles on military epidemiology.

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EPIDEMIC-CONTROL WORK AMONG THE PUBLIC IN THE ZONE OF COMBAT OPERATIONS ON
THE WESTERN (THIRD BELORUSSIAN) FRONT

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5,
May 80 pp 17-21

[Article by T. T. Pozyvay]

[Text] One of the most pressing problems of epidemic security of the troops during the Great Patriotic War was to organize epidemic-control work among the public in regions of combat operations. The forms and methods of organizing this work on the Western (3d Belorussian) Front were consistent with the combat and epidemic situation.

During the period of the war, as the Western Front withdrew to the east, covering the main direction of Moscow, then during the 2-month battle in Smolensk and near Moscow, the military units (chasti) operated on Soviet territory, which was in a good situation with regard to epidemics, and for this reason the problem of epidemic-control work among the public did not exist.

In early December 1941, the Western Front changed to the offensive, which ended with the utter defeat of the Germans near Moscow. The strategic initiative in the direction of Moscow moved after this time to the troops of the Western Front. The epidemic situation deteriorated in areas of combat operations during the counterattack. As the troops advanced to the west, an increasing number of typhus sites was detected in the liberated settlements. From December 1941 to April 1942, a total of 2146 typhus cases were found. Cases of typhus started to penetrate into military units from the civilian epidemic sites. The liberated regions near Moscow were occupied from October 1941 to March 1942. The spread of typhus within such a short time to the vicinities of Moscow, which had no epidemic problems before the German occupation, is attributable to lice-infested German troops, among whom there were many cases of typhus, who brought the infection in. At the same time, the very difficult sanitary and living conditions, concentration of local population who found shelter in the buildings that had not been destroyed were also instrumental in the spread of typhus in occupied areas.

At first, epidemiologists who conducted sanitary and epidemiological reconnaissance merely posted signs on buildings, if infectious patients were found there, forbidding billeting of servicemen there. However, it soon became

clear that this method of preventing infectious diseases from being carried in from endemic sites among the public did not yield the required results. The only effective method was eradication of the epidemic sites. Patients were hospitalized in local hospitals or army infectious mobile field hospitals (IMFH). At the same time, the sites were processed using the manpower and resources of the epidemic-control service, mainly of the army. This is how a new problem emerged, that of epidemic-control work among the civilians. As a result of implementation of a set of epidemic-control measures among the public and in the troops, the incidence of typhus did not reach epidemic proportions on the front.

In April 1942, offensive battles stopped and a prolonged intercombat period began. At this time, epidemic-control work was done more actively and on a broader scale.

As they retreated in the battle near Moscow, the Germans were detained to the spring of 1943 on the Rzhev-Vyazma base of operations [staging area] which extended over 300 km along the front and 100-120 in depth. This base of operations consisted of a projection of the German defense, which was situated relatively close to Moscow, about 100 km in a straight line. The Rzhev-Vyazma offensive operation, which took place in March 1943, occurred during the bad-road season, which affected the speed of attack of the Western Front troops, which did not exceed 6-7 km per day.

The sanitary and living conditions presented a grim picture for the inhabitants of liberated regions. There, the occupation had lasted 3 times longer than in the vicinity of Moscow--17 months. We already encountered a major typhus epidemic in cities--Rzhev, Vyazma, Gzhatsk and others, as well as rural areas. During the offensive battles, a total of 2453 typhus cases were discovered by the sanitary and epidemiological reconnaissance service, mainly that of the army. Epidemic-control work was done under the difficult conditions prevailing because of seasonally bad roads. A total of 2120 patients were hospitalized in army IMFH and the others were evacuated to local hospitals. At the same time, 43,223 people were submitted to sanitary processing in the epidemic sites, including disinfection of clothing and bed linens.

The epidemic spread of typhus among the public in occupied territory started in 1941, progressed in 1942 and had the greatest rise in 1943. According to sanitary and epidemiological reconnaissance data, in the strip of combat operations on the Western Front there were 7931 cases of typhus in settlements in 1942 and 11,371 in 1943. Typhus became the principal and terrible danger to the troops.

As compared to the number of cases of typhus, there was 1/15th of that number of typhoid fever cases among the population and 1/8th that number of dysentery cases in 1942, the figures for 1943 being 1/20th and 1/50th, respectively. In 1944, there was a change in structure of infectious morbidity: 4002 cases of typhus, 1288 cases of typhoid fever and 1028 of dysentery were recorded. Typhus continued to be in first place, but its share in relation to all the main infectious diseases diminished, constituting 63.3% in 1944, versus 92.6% in 1943. Conversely, the share of intestinal infections increased by more than 5 times in 1944, as compared to 1943.

We submit below data on changes in the structure of infectious diseases observed during the Belorussian offensive operation in the summer of 1944, which was characterized by rapid advance of our troops (at times 20-30 km per day to a depth of 500-600 km).

At the start of the operation, while the troops waged battle in regions of Belorussia, typhus continued to be in first place. Sanitary and epidemiological reconnaissance of liberated settlements revealed 1264 cases of typhus--95.6% of all cases of this infection detected during the entire Belorussian operation. After the liberation of Minsk and passage of the frontline troops to the territory of Lithuania, 57 cases of typhus were found (4.4%). Such a difference in incidence of typhus in Belorussia and Lithuania can be attributed to the fact that the liberated regions of Belorussian (part of Minsk and Vitebsk Oblasts) were in the region of the front for a long time; there were large concentrations there of German troops, and the civilians found themselves under the most adverse conditions, which led to a major typhus epidemic. The combat and epidemic situation developed differently in Lithuania, in particular, in Vileyka and Vilnius, as well as Kaunas Oblasts. Relatively small German garrisons were located there. Moreover, there was prevalence of the farmstead ["khutor"] type of settlements in Lithuania, so that there was limited contact between the rural population, which prevented the spread of typhus. However, the poor sanitary and living conditions, as well as utterly inadequate medical care of the public in occupied Lithuania led, in the summer of 1944, to the spread of dysentery and typhoid fever, the incidence of which was already higher than that of typhus (by 4 times).

Table 1. Nature and extent of epidemic-control work among the public in 1942-1944

Measure	1942	1943	1944	Totals
Inspection of settlements	1 005	10 160	22 478	33 643
Hospitalization in IMFH of civilian infectious patients	3 485	4 015	2 925	10 425
Sanitary processing of public	394 483	1 055 577	315 111	1 465 171
Disinfestation of clothing	576 129	1 957 751	706 128	3 140 006
Restoration of hospitals	40	60	75	175
Outfitting baths	481	1 966	171	2 618
Outfitting disinfection chambers	575	415	182	1 172

The epidemic-control service continuously strived for maximum coverage of cities and villages with reconnaissance and epidemiological observation. From year to year, more complete information was gained about the sanitary and epidemic conditions of the areas of combat operations, and this served as the basis for improving the quality of all epidemic-control measures among both the civilian population and troops.

In the period when German occupationists were being driven out of the Soviet Union, in the strip of combat operations of the Western (3d Belorussian) Front, a total of 10,425 infectious patients referable to the civilian population were hospitalized mainly in army IMFH. Of all the infectious patients

discovered in liberated settlements, the following numbers were hospitalized in IMFH: 36.6% in 1942, 32.2% in 1943 and 46.2% in 1944. The rest of the cases were evacuated to local hospitals, some of which were restored with the participation of the frontline epidemic-control service. On the Western (3d Belorussian) Front, tactics involved complete eradication of detected epidemic sites among the civilian population within the shortest time, whereas localization of epidemic sites was considered a half-measure, which did not guarantee prevention of transmission of infectious diseases to the troops and, for this reason, was allowed as an exception.

Since the vast majority of infectious cases among local residents was referable to typhus, sanitary processing with extermination of insects in clothing for individuals who had been in contact with the sick occupied a prominent place in the epidemic-control work done in settlements. The largest number of people submitted to sanitary processing (over a million people) and disinfection (about 2 million sets of clothing) is referable to 1943 (see Table 1), when a major typhus epidemic was discovered among the public.

There was a change, starting in 1943, in the form of joint work of military medical agencies with public health agencies, which became appreciably more active. Not limiting itself to contact with local rayon and oblast health departments, the frontline Military Medical Directorate established direct contact with the RSFSR Narkomzdrav [People's Commissariat of Public Health]. This made it possible to expand the work and make it better organized. Without dwelling on details, let us mention the following: rayon health departments were created at rayon centers as early as 3-5 days after their liberation and hospitals were deployed that were capable to receiving infectious patients for treatment; previously trained brigades of civilian physicians and paramedical personnel were used for epidemiological inspection of settlements with every new advance of the troops.

The structure of infectious morbidity in the strip of combat operations on the front differed significantly among civilians and in the troops (Table 2).

Table 2. Summary data for 1942-1944

Infectious diseases	Among civilians	In the troops
Typhus	82.7	15.5
Dysentery	8.3	37.8
Typhoid fever	8.0	7.5
Tularemia	1.0	39.2

The epidemic situation in the troops was not a mirror image of that of the civilians in whose territory they operated. This was attributable to several circumstances. In the first place, of importance was epidemic-control work, thanks to which typhus did not spread in the troops, whereas among civilians it assumed the features of a major epidemic. In the second place, the large share of dysentery in infectious morbidity on the front was attributable to the difficulty of controlling this infection, the source of which was the

fact that its reservoir was almost exclusively confined to the military units. Finally, the large share of tularemia in the structure of infectious diseases in the troops was attributable to the following: by the end of the summer of 1942, when grain was ripening and had not yet been harvested, the troops of the Western Front had occupied the defense lines; the local inhabitants, who had been relocated from the area of the forward-position line and were in the rear area of the troops and army, gathered and threshed the grain, as a result of which the conditions for mass reproduction of rodents were absent from settlements. However, on the forward-position line of defense and in the neutral strip, which was covered with fire from both sides, where grain had also not been harvested, conditions were there for reproduction of rodents (common voles) and then the spread of epizootic among them. With the onset of winter cold, the rodents sought warmer areas and headed for mud huts and dug-outs. In December 1942, there was a rise in incidence of tularemia in military units located on the forward-position line of defense. Tularemia was soon eradicated from the troops as a result of the steps that were taken.

The data submitted in this article are of more than historical significance. The knowhow gained in that war with regard to epidemic-control work must be used creatively to work out measures that would conform to the requirements of a modern war. Since questions of modern war are not the topic of this work, we shall merely mention one circumstance. With use of bacteriological (biological) warfare by the enemy, infectious diseases will appear, which it is expedient to consider as infectious combat pathology, by analogy to the fact that surgeons call gunshot wounds surgical combat pathology. Combat infectious pathology is considered as a category of military field epidemiology.

Epidemic security of the troops in action during the described period of the war consists of two main interrelated directions of work in the troops and among the public in regions of combat operations. In the specific combat and epidemic situation that was formed at the time the German occupationists were driven away from the territory of our country that they had temporarily captured, epidemic-control work among civilians liberated from fascist German occupation acquired particular acuity. By virtue of the intensive epidemic-control work among civilians in the strip of combat operations of the Western (3d Belorussian) Front, the epidemic-control service and medical service of units, "soyedineniya" [units] and hospitals achieved well-being in the troops with respect to epidemics.

In celebrating the 35th anniversary of the great victory of the Soviet Army over the fascist German aggressor, we cannot fail to mention kindly the performance of a large detachment of specialists in the epidemic-control service. Special mention should be made of such specialists as I. S. Bezdenezhnykh, P. A. Vavilin, N. K. Vladimirova, G. S. Vol'fson, B. S. Grabovskiy, V. S. Dmitriyeva, N. V. Zagorskiy, M. V. Zemskov, M. A. Komarov, K. P. Mochalov, Yu. S. Musabekov, P. V. Ostapenya, K. N. Popov, K. F. Pugachev, Ya. I. Rautenshteyn, G. P. Rudnev, Ye. Ye. Serkova, M. A. Sibiryakov, P. G. Tkachev, G. P. Tribulev, B. O. Ugryumov and many many others.

This was the first time in the history of wars that such large-scale epidemic control measures were effected in the theater of war, as they were on the fronts of the Great Patriotic War. Epidemic-control work of the military

medical service of the Soviet Army among the civilians in regions of combat operations constitutes an exceptional phenomenon in the history of Russian and Soviet medicine, which merits further comprehensive investigation.

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EFFECTIVE METHOD OF DISCOVERING TULAREMIA EPIZOOTICS IN THE WINTER

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[Article by I. V. Mikhaylovskiy, Z. I. Litvinova and R. N. Sadovnikova, Ryazan Oblast Sanitary and Epidemiological Station]

[Text] Observations of endemic tularemia sites by oblast sanitary and epidemiological stations are pursued mainly in the summertime. In the winter, epizootics are demonstrable in ricks of straw in the meadow-field zone of the sites. In particular, we have conducted major work annually to organize mass scale collection of murine rodents in the wintertime when straw is transported to the oblast's kolkhozes and sovkhozes. As a result, we have made a relatively good study of enzooticity of the territory of meadow-field site regions. In virtually every region in the vicinity of 5-10 settlements, we recorded tularemia epizootics.

We have not studied enough the zone of backwater-swamp sites (about half the oblast's area). In the wintertime, over all of the years such work was done, only one culture of *F. tularensis* could be isolated from the common redbacked vole (January 1976, Ryazanskiy Rayon, Polyany).

This year, we tested a new, very effective method of detecting winter epizootics of tularemia by means of bacteriological tests on fragments of pelts of murine rodents.

Material and Methods

In late February 1979, fragments of pelts from murine rodents were gathered for the first time in the Oka River floodplain (Konstantinovo village, Rybnovskiy Rayon).

Our method consisted essentially of the following. Predatory birds, as well as crows and magpies, tear off the pelt of a caught rodent in shreds before they eat it. These shreds of pelts can be found more often under isolated trees and bushes in fields and meadows, under surveyor stakes, in stacks and piles of straw, hay and in general on various elevations. Magpies catch rodents more often in willow bushes, while crows and predatory birds catch them more often in open areas. In the wintertime, predatory birds are seldom encountered, and they are referable mainly to the rough-legged buzzard (*Buteo*

lagopus Br.) and short-eared owl (*Asio flammeus* Pon.). One can see 1-2 buzzards during an entire day of inspecting the floodplain, and short-eared owls are encountered even less often. In the wintertime, the main enemies of mouse-like rodents among birds are those in the *Corvidae* family.

The results of bacteriological examination of fragments of pelts revealed that this method is many times more effective than examination of rodents caught in traps. The traps are usually placed over a limited territory and primarily the more active, i.e., healthier, rodents get into them. Birds catch primarily weak, less active and, consequently, sick rodents, and moreover they also pick up their carcasses.

If traps are used to inspect some region, at least 2 days are required, whereas with the new method one can inspect a much larger territory in 1 day. One can travel 10-15 km per day on skis, and examine places that are inaccessible in other seasons. In only 3 work days, in March 1979, one zoologist from the department collected pieces of pelts from about 210 common voles. A total of 18 biotests were made with these pelts and 16 cultures of *F. tularensis* were isolated. This material was collected in the meadows of the Oka River floodplain, in the vicinity of Konstantinovo, Rybnovskiy Rayon and the villages of Solotcha and Zaokskoye in Ryazanskiy Rayon.

In regions where the rodent population is small in size there are no shreds of pelts. For example, during the same period, in March 1979, the floodplain of the Oka River was inspected from Ryazan to Spassk and no pieces of pelts were discovered.

On the pelts there are sometimes traces of blood, frozen drops of which can also be detected on the snow. At first, we limited ourselves only to collection of pelts, but near Zaokskoye, we also collected frozen drops of blood with the pelts. It is best to collect these frozen blood drops and blood-soaked snow separately. Perhaps, it will also be possible to isolate cultures of *F. tularensis* from this frozen blood.

Biotests of pelt shreds were prepared in the following manner: the pelts were shredded finely with scissors, covered with saline, mixed well, pressed down with a pestle and then this material was used for hypodermic infection of white mice. When the result was positive, the rodents usually died on the 5th-6th day.

Having positive results from testing the pieces of pelts, we can determine exactly when and where there is an epizootic. The fact of the matter is that the pelt fragments were gathered fresh after each snowfall or blizzard. They were collected from many points, because the birds consume each trapped rodent in the vicinity.

Results and Discussion

It is apparent from the data we obtained (see Table) how reliable our method is in discovering tularemia epizootics in the winter. For example, on 9 March 1979, a zoologist traveled 10 km on skis in the meadows of the Oka River

floodplain, near Solotcha, and gathered pieces of pelts from about 130 common voles, with which 10 biotests were performed with positive results. If the test results had been negative in this case, it could have been stated almost with certainty that this territory has no tularemia problem, because both birds catch rodents at random and the route of the floodplain inspect was laid at random. We believe that it would be difficult to miss an epizootic in such a case.

Results of collecting and examining fragments of rodent pelts

Locality	Date	Approx. number of rodents from whom pelt shreds were collected	Quantity of biotests	Quantity of <i>F. tularensis</i> cultures isolated
Rybnovskiy Rayon, Konstantinovo	27 Feb 79	20	2	2
Ryazanskiy Rayon, Solotcha	9 Mar 79	130	10	10
Ryazanskiy Rayon, Zaokskoye	24 Mar 79	60	6	4

All of the cultures isolated from pelt fragments presented the typical properties. They did not differ in any way from cultures that were isolated from organs of trapped rodents.

The tularemia site found in the Oka River floodplain is 50-60 km in length, from the boundary of Moscow Oblast to Ryazan. There, the epizootic occurred against the background of a large number of common voles.

In these regions, the Oka floodplain is up to 5-8 km, with numerous lakes, oxbows, streams with abundant aquatic vegetation. There are bush thickets along the shores of the lakes and oxbows, and small sections of forest. The open areas are covered with hayfields. Near the villages of Solotcha and Konstantinovo, considerable parts of the meadows are covered with pasturage for cattle.

The composition of mammals in the floodplain is quite diversified. Over a period of several years of surveying work, the following species were trapped: common vole (*Microtus arvalis* Pal.), root vole (*Microtus oeconomus* Pal.), water rat (*Arvicola terrestris* L.), muskrat (*Ondatra zibethica* L.), common redbacked vole (*Clethrionomys glareolus* Schr.), common field-mouse (*Apodemus sylvaticus* L.), harvest mouse (*Micromys minutus* P.), brown rat (*Rattus norvegicus* Ber.), common shrew (*Sorex araneus* L.), small shrew (*Sorex minutus* L.) and water-shrew (*Neomys fodiens* Pen.). The predominant species were the common vole, root vole and common field-mouse. The natural enemies of mouse-like rodents that we encountered included the weasel (*Mustela nivalis* L.), ermine (*Mustela erminea* L.), European polecat (*Mustela putorius* L.) and fox (*Vulpes vulpes* L.).

The opinion is held [1] that infection is maintained primarily by the water rat in endemic tularemia sites of the floodplain type. However, our data

indicate something different. The intensive winter epizootic of tularemia occurred in common voles. At the same time, the number of water rats was low in recent years in all areas. During the spring high waters, a route was laid for registration of water rats in the territory of the site we discovered (meadows near Solotcha). None was found over a 3-km distance. Local fishermen and hunters also noticed that there were very few water rats. In July 1978, when a "census" of water rats was taken in the same places, a small number was also found: 2% incidence of trapped animals per 100 traps per day.

Conclusions

1. Bacteriological testing of fragments of rodent pelts demonstrated the predominant role of the common vole, root vole and common field-mouse in enzootic tularemia sites of the floodplain type.
2. The water rat is not the chief source of epizootics in enzootic floodplain sites of tularemia.

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BACTERIOLOGICAL AND SEROLOGICAL METHODS USED TO STUDY EPIZOOTICS OF TULAREMIA
IN RODENTS IN AN ENZOOTIC SITE OF THE MEADOW-FIELD TYPE

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Mar 81 (manuscript received 15 Jan 80) pp 79-83

[Article by I. S. Meshcheryakova, P. M. Baranovskiy and Yu. V. Okhotskiy,
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[Text] The principal method of studying enzootic tularemia sites and detection of epizootics is bacteriological examination of small mammals, ticks, blood-sucking insects and environmental objects in order to isolate the pathogen of infection. However, a bacteriological examination is not always effective in the case of epizootics that have a sluggish course, it requires much labor and time to inspect extensive territories. Moreover, the test must be done very soon after material is collected, in specially equipped laboratories.

At the present time, serological methods are acquiring much importance. The most effective of these is the antibody neutralization reaction (ANR). It permits demonstration equally well of live and killed bacterial cells and antigen in any material; it is suitable for testing decayed and mummified carcasses, bony remains of rodents, nest masses and other objects when isolation of *F. tularensis* is difficult or impossible [12, 14, 15].

In recent years, the ANR has been used extensively for testing bird pellets and droppings of carnivorous mammals for tularemia [2]. This methodological procedure can be used for early and retrospective detection of an epizootic, definition of its range and animal species involved in the process of pathogen circulation. It is possible to examine bird pellets in virtually all types of endemic sites for tularemia and in unexplored territories [3-7, 9]. Organization of commercial production of tularemia antigen erythrocytic diagnosticum and availability thereof to scientific and clinical institutions enables us to make wide use of serological methods for inspection of endemic tularemia sites.

Our objective here was to make a comparative evaluation of the efficacy of the bacteriological and serological methods of examining rodents, which we made in 1977-1978, during a period of an intensive fall-winter tularemia epizootic in an endemic site of the meadow-field type.

Material and Methods

These studies were conducted in the southern part of Moscow Oblast within the limits of Stupinskiy Rayon. Under observation was a section about 5 km² in area, with alternation of fields, meadows and forests (about 30%) adjacent to the small Samorodinka River (tributary of Lopasnya). The most common rodents in the fields and meadows of this section are common voles, with a small number of harvest mice, common redbacked voles and striped field-mice. There are considerable fluctuations in number of common voles and other small mammals. This locality is known for its epizootic and epidemic manifestations of tularemia from at least 1938 (when regular observations were started). No cases of tularemia among people have been noted since 1952 (the public is immunized and reimmunized), but periodically there have been epizootics in stacks of straw, which was indicative of persistent preservation of endemic tularemia sites in the southern part of Moscow Oblast.

A rise in number of common voles was noted in the fall of 1977 (2316 burrows per hectare field in September). Their number continued to rise thereafter, which led to outbreaks of tularemia epizootics.

The material for our studies consisted, in the first place, of 75 common voles delivered to the laboratory between October 1977 and February 1978. Each animal was tested individually with the ANR and the method of direct culture on special nutrient medium of fresh fish hydrolysate with cystine, glucose and blood, to which we added antibiotics (ampicillin and polymyxin) to prevent growth of extraneous microflora. In the second place, we examined 310 rodents, mainly common voles, 120 of which were live specimens and 190 carcasses, discovered in the course of examining four experimental straw stacks in February-March 1978 during expeditionary work. When examining carcasses, each was tested individually both with the ANR and bacteriologically (cultures and biological test). Trapped animals were submitted to bacteriological examination only after detection of tularemia antigen in their organs with the ANR. Finally, 79 animals were also tested for antibodies to *F. tularemia* using the passive hemagglutination reaction (PHAR). For this purpose, we used washings from the thoracic cavity prepared by the method of Marin et al. [11].

In addition, during the period of our work from October 1977 to April 1978, we gathered bird pellets in the same points of the inspected territory (total of 3 collections). The pellets were tested in the ANR using a previously described method [8]. The pellets were not submitted to bacteriological examination, since it was experimentally shown that there was rapid extinction of pathogen in them (1st-2d day) [2].

Results and Discussion

Already the first episodic tests on rodents delivered to the laboratory enabled us to demonstrate antigen of *F. tularensis* in the ANR, in high titers--1:80-1:5120 (geometric mean titer was 1:1280)--in 14 out of 75 cases. Using the method of direct inoculation on nutrient medium, we isolated *F. tularensis* cultures from organs of nine rodents (Table 1). The first finding of antigen in rodents and isolation of culture were recorded in early November 1977.

Table 1. Results of bacteriological and serological testing of 75 common voles for tularemia

Test method	Number of rodents	Number of rodents with positive ANR							
		total	titer						
			1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
ANR	75	14 (18,6±4,4%)	1	2	2	1	2	2	4
Bacteriological	75	9 (12,0±3,7%)	—	2	1	—	2	1	3

Examination of rodents collected in experimental stacks (Table 2) revealed an extremely high percentage of demonstration of *F. tularemia* in rodent carcasses: 78 (65%) positive ANR out of 120 and 26 (21.6%) isolated cultures. Antigen titers in the ANR constituted 1:80 to 1:81,920, with a geometric mean of 1:10,240. Examination of 190 live animals revealed *F. tularensis* antigen in the ANR in 11 (5.7%) cases, and ANR titers constituted 1:80 to 1:40,960, with a geometric mean of 1:5120; cultures of the pathogen were isolated from 2 (1.05%) animals. In all cases of isolation of cultures there were positive serological reactions. When cultures were isolated from rodent organs, ANR titers were most often high--1:5120-1:81,920; however, in 2 cases cultures were isolated with ANR titers of 1:80 and 1:160 (Table 3).

Table 2. Results of testing rodents from experimental stacks

Material examined	Rodent species	Number of rodents	Incidence of positive ANR		Incidence of isolation of cultures	
			total	%	total	%
Carcasses	Common vole	109	73	66,9±4,5	25	22,9±4,0
	Harvest mouse	5	2	40,0±22,0	—	—
	Striped field-mouse	1	—	—	—	—
	Common redbacked vole	5	3	60,0±22,0	1	20,0±18,0
	subtotals	120	78	65,0±4,3	26	21,6±3,5
Live animals	Common vole	180	11	6,11±1,75	2	1,1±2,6
	Harvest mouse	6	—	—	—	—
	Striped field-mouse	—	—	—	—	—
	Common redbacked vole	4	—	—	—	—
	subtotals	190	11	5,7±1,65	2	1,05±2,4
	Totals	310	89	28,7±2,6	28	9,03±1,5

Over the entire period of the study, we isolated and examined 37 *F. tularensis* cultures: 18 were obtained by direct inoculation on nutrient medium and 19 by

Table 3.
Correlation between ANR titers and frequency of isolation of *F. tularensis* cultures from experimental stacks

ANR titer	Incidence of isolation of cultures	
	car-casses	live rodents
1:80	3/1	1
1:160	3/1	
1:320	3	1
1:640	2	
1:1 280	2	1
1:2 560	2	1
1:5 120	16/1	1
1:10 240	10/7	1
1:20 480	8/3	3/1
1:40 960	13/5	2/1
1:81 920	16/8	
Totals	78/26	11/2
Geometric mean titer	1:10 240	1:5 120

Note: Numerator--number of positive ANR, denominator--number of cultures isolated with corresponding ANR titer.

sensitive animals. There are sparse data in the literature concerning detection of specific antibodies in animals of the first group [1, 5, 13, 15]. However, there were few animals with prior infection, ranging from 0.3-0.9 to 4% of all animals tested. We failed to demonstrate rodents with specific antibodies during the intensive fall-winter epizootic in 1977-1978, which could perhaps be attributable to the small number of animals tested.

We collected 908 bird pellets for early discovery of a tularemia epizootic and subsequent investigation thereof. The pellets were gathered at the same points at three different times: October, November and April (Table 4). As we know, inspection of such material increases drastically the probability of detection of the pathogen of infection and permits reliable determination of the time and intensity of an epizootic. Already in the material collected on 11-14 October 1977, we demonstrated *F. tularensis* antigen in 26 out of 319 pellets, which was the first sign of the start of a tularemia epizootic. In the pellets collected in November, *F. tularensis* antigen was demonstrable consistently in a study of 189 specimens; the percentage of positive findings and mean ANR titers grew. In the spring of 1978, we made a thorough collection of pellets in places where there had been a tularemia epizootic in the winter. Tularemia antigen was found in high titers in 282 out of 400 pellets. One can assess the intensity of a prior epizootic on the basis of number of positive findings.

means of the biological test on white mice. All of the cultures were found to be quite typical in the main biological properties--morphological, cultural, tinctorial and agglutinability with specific tularemia serum to a titer of 1:1600. Most of them (31) were sensitive to erythromycin (as determined by the disc method) and classified as biotype I according to this property; 6 cultures were resistant to erythromycin and classified as biotype II. A study of a sampling for pathogenic properties revealed that the isolated cultures were quite similar in virulence to animals that are highly sensitive to tularemia--white mice and guinea pigs. The animals died of tularemia after hypodermic injection of isolated bacterial cells, presenting typical pathoanatomical signs and with isolation of pathogen from internal organs. Virulence of cultures obtained by direct inoculation did not differ from the virulence of cultures isolated by the biological method with white mice.

We examined 79 trapped live rodents in order to test the animals for antibodies to *F. tularensis*, which would be indicative of prior infection in highly

They reflect indirectly the quantity of infected specimens in the animal population, from which the sample was taken. Although the ANR titer is only a relative indicator of amount of antigen in a pellet, the high titers we demonstrated served as proof of an intensive and recent tularemia epizootic.

Table 4. Results of ANR testing of bird pellets for *F. tularensis* antigen

Date of collection	Quantity of pellets	Quantity of ANR-positive pellets										Geometric mean titer
		totals	titer									
			1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560		
1—14/X 1977	319	26 (8,15±1,5%)	11	3	4	4	3	1			1:57	
14—16/XI 1977	189	42 (22,2±2,9%)	7	11	12	5	1	1	5		1:86	
10—14/IV 1978	400	272 (68,0±2,3%)	58	78	48	9	23	18	20	18	1:98	

Analysis of pellet contents revealed that they contained mainly residue from the common vole. A total of 908 pellets contained considerably more animals than were collected or trapped over the entire period of the study.

Thus, our results demonstrated convincingly that the serological method was highly effective in detecting and studying tularemia epizootics in a site of the meadow-field type. Serological examination of trapped and dead rodents enabled us to determine that considerably more animals were stricken with tularemia than can be demonstrated by traditional bacteriological methods. Thus, over the entire period of our study, *F. tularensis* antigen was found in 103 (26.6%) out of 385 small mouse-like rodents and the pathogen was isolated from 37 (9.6%) animals. An epizootic was discovered for the first time by serological examination of bird pellets collected in early October. Thus, this method was found to be the most effective for early detection of an epizootic. Analysis of the pellets collected in the spring enabled us to retrospectively assess the scope and intensity of the epizootic and obtain a fuller description of the epizootic process. When it is impossible to organize trapping of small mammals in the fall and winter, testing of bird pellets, the collection of which is less time-consuming, for *F. tularensis* antigen alone makes it possible to determine quite conclusively the onset of an epizootic, to evaluate its intensity, identify the species and number of animals involved in the epizootic. This, in turn, facilitates early institution of preventive measures in an enzootic site.

Conclusions

1. A study of a fall-winter tularemia epizootic, which was recorded in 1977-1978 in an enzootic site of the meadow-field type in the southern part of Moscow Oblast, revealed that the serological method of testing rodents and their carcasses for *F. tularensis* is more effective than bacteriological methods: testing of 385 rodents revealed *F. tularensis* in 26.6% of the cases using the serological method and only 9.6% of the cases by the bacteriological one.

2. Examination of bird pellets made it possible to discover an epizootic at an early stage, with minimal expenditure of labor, as well as to track its development, retrospectively assess its intensity, animal species involved in the epizootic, territory involved and gain a more complete idea about the epizootic process.

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EFFECT OF PULSED MAGNETIC FIELD ON SHIGELLA FLEXNERI

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[Article by V. I. Ryabtseva and B. N. Kuz'minskiy, Voroshilovgrad Sanitary and Epidemiological Station]

[Text] This study determined the possibility of controlling growth and reproduction of microorganisms by means of magnetic fields and use of results of these studies in bacteriological practice. We tested the effect of a pulsed magnetic field (PMF) on rate of growth and biochemical activity of *S. flexneri*. The studies were conducted in the horizontal pulsed magnetic field of a solenoid. We used the method of counting live microorganisms: a 24-h agar culture, diluted in accordance with the GKI [State Control Institute of Medical Biologicals imeni L. A. Tarasevich], was "magnetized" in the PMF, then inoculated on Ploskirev medium, and after 1 day of growth in the incubator we counted the colonies that grew in experimental and control dishes. We also tested the effect of PMF on biochemical properties of *Shigella* with respect to capacity to ferment glucose, lactose, mannitol, saccharose, production of indole, hydrogen sulfide, lysis of urea, growth on Simmons, acetate and polytropic media.

We conducted three series of experiments. In the first we tested the effect of PMF with 100 G for 1, 2 and 3 h on growth rate of the microorganism; in the second we tested the effect of fields differing in induction--10, 50, 100, 150 and 200 G--for 2 h also on growth and reproduction of a test strain; in the third we tested the effect of 150 G induction for 3 h on biochemical and antigenic properties of *Shigella*.

It was shown that 1-h exposure to a field with induction of 100 G did not alter the growth rate of the test microorganisms ($P > 0.05$); with 2-h exposure, the number of viable cells increased by 13.2% ($P < 0.01$) and with 3-h exposure it increased by 40.8% ($P < 0.01$). Thus, the magnetobiological effect depended on "magnetization" time.

We also demonstrated that the magnetobiological effect depended on the induction of PMF with the same exposure time. Thus, with exposure to a field with induction of 10 and 50 G, we observed depression of microorganism growth ($P < 0.01$), with 100 G there was 13% stimulation of growth, with 150 G 51% stimulation and with 200 G 25.5% stimulation.

There was no change in biochemical or antigenic properties of *S. flexneri* after 30-h exposure to a field with induction of 150 G.

Thus, by varying the level of PMF induction and time of exposure to the field, one can control the rate of microorganism growth. A pulsed magnetic field with induction of 150 G can be used in the practical work of bacteriological laboratories in order to diagnose dysentery faster, since magnetic fields with the above parameters used for "magnetization" for 2-3 h stimulated growth of *S. flexneri* without altering its biochemical and antigenic properties.

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RESULTS OF IMMUNOLOGICAL SCREENING OF THE PUBLIC FOR TULAREMIA IN THE EASTERN SECTION OF THE BAYKAL-AMUR RAILROAD

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[Article* by L. S. Kamennova, V. I. Timoshenko, Ye. V. Ananova, V. D. Baranchikov, M. I. Lev and A. Ye. Vasyanovich, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] Tularemia in the Far East has not been sufficiently investigated. There are only a few reports in the literature dealing with this question [1, 3-6, 8]. A need arose to make a thorough study of this infection in that region in connection with construction of the BAM [Baykal-Amur Railroad], in order to validate preventive measures. This study was conducted in 1976-1979 by an expedition from the Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences (expedition head--E. I. Korenberg).

Material and Methods

The principal method we used was a serological and allergy screening of the inhabitants, with additional comprehensive interrogation. This work was conducted along the BAM route in Komsomolskiy, Solnechnyy and Verkhnebureinskiy Rayons of Khabarovsk Oblast. The public was divided into the following groups: 1) indigenous--individuals who were born in Khabarovsk and Maritime Krays and lived in the above rayons; 2) new arrivals--individuals born elsewhere and had lived in this territory for different periods of time (over 10 years, 4-10 years, less than 4 years). We made a separate study of organized groups of people who had worked in the BAM territory for 1 month to 2 years.

In all we examined 2569 people from 18 settlements: 955 indigenous residents (562 women and 393 men, of whom 235 were 15-19 years old, 652 were 20-50 years old, 67 over 50 years) and 1614 new arrivals. Individuals who had been re-tested within 4 years were recorded once.

In this region, the indigenous population had not been immunized against tularemia. The new arrivals consisted of individuals who came from republics

*Reported on 20 December 1979 to a scientific conference of the Department of Endemic Infections, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, dedicated to the 50th anniversary of the Tularemia Laboratory.

and oblasts where there were active endemic tularemia sites and mass-scale immunization against this infection was effected.

The screening was conducted by means of the tularin skin test and serological examination of serum. The skin tests were evaluated after 48 h. In some cases, observations were pursued at a later time (72-96 h). Serum was tested 2-4 months after taking blood samples. They were stored in frozen form. The serological reactions were run by conventional methods [2, 7], starting with the following dilutions: 1:10 for agglutination test (AR), 1:20 for passive hemagglutination (PHAR) and 1:5 for immunofluorescence test (IFR). Highly sensitive diagnosticum derived from a Nearctic strain for the AR and erythrocytic diagnosticum were prepared at the Institute imeni Gamaleya by Meshcheryakova, candidate of biological sciences. Any positive AR and PHAR results with low titers (1:10, 1:20) without reciprocal confirmation, negative IFR, RTPGA [inhibition of passive hemagglutination?] and tularin tests were excluded as being questionable.

Results and Discussion

According to the aggregate of positive skin test and serological results, we found 41 ($4.2 \pm 0.65\%$) immunopositive individuals among the indigenous inhabitants and 240 ($14.8 \pm 2.29\%$) among the others. The tularin test was positive in 4% of the indigenous population, AR in titers of 1:10-1:160 in 4.1%, PHAR in titers of 1:10-1:320 in 3.9% and IFR in 3%. Geometric mean titers were 1:22 in the AR, 1:39 PHAR, 1:16 IFR. Among those from other regions, positive tularin tests were recorded in 12.3% and serological reaction in 14.8%. Previously published data for the same territory [6], showing 17% immunopositive individuals for tularemia, refer to a group that consisted of both indigenous and nonindigenous population.

We selected 36 people (8 indigenous residents and 28 arrivals) for a second examination in order to determine the reliability of negative skin test results in individuals with positive serological reactions for tularemia. One year later, we demonstrated coinciding positive results of both the skin test and serological reactions in the same individuals, with the exception of one (outsider). In 3 cases the skin reactions were rated as strongly positive, in 24 as positive (with infiltrates up to 1.5 cm in size) and in 8 cases as weak (0.5 cm infiltrates, hyperemic ridge around the scarifications or 2-3 vesicles along their course).

In order to determine the distinctions of immunological reactions of the indigenous population, we screened 168 individuals referable to local ethnic groups (119 Nanaians, 32 Evenks, 9 Yakuts, 5 Koreans, 2 Ul'chi and 1 Udegeyets) and 787 indigenous inhabitants referable to the Russian, Ukrainian, Belorussian and other ethnic groups. Immunity was found in 39 cases, including 8 Nanaians, 1 Yakut, 1 Udegeyets, 1 Ukrainian and 28 Russians, with coinciding positive skin test and serological reactions. Only two indigenous residents (Russian) presented a negative tularin test with positive serological reactions. Among the newcomers, a negative tularin test in seropositive individuals was observed in 2.5% of the cases. All of the described negative tularin tests were evaluated after 48 h, and it was not possible to observe them further. However, in the course of the screening, in some cases tularin skin reactions appeared at a later time, after 72 and 96 h.

Our findings did not confirm the conclusions that indigenous inhabitants presented weak allergic reactivity of the skin [5]. It may be assumed that the negative skin tests in individuals with positive serological reactions during the mass-scale screening were attributable to technical flaws in running the test, insufficient observation time and incorrect evaluation of mildly positive tests as negative.

The size of the immune stratum among the indigenous population varied in different settlements. In Solnechnyy Rayon, the largest number of immune individuals was recorded in the villages of Kondon (7.8%) and Evoron (7%), which are located in the Devyatka River valley near Lake Evoron. This data coincide with the results of an epizootiological inspection of the territory. In 1977, cultures of *F. tularensis* were isolated from the northern redbacked vole and Ungur vole trapped in the region of Lake Evoron [6]. In adjacent regions, in the valleys of Amgun and Gorin Rivers, there were appreciably fewer immune individuals in villages (1.7-3.4%). In Verkhnebureinskiy Rayon, the largest number of immune people (12.1%) was found in the village of Sredniy Ural, which is situated in the Ural River (Bureya tributary) valley. There were 4.8-6.2% positive reactions among the inhabitants of nearby villages, whereas in settlements far from the river only 1 positively reacting individual was found out of 118 tested.

We failed to observe appreciable differences in size of the immune indigenous stratum as a function of occupation and activities--forestry, agricultural, transport workers, casual laborers [or handymen] and white-collar workers (4.3 to 5.7%). The immune stratum was found to be higher (13.6%) only in hunters and fur farm workers. A positive immunological reaction was found in only 1 out of 191 school children tested.

Immunopositive men were encountered about 1.5 times more often than women-- 5.1 ± 1.1 and $3.7 \pm 0.79\%$. With increase in age, there was uniform increase in the immune stratum. Immune individuals constituted 0.9, 4.6 and 13.4% in the tested age groups of 15-19, 20-50 and over 50, respectively. We failed to demonstrate any appreciable differences between outsiders living in the BAM territory for different periods of time with regard to immunological structure.

Retrospective analysis of serum revealed diagnostic titers of antibodies in isolated cases. Our recent discovery of a 16-year-old school girl, who lived in the village of Evoron and had had tularemia, merits attention. Demonstration of diagnostic titers of antibodies (1:60 for AR and 1:320 for PHAR) combined with strongly positive tularin test and severe sore throat in her history were indicative of recent tularemia. A second case was discovered in Berezovyy, where a new arrival (woman) who had apparently been immunized in the past presented a positive tularin test and low antibody titer (1:20 for PHAR with negative AR) in the first screening. When checked 1 year later, we established fresh tularemia infection, which had caused a febrile illness with high temperature, enlargement of cervical lymph nodes, elevation of antibody titers in serological reactions (1:160 for AR, 1:1280 for PHAR and 1:320 for IFR) and strongly positive skin test for tularin. The reliability of a disease of tularemic etiology was confirmed by demonstration of specific IgG in a titer of 1:160 (Meshcheryakova) in her blood serum. It is quite likely that infection occurred through water in both cases.

It can thus be considered proven that the inspected segment of the BAM has a tularemia problem. However, the epidemiological activity of the existing endemic sites is minimal, as indicated by the relatively small share of indigenous population with a history of tularemia, as well as the small number of fresh cases of the disease, which we discovered in the course of our work. The low activity of these sites was also confirmed by the results of screening an organized group of 614 people, who had come to work on BAM construction and had been under constant medical supervision for 2 years. Of this group, 195 people were checked twice at a 1-year interval. However, no fresh cases of the disease were found at either the first or second examination.

The results of our survey warrant the recommendation that immunization against tularemia be performed for limited groups: hunters, foresters, fur farm workers, shepherds, residents of the most dangerous territories, in accordance with Order No 133, dated 5 February 1976, of the USSR Ministry of Health. In addition, there must be provisions for nonspecific preventive measures: sanitary protection of water sources, decontamination of water for drinking and household purposes, health education.

Conclusions

1. In the eastern segment of the BAM route, 4.2% positive serological and allergic reactions for tularemia and isolated fresh cases of the disease were recorded among the indigenous population; however, the small size of the immune stratum is indicative of minimal activity of endemic sites of this infection in the territory in question.
2. Regions of maximum contact of the inhabitants with endemic sites were found in the area of Lake Evoron and upper reaches of Bureya River.
3. Routine contact of inhabitants of villages near stations [trading posts?] with the forest causes a comparable risk of tularemia for representatives of most occupations and activities; it is somewhat higher among hunters and fur-farm workers
4. Similar indicators of immune stratum among the indigenous population were found with use of known serological reactions and the tularin skin test.

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LEUKOCYTOLYSIS AND NEUTROPHIL DAMAGE TEST AS METHODS OF DEMONSTRATING ALLERGY TO FRANCISELLA TULARENSIS

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[Article by N. A. Sukhanov, I. I. Osipenko and D. I. Brikman, Irkutsk Scientific Research Plague-Control Institute of Siberia and the Far East]

[Text] We investigated here the possibility of using the indicator of neutrophil damage (IND) and leukocytolytic reaction for demonstration of bacterial allergy in the course of the tularemia vaccinal and infectious process, as well as determined the correlation between IND, lysis of leukocytes and results of serological tests. We ran the specific leukocytolysis reaction and IND using a modification of the Fradkin method; antibody titer was determined by the agglutination reaction (AR) and passive hemagglutination reaction (PHAR).

This study was conducted on 20 chinchilla rabbits, 12 of which were infected subcutaneously with a highly virulent strain of *F. tularensis* (1000 bacteria/ml) and 8 were immunized with *F. tularensis* vaccine strain No 15 (2000 bacteria/ml). In addition to rabbits, we used 8 guinea pigs immunized with *F. tularensis* No 15 (1000 bacteria), in whose blood we only determined the leukocytolysis reaction. IND and leukocytolysis were also determined in 7 people immunized against tularemia at different postvaccinal times--10, 5, 2 years and 2 months--and in 5 people who had not suffered from tularemia and had not been immunized against it. Blood was taken from animals on the 3d, 7th, 15th, 21st, 28th, 42d, 54th and 120th postinfection and postvaccination days.

It was determined that leukocyte lysis and IND were positive already on the 3d day. The leukocytolysis indicator constituted an average of 21% and IND was 0.123 in infected animals; the mean figures for immunized ones were 20% and 0.13, respectively. Highest parameters of these reactions were noted in the 3d-4th week: leukocytolysis 34-40%, IND 0.23 in infected animals, 32-42% and 0.18-0.20 in immunized ones. At later stages (35th, 42d, 54th and 120th days), when the serological parameters dropped drastically or were negative, the allergic indicators in vitro remained stable: leukocytolysis 30%, IND 0.18.

Neutrophil alteration was referable primarily to first grade, with the exception of infected animals, in whom we observed second grade damage on the 21st-35th day and occasionally even third grade.

Guinea pigs presented the same pattern of leukocytolysis as rabbits immunized against tularemia.

Among the people immunized against tularemia, the highest percentage of leukocytolysis (44-50) was found 1-2 months after vaccination. In the other subjects, lyzed leukocytes ranged from 25 to 40%. In the five individuals who were not immunized against tularemia and not been in contact with this infection, the quantity of leukocytes in the experiment and control, where specific allergen was replaced with saline, was the same, i.e., leukocytolysis constituted zero percent.

When we tested the blood of rabbits immunized against tularemia with brucellin and pestin, as well as blood of intact animals with tularin, we obtained negative results.

Thus, dynamic observation of immunoallergic parameters in the course of the infectious and vaccinal processes referable to tularemia was indicative of existence of allergic alteration in experimental animals, which was demonstrable from the 3d day and reached a maximum on the 21st-28th day.

The results of these studies revealed that the IND and leukocytolysis reaction are highly specific for tularemia, as well as that they can be used to assess specific sensitization in the course of the infectious process and with immunization.

As a rule the parameters of leukocytolysis and IND were correlated and they verified one another. The advantage of these tests is that they are performed without introduction of specific allergen, which makes it possible to perform them repeatedly and assess the reaction several hours after taking blood, rather than after 2 days, as is the case when allergen is applied on the skin. These methods can be used well for studying the allergic status of experimental animals. It is very difficult to conduct skin and intracutaneous tests on animals, and they do not always yield positive answers. Moreover, the skin test for allergy is qualitative, whereas allergic alteration of the organism is evaluated quantitatively. Of course, allergy skin tests have their advantages, the main one being the simplicity of performing and evaluating them. For this reason, the method we recommend does not presume to replace conventional methods of testing for allergy with reference to tularemia; however, it can be recommended as an option.

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ACHIEVEMENTS IN THE CONTROL OF THE MOST IMPORTANT INFECTIOUS DISEASES IN
ARMENIAN SSR IN THE YEARS OF SOVIET POWER (1920-1980)

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[Article by K. M. Dekhtsunyan, Armenian Scientific Research Institute of
Epidemiology, Virology and Medical Parasitology imeni Aleksanyan and Yerevan
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[Text] There was no epidemiological service as such in prerevolutionary
Armenia. Economic backwardness, poor sanitation, lack of amenities in
settlements, extremely unsatisfactory medical care, to which were added such
social upheavals as war, mass migration of people, starvation, poverty, were
instrumental in onset of epidemics of malaria, cholera, plague, typhus and
relapsing fever, childhood infections and many other diseases. Epidemic
diseases reached particularly significant proportions during the period of
domination of the Union of Action [Armenian nationalist party] (1918-1920),
when the population was literally becoming extinct.

From the very first days of establishment of Soviet power in Armenia, this
republic's public health agencies concentrated on development of measures
against epidemic diseases, and this presented major difficulties due to the
lack of qualified epidemiologists, bacteriologists, infectious disease special-
ists, health inspectors [physicians] and other specialists.

In 1923, on the basis of a decision of the republic's council of people's
commissars with the help of Russian scientists (V. I. Kedrovskiy, K. I.
Skryabin, Ye. N. Pavlovskiy, P. P. Popov, N. P. Popov and others), the first
scientific research institution in this republic's public health system was
organized--the Tropical Institute, whose staff implemented some rather
valuable steps for the study and organization of control of both malaria and
other parasitic diseases. Then, within a relatively short time, an abundant
network of practical malaria-control institutions (stations, dispensaries,
centers) was developed, which did much work for the control of malaria and
prevention of this disease in this republic.

In the course of comprehensive investigation of epidemiology, symptomatology,
therapy and prevention of malaria, the institute's scientists in collaboration
with clinical physicians analyzed and summarized some valuable data, which

served as the basis for working out scientifically validated sets of malaria-control measures. As a result of their implementation, by 1953 tropical malaria was eradicated, whereas other forms of malaria were eliminated from the republic in 1963 [2, 11, 12, 16, 28, 29, 34].

At the present time, in order to reinforce the advances made in the control of malaria, this republic's scientists are concerned with problems of prevention, since the possibility cannot be ruled out of penetration of infection from foreign countries where there is a malaria problem.

At the same time, studies were pursued of epidemiology, symptomatology and ecology of carriers of leishmaniasis and phlebotomus fever, which made it possible to organize effective control of these diseases, leading to their eradication [17, 26, 35]. As a result of an in-depth study of protozoan intestinal infections in this republic, many unclear aspects of their epidemiology, pathogenesis, symptomatology and laboratory identification were elucidated; a set of measures was elaborated, introduction of which lowered morbidity and incidence of the diseases among the public [8, 21, 30, 32]. However, there are still some unanswered questions in this area, so that further studies are needed. In particular, it is interesting to determine the incidence of disease in different geographic zones of this republic, epidemic significance of different forms of pathogens, causes of the phenomenon of inconsistency between carriers and morbidity, etc.

Studies were also conducted in the area of helminthic invasions, which showed that there is a diversified helminth fauna in Armenia (20 species of parasitic nematodes). Such helminths as *Trichostrongyles axici* (1928) and *Trichostrongyles skrjabini* Kalantarian (1932) were discovered for the first time in the world. Thanks to the active work of helminthologists in the epidemic-control service of this republic, it was possible to lower drastically invasiveness of the public within a historically short time. Thus, while ascariasis and trichuriasis struck virtually everyone (98-99.5%) in the early years of establishment of Soviet power, at the present time these indicators dropped to 1/10th-1/14th the former level. A galaxy of scientists developed in this republic, which became the worthy successors of the departed older generation of scientists and are presently working fruitfully in the area of helminthology. The main direction of scientific research is immunodiagnostics of a number of helminthiases (echinococcosis, toxoplasmosis and others).

In 1930 the Institute of Sanitation and Hygiene was organized and in 1936 it was renamed as the Sanitary and Bacteriological Institute, and somewhat later the Institute of Epidemiology and Hygiene. This institute has performed enormous work in the area of investigation of regional infectious pathology--diphtheria, typhoid fever, dysentery, typhus, brucellosis, endemic diseases, as well as problems of sanitation and hygiene.

In accordance with a decision made by directive agencies (following the example of RSFSR and UkSSR), major work started for the first time in Armenia in 1935-1936 to organize sanitary and epidemiological stations in this republic's cities and regions, which are well-supplied with specially equipped laboratories for epidemiological, bacteriological, hygienic and other studies. Constant concern was displayed about supplying these stations with specialists,

which was aided by organization of the health [sanitation] and hygiene faculty at the Yerevan Medical Institute in 1930.

Particularly great advances were made in the study of diphtheria. Thanks to the efforts of Prof A. B. Aleksanyan, academician of the USSR Academy of Medical Sciences [3, 4], who spent decades on scientifically validated control of this infection and implemented measures to prevent it, by 1970 diphtheria was eradicated in this republic. A. B. Aleksanyan proposed an intensive method of preventive immunization against this infection, which played a major role in eradicating it from the USSR.

Much work was done in Armenia to control smallpox. Thus, while one frequently encountered localized outbreaks of smallpox in many settlements in the early years of establishment of Soviet power, later on, as a result of mass vaccination and other measures, there were only sporadic cases of this infection. The task of total eradication of smallpox in this republic was completed by 1935.

In this republic, systematic studies are pursued on the prevention of plague (although the last cases of this disease were recorded in 1920 and 1921). In 1943, a plague-control station was created in this republic, which conducts epidemiological, bacteriological, entomological and epizootiological studies in this aspect. The results of these studies revealed that *Y. pestis* persists in highland regions of the republic in common voles and fleas. This finding is of great epidemiological significance, and it must be taken into consideration when organizing and implementing targeted measures against rodent plague.

After discovery of the pathogen of tularemia in the USSR, this infection began to attract the attention of Armenian public health agencies. In subsequent years, rather large outbreaks of tularemia were noted in different parts of this republic, for which reason a study was begun of the role of different rodent species and bloodsucking arthropodes (ticks, fleas, etc.) and their biology, as well as the role of sheep and crabs as new sources of tularemia infection. A comprehensive study of epizootiological and epidemiological situations in different localities made it possible to type the tularemia sites in Armenia, work out necessary measures and use them with success [15].

Scientific research on the problem of intestinal infections is conducted in this republic mainly with local material, so that it permits solving specific epidemiological problems.

Extensive studies were made of the carrier state with reference to typhoid-paratyphoid fever, duration thereof and epidemiologically dangerous types. Studies of epidemiology of typhoid fever and paratyphoid fever in Armenia made it possible to define and clarify several important factors, which made it easier to develop and implement effective preventive and therapeutic measures [5, 23]. It was determined that 19 phagotypes of *S. typhi* circulate in Armenia, among which there is prevalence of F, A, B and others [10, 25]. The results of studying *S. paratyphi* cultures revealed that they are referable to seven phagotypes, among which there is prevalence of the Taunton phagotype.

Extensive investigations were conducted of reactogenicity and immunological efficacy of some typhoid vaccines. The results made it possible to rate Soviet vaccine as a product that conforms with the best international standard with regard to main properties and efficacy. By virtue of scientifically validated epidemic-control measures, typhoid and paratyphoid fever is currently recorded in this republic in the form of sporadic cases.

In this republic, numerous studies have been made of epidemiology of dysentery and dysentery-like diseases. As far back as 1930, it was proven on concrete examples that the water factor, whose significance was denied in those years by many researchers, plays a major role in onset and spread of bacterial dysentery. Much attention was devoted to the study of chronic dysentery in children and effect of mountain-climate factors on its course [9], as well as the correlation between amebiasis and bacterial dysentery. The extensive experience in mass scale phage prophylaxis in dysentery sites was summarized, and its beneficial epidemiological effect was demonstrated. Etiological distinctions of dysentery were studied in detail [24].

In recent years, there has been a drastic decline in share of dysentery cases among all intestinal diseases. There has also been a drastic decline in percentage of laboratory-confirmed cases. This is attributable mainly to the significant changes in etiological structure of dysentery.

In 1965, work was started in this republic on the study of episomal heredity of enterobacteria, in particular, colicinogenicity. Studies were made of the incidence of colicinogenicity and colicin sensitivity among *Salmonella* and *Escherichia* strains [18].

The scientists of the Institute of Epidemiology and Hygiene proved that children suffer from colienteritis due to pathogenic serotypes of *E. coli* 055, 026 and 0111. The composition of H antigens of pathogenic serovars of *E. coli* circulating in Yerevan was studied and identified. This work was of great importance to diagnosis, treatment and prevention of this disease in infants [18, 31].

Prevention of intestinal infections requires a set of measures that are implemented in this republic not only by public health agencies, but all concerned economic, cultural and public organizations.

As a result of studying the epidemiology of brucellosis, determination was made of the distinctions of this infection in different geographic zones of the republic [20, 27, 33]. It was established that small and large cattle are the main source of infection. Infection occurs the most often via the alimentary and contact routes. Investigation of territorial distinctions of incidence of brucellosis in the epizootiological and epidemiological aspects made it possible to type the sites, which facilitated the use of a differentiated approach to elaboration of preventive measures. Introduction of the latter resulted in the fact that only isolated new cases of the disease are currently observed.

S. V. Rostomyan studied leptospirosis in man because of its wide distribution in this republic since 1959 among livestock. It has been determined that

the main source of *Leptospira* infection of humans in this republic is cattle. It was proven that the etiological structure of leptospirosis in man and domestic animals is identical, and the geographic distribution of different serovars was determined. For the first time, endemic leptospirosis sites were also discovered, where the common vole and striped field-mouse are harborers of the pathogen.

Numerous studies were concerned with relapsing fever and typhus [15, 18], as well as rat-bite fever [sodoku]. Sites of Q fever were found in different climate and geographic regions of this republic; the sites were typed and scientifically validated measures were worked out for control and prevention thereof.

As a result of studying epidemiological and immunological patterns of measles in Armenia SSR, in the prevaccinal period and that of mass scale immunization, the epidemiological efficacy, reactogenicity and immunogenicity of different vaccines produced in the USSR were determined, and live measles vaccine derived from strain L-16 was deemed the best. Mass inoculations have resulted in a drastic decline of measles, as well as lethality and mortality referable to this disease [13] with, however, persistence of its periodicity.

For several years, studies were conducted in this republic of epidemiology and specific prevention of pertussis and scarlet fever [6, 7]; some distinctions of the epidemic process associated with these infections were identified. It was concluded that ADTP and DTP vaccines are immunogenic, reactogenic and epidemiologically effective. In recent years, a comprehensive study was made of immunological and epidemiological forecasts of diphtheria and tetanus after reinoculation with lower doses of toxoids. The preliminary results confirmed the efficacy of booster [diminished] doses of toxoid [31].

The influenza problem is being worked on actively in Armenia. Determination was made of etiological structure of this infection, distinctions of outbreaks, epidemiological patterns of spread thereof in this republic, role of collective immunity in periodicity of outbreaks, epidemiological and immunological efficacy of various influenza vaccines [1, 10].

In recent years, studies have been made of the role of meningococcus in etiology of suppurative meningitis, nasopharyngitis, antigenic structure of meningococcus, as well as incidence of meningococcus carriers among the public.

Arboviruses started to be studied in Armenia in 1971. In this republic, Bhanja, Dhorl and KG viruses were isolated, and Bhanja was isolated in Armenia for the first time in the USSR [19, 22]. Viruses new to science, such as Razda-Artashat, were also isolated and studied [13]. In 1980, serological exploration was pursued to detect reservoirs of this virus in nature, among animals, as well as people.

By order from the Armenian Ministry of Health, a scientific research laboratory (NIL) for intramural suppurative-inflammatory diseases (SID) was organized in the department of epidemiology and medical parasitology of Yerevan Medical Institute (headed by Prof K. M. Dekhtsunyan), since these diseases occupy a significant place in infectious pathology and are very detrimental to public health; this laboratory has been functioning since 1976. In 1976-1980,

in-depth studies were made of the share of SID in different types of hospitals. In order to determine the etiological structure of SID in surgical and maternity institutions, patients, service personnel and environmental objects were submitted to epidemiological and microbiological examination, and it was established that pathogenic staphylococci were the cause of SID in 58.6% of the cases among surgical and maternity patients, Gram-negative microorganisms in 33.9%, mixed microflora in 7.5% and Streptococcus in isolated cases; in 85-90% of the cases, the isolated microflora was found to be resistant to the antibiotics widely used at the hospitals.

Mandatory registration and reporting of SID was introduced in medical and preventive institutions for the first time in this republic, at the initiative of the department and NIL staff, which was reflected in an order issued by the Armenian Public Health Minister (3 February 1977); a system was elaborated for registration of SID, which is instrumental in early detection of post-operative and postpartum complications.

Some interesting work has been done in the Department of Epidemiology (headed by Prof V. A. Aleksanyan) of the Yerevan Institute for Advanced Training of Physicians in the area of nosogeography of infectious diseases, listeriosis, etc.

This republic's epidemiologists celebrated a noteworthy date, the 60th anniversary of Soviet power in Armenia, with new achievements, introduction of which will no doubt aid in lowering the incidence of infectious and invasive diseases there.

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TOPOLOGY OF ENDEMIC TULAREMIA SITES IN KAZAKHSTAN AND CENTRAL ASIA

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[Article by O. A. Baytanayev, M. A. Aykimbayev and O. B. Chimirov, Central Asian Scientific Research Plague-Control Institute, USSR Ministry of Health, Alma-Ata]

[Text] The topology, or spatial structure, of endemic tularemia sites in southern and eastern parts of the USSR has not been studied thoroughly enough, and there is no special research on this subject. We have undertaken here an attempt to formulate an hierarchic classification of spatial categories of tularemia sites in Kazakhstan and Central Asia.

Material and Methods

Our material consisted of the results of our many-year (30 years) study of endemic tularemia sites. We used conventional methods [1, 3, 4, 8] and were governed by the suggestion of Olsuf'yev and Dobrokhotoy [6], to the effect that the system for spatial division of tularemia sites should include the following main parameters: 1) range of endemic sites (pathogen), 2) region of concentration, 3) province of concentration, 4) landform complex, 5) district or mesofocus, 6) local site [focus] or microfocus, 7) elementary site.

Results and Discussion

In Kazakhstan and Central Asia there are four types of endemic [enzootic] tularemia sites [1, 3, 4, 8]: backwater-swamp, foothill-stream, steppe and tugay landform-biocenotic. backwater-swamp sites of tularemia are distributed in Kazakhstan in the basins of central and lower reaches of Irtysh, Ishin, Tobol and Ubagan, Nura, Turgay, Ural, Big and Little Uzeney, Karatal, Sarysu and Kengir Rivers, as well as the Volga delta. They also include the tularemia sites along the shores of Alakol, Sasykkol and Usek Lakes. The foothill-stream sites of this infection are situated in central mountain areas of Dzhungarskiy and Zailiyskiy Alatau, Tarbagatay and Altay. They are near small streams and brooks originating from springs.

The only steppe site of tularemia is in the steppe zone of the left shore of the Transural area. Tugay [vegetation covered bottomland] tularemia sites

have been discovered in the desert zone of Kazakhstan and Central Asia, within the limits of tugay forests along the lower reaches of Ili, Chu, Amudarya Rivers and Syrdarya delta.

The present range of *Francisella tularemia* essentially covers the Holarctic region and in Eurasia it has an independent region of concentration, which is subdivided into provinces [3, 6]. Endemic sites of this infection in Kazakhstan and Central Asia are contained in the East European, West Siberian-North Kazakhstan and Central Asian concentration provinces.

The first province within the limits of Kazakhstan contains only backwater-swamp sites of *tularemia* in the basin of the Ural River and Volga delta.

In the Central Asian province, a distinction is made of three landform complexes: backwater-swamp, foothill-stream and tugay [6]. Yet, on the basis of topological analysis combined with our findings from an epizootiological inspection of the republic's territory, the above province could be divided into three subprovinces: Central Kazakhstan, Southern Kazakhstan-Central Asian and Southeastern Kazakhstan. In distinguishing the subprovinces, we took into consideration the typology of the sites in them, degree of their uniqueness, autonomy and relative definition of boundaries. For example, the boundary between the West Siberian-North Kazakhstan and Central Asian provinces (Central Kazakhstan subprovince) passes along the interfluvium of the Nura and Sarysu Rivers. The arid Betpak-Dala desert lies between the Central Kazakhstan and Southern Kazakhstan-Central Asian subprovinces.

We included in the Central Kazakhstan subprovince the landform complex of stagnant backwater-swamp sites of *F. tularensis* in the basins of Sarysu and Kengir Rivers.* The South Kazakhstan-Central Asian subprovince contains the tugay type and, finally, the Southeastern Kazakhstan subprovince contains backwater-swamp and foothill-stream landform complex types of sites.

Olsuf'yev and Dobrokhotoy [6] use the term endemic district to refer to individual, relatively large parts of the landform complex that are relatively isolated from one another and consist of a system of local sites. Thus, the Volga (delta) and Ural districts of concentration are contained in the East European province in the limits of Kazakhstan. We distinguished the following six districts of endemicity in the West Siberia-North Kazakhstan province, which are outlined by the hydrogeographic network along the basins of the following rivers: Turgay, Tobol, Ubagan, Nurina, Ishim and Irtysh. In the Central Kazakhstan and Southeastern Kazakhstan subprovinces of the Central Asian province, a distinction is made of the Sarysu-Kengir, Alakol-Sasykkol and Usek districts of endemic *tularemia* sites. In addition, in the last subprovince mentioned, within the limits of the foothill-stream landform complex there are the following endemic districts: Zailiyskiy, Dzhungara, Tarbagay [5] and South Altay. The South Kazakhstan-Central Asian subprovince (according to the data of Olsuf'yev and Dobrokhotoy [6], as well as our own) is divided into the following endemic districts: Syrdarya, Chu, Ili and Amudarya.

*Thus far this microorganism has not been found there, however we know of one human case of *tularemia* (Sarysu) and specific antigens were found in myophagous bird pellets [2, 7].

As we know, local tularemia sites are detected by means of many years of permanent epizootiological observations and they include the smallest endemic site unit that is relatively persistent in time. Tularemia epizootics are found there for many years. In most of the endemic tularemia sites we discovered in Kazakhstan on the basis of epizootics there, the local sites were not the subject of our comprehensive investigation. However, we noticed that, in a number of instances, the boundaries of a local site could coincide with the boundary of the endemic site, which we observed in the tugay site in the lower reaches of the Chu River (between the villages of Furmanovka and Karabugut), where the intrazonal habitats of background mammalian species--Tolay hare [*L. capensis* L.], tamarisk gerbil and house mouse--were the area of epizootics that occurred among them.

The next objective of investigating the topology of endemic tularemia sites in Kazakhstan and Central Asia should be to define the localization of *F. tularensis* in space (mapping local sites of infection), as well as detailed epidemiological zoning of the sites, which are needed to succeed in lowering the incidence of this infection.

Conclusion

Topologically, the Central Asian province of tularemia sites is divided into the Central Kazakhstan, Central Asian-Southern Kazakhstan and South-eastern Kazakhstan subprovinces, in which 18 site districts were singled out.

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FURTHER INVESTIGATION OF INTRASPECIFIC TAXONOMY OF FRANCISELLA TULARENSIS

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[Article by N. G. Olsuf'yev and I. S. Meshcheryakova, Scientific Research Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] Soviet researchers [1, 9, 12] have elaborated the intraspecific taxonomy of *F. tularensis*, which is comprised of three subspecies or geographic races: Holarctic (*Francisella tularensis* subsp. *holarctica* Ols.), which is widespread in Europe, Asia and North America; Nearctic (*F. tularensis* subsp. *nearctica* Ols.) inherent in North America, and Central Asian (*F. tularensis* subsp. *mediaasiatica* Aikimb.) encountered only in Central Asia. The Holarctic subspecies includes the Japanese variety or, more correctly, biovar (biovar *japonica* Rod.) which is distributed on the Japanese islands. These taxons can be well-distinguished, not only by their ranges, but certain biochemical properties, for example, ability to ferment glycerin and citrulline, whereas the Nearctic subspecies is also distinct from others in that it is highly pathogenic for domestic rabbits and man. Other finer biological differences have also been discovered between the different subspecies, but serological differences between them have not been established. In recent years, differences were determined between strains of the Holarctic species in their sensitivity to antibiotics of the macrolide group and lincomycin [2, 4-6, 17, 23]. These differences are particularly graphic with regard to erythromycin, and according to this property we [6, 10] singled out 2 biovars (biotypes): erythromycin-sensitive (biovar I or ery^S) and erythromycin-resistant (biovar II ery^R). The former is distributed in Eurasia and America, the latter only in some parts of Eurasia [6, 10].

The proposed intraspecific taxonomy of *F. tularensis* raised no objections on the part of Soviet researchers, and it is also used to some extent or other in foreign publications. Thus, American researchers [19, 21] consider it proven that the two forms of *F. tularensis* we described--Nearctic and Holarctic--which differ distinctly in virulence and ecology, are distributed in North America. Jellison and Bell prefer to designate them with the letters A and B, but in his latest publication [22] Jellison also mentions our classification of intraspecific taxons. In the 8th edition of the Bergey manual [18], both forms are included and described as varieties of *F. tularemia*.

At the suggestion of Dr Mair (N. Mair, London), chairman of the Subcommittee for Taxonomy of *Pasteurella*, *Yersinia* and *Francisella* of the International Committee for Bacterial Systematics, in 1975 we sent strains of the three subspecies to Dr Karlsson in Sweden (K. A. Karlsson, State Institute of Veterinary Medicine, Stockholm) and to Prof Jamanaka in Japan (M. Jamanaka, Medical College, Osaka). Both researchers in their written conclusion evaluated these forms as biochemical varieties and noted that they can be differentiated by the traits we mentioned, but not serologically, which coincides with our data. But both researchers failed to mention in their conclusion any appreciable differences in the Nearctic subspecies with regard to its high virulence, nor clearcut differences between different taxons according to their geographic distribution (range). But a conclusion we recently received from the above-mentioned subcommittee (letter from Dr G. Wauters, secretary, dated 29 December 1979) is in overt contradiction to all of the foregoing; it stated that "in the opinion of the Subcommittee, the *F. tularensis* species is quite homogeneous, and the geographic, pathological and biochemical distinctions of the strains are not sufficient to establish intraspecific taxons."

Through the kindness of Dr J. Bell (Rocky Mountain Laboratory, Hamilton, United States), in 1975-1976 we received a collection of American strains, for which we are grateful. In all, he sent 6 Nearctic and 13 Holarctic strains. This enabled us to study them in greater detail as compared to numerous newly obtained Eurasian strains, including those isolated in the USSR. The purpose of our study was to determine the validity of intraspecific taxonomy of *F. tularensis* described above. We previously had the opportunity to study only 6 American strains, including 4 Nearctic and 2 Holarctic ones [11, 12]. The study we describe below is limited to strains of the Nearctic and Holarctic subspecies, but not the Central Asian, which had been studied previously quite comprehensively on a large number of strains [1, 7, 16]. We do take into consideration the data on properties of this subspecies in our discussion.

Material and Methods

We studied 174 strains of *F. tularensis*. In addition to the above-mentioned 19 American strains, we studied 155 Holarctic strains, including those isolated in the USSR (123), France (6), Yugoslavia (1), Sweden (2), Poland (19), Japan (3) and Mongolia (1). We express our thanks for sending the strains to Prof Mollare (France), Prof Borchich (Yugoslavia), Dr Karlsson (Sweden), Dr Dombrovski (Poland) and Dr Ohara (Japan), as well as to staff members of Soviet institutions-- departments of particularly dangerous infections at the Moscow, Ivanovo, Yaroslavl, Ryazan, Kalinin, Gorkiy, Novgorod and Bryansk Oblast sanitary and epidemiological stations and the Irkutsk Plague-Control Institute. We isolated 51 strains from common voles delivered to us in recent years from Moscow Oblast. Like other researchers, we recovered most strains by means of passage of material through biotest animals, but we succeeded in isolating 11 strains by direct culture on nutrient media in studies of organs of dead common voles.

Among the strains studied, 8 were isolated from sick people (1 strain by direct culture from a skin ulcer), 104 from rodents, 12 from hares, 12 from Ixodes ticks, 31 from water, 2 from birds and 5 from other objects. The strains were examined in the following tests: growth and morphology of cells on McCoy coagulated yolk medium and Yemel'yanova blood agar, fermentation of glycerin

on Downs medium and citrulline by the method of Rodionova [15], agglutinability in the agglutination reaction with rabbit or commercial horse antiserum, sensitivity to erythromycin by application of a disc with antibiotic on medium with thick culture of a strain. We tested pathogenicity (according to lethality) for white mice and guinea pigs of hypodermic injection of doses of 1 and 10 bacterial cells according to the bacterial standard of cloudiness of the State Control Institute of Serum and Vaccines imeni Tarasevich and by the sampling method on domestic rabbits. In the latter case, rabbits were given hypodermic injections of 1 and 10 bacterial cells of a Nearctic strain or 10 and 100 million of a Holarctic strain. The mice and guinea pigs were usually infected using three animals per dose and rabbits, two per dose. The animals were followed up for 30 days. We did not test the pathogenicity of the strains from Poland in view of their prolonged storage at the museum. However, two of them (ZgS and LM), which had been sent earlier by Prof Skrodzkiy, were tested for virulence and they were highly pathogenic for white mice in low doses, but did not cause death of rabbits in doses of 10 million to 1 billion bacterial cells injected subcutaneously [4].

Results and Discussion

The Table lists the main results of our study. Let us mention the complete coincidence of the new data to those we obtained earlier. Strains of Eurasian origin, as well as American strains that we referred to the Holarctic subspecies, were moderately pathogenic for rabbits (the complete lethal dose with hypodermic injection was above 100 million bacterial cells) and highly pathogenic for white mice and guinea pigs (completely lethal dose 1 and 10 bacterial cells); none of the strains fermented glycerin and citrulline. We should stress the complete identity of the American and Eurasian Holarctic strains with regard to the main biological properties. In contrast, six nearctic strains, including five from the United States and one from Canada, differed distinctly from the preceding ones in that they had the same high pathogenicity for rabbits, guinea pigs and white mice. The completely lethal dose with hypodermic injection constituted 1-10 bacterial cells for any of the above-mentioned animal species; all of the strains fermented glycerin and citrulline. The three Japanese strains, like those studied previously [13, 24], failed to demonstrate fermentation of glycerin on Downs medium, but it was demonstrated by the Rodionova method [14]. We were impressed by the stability of the glycerin trait in some taxons. Previously, in a study of 674 strains isolated in different regions of the USSR, from the Baltic in the west to eastern Siberia in the east, fermentation of glycerin was never demonstrated, whereas a sampling check of virulence showed that the strains were quite pathogenic for white mice and guinea pigs [3]. Studies of more than 60 strains of the Central Asian subspecies demonstrated fermentation of glycerin in all cases [1, 7].

All 174 strains we studied for the first time presented no appreciable differences in cultural and morphological properties, regardless of their subspecies, when cultivated on coagulated yolk medium or blood agar of Yemel'yanova. The strains also failed to differ in serological activity. They were distinctly agglutinated, with the Vi type of agglutination, by rabbit and horse antisera to a titer of 1:1280-1:5120. The only exception was a dissociated strain from Poland, which was agglutinated to a titer of 1:160 with O-agglutination type.

Biological properties of strains of *F. tularensis* of Holarctic and Nearctic subspecies from different countries and oblasts

Subspecies	Countries, oblasts Quantity of strains given in parentheses (174 strains in all)	Years isolated	Source of isolation and quantity of strains	Fermentation of glycerin	Ferment of citronelline	Sensitivity to erythro- mycin*	Agglutination - lity in ag- glutination	Pathogenicity for		
								white mice	guinea pigs	domes- tic rabbits
Holarctic	USSR (123)	1975-1981	Man--1 (direct culture) Common voles & their nests-- 73 (11--direct culture from voles), other rodents--9, shrew--1, ixodes ticks--1, water--31 and others	—	—	S-74 R-49	1:1280 1:5120	High	High	Mod- erate
	Moscow Oblast (68)									
	Gorkiy Oblast (2)									
	Ivanovo Obl. (15)									
	Yaroslavl Obl. (13)									
	Ryazan Obl. (2)									
	Novgorod Obl. (15)									
	Kalinin Obl. (1)									
	Bryansk Obl. (2)									
	Taymyr (1)									
Holarctic	Yakutsk ASSR (1)	1976-1980	Siberian & Arctic lemmings, muskrat--2, root vole	—	—	S	1:1280 1:2560	"	"	Same
	Kamchatka (3)									
	France (6)	1974-1975	Hares--2, common field- mice--2, Ixodes ticks--2	—	—	S	1:1280	"	"	"
	Yugoslavia (1)	1978	Ixodes ticks	—	—	R	1:2560	"	"	"
	Sweden (2)	1974	Hare, owl	—	—	S	1:1280	"	"	"
	Poland (19)	1952-1958	Man--2, hares--8, common voles--4, root vole--1, Ixodes ticks--4	—	—	S-2	1:2560	"	"	"
						R-17	1:5120 1 strain			
	Japan (3)	1973-1975	Man	+	—	S	1:160 1:1280	"	High (low in 1 strain)	"
	Mongolia (1)	1975	Long-tailed suslik	—	—	S	1:2560	"	"	"
	United States (13)	1941-1976	Muskrat--5, other rodents-- 4, Ixodes ticks--3, hazel hen--1	—	—	S	1:1280 1:2560	"	High (low in 1 strain)	"
Nearctic	United States (5)	1926-1972	Man (B399A Cole), hare (Nevalla 14), green lace- wings (B399 E 261), man (O-402), unknown (O-419)	+	+	S	1:1280 1:2560	High	High	High
	Canada (1)	1935	Hemaphysalis ticks (O-328)	+	+	S	1:2560	"	"	"

Key: S) erythromycin-sensitive strains

R) erythromycin-resistant strains

We should mention the relative homogeneity of strains with regard to high pathogenicity for white mice and guinea pigs. But we detected diminished virulence for guinea pigs with high virulence for white mice in two holarctic strains. Thus, all 6 animals survived after infection with doses of 1 and 10 bacterial cells of the Japanese Ischijo strain, which was isolated from a person in 1973; 4 out of 6 animals survived after being given the same doses of American strain 425M, which was isolated in 1941 from *Dermacentor andersoni* ticks. Immunity was formed in the surviving guinea pigs. Perhaps, the diminished virulence of these strains for guinea pigs was not always present, and partial attenuation occurred during storage under laboratory conditions for several years. We failed to demonstrate differences either in pathogenicity for laboratory animals or other properties between strains isolated by direct culture or with the biotest.

All of the Nearctic strains were sensitive to erythromycine, but the Holarctic strains were represented by two biovars for this trait: I and II. When biovar I strains were cultured, a transparent zone (absence of growth) 2-3 cm in diameter was demonstrable around the disc with antibiotic; when biovar II strains were cultured this zone was absent. Both biovars have been found among strains from central regions of European SSR, with prevalence of biovar I. Strains from northern and eastern Siberia, the Far East, Sweden, France, Mongolia and the United States are also referable to this biovar. Virtually all of the strains from Poland and one from Yugoslavia are referable to the second biovar, and this was demonstrated for the first time. The new information obtained about the geographic distribution of both biovars enlarge upon previously published data and generally coincide with them [5, 6, 10]. We should merely mention that the trait of sensitivity to erythromycin is rather stable in the population of *F. tularensis* subspecies, and it is valid for intraspecific taxonomy below the subspecies level or as an addition to its characteristics.

Thus, the results of our new study of a considerable number of *F. tularensis* strains of Eurasian and American origin, together with data in the literature, including information about Central Asian strains, confirm the heterogeneity of the species with respect to some biological properties, which justifies intraspecific taxonomy. In biosystematics, including systematics of bacteria, singling out a given taxon is based on any stable traits (properties) that distinguish it from similar taxons. The justification for distinguishing subspecies of *F. tularensis*--Holarctic, Nearctic and Central Asia--is that they have several very distinct and stable distinguishing features--biochemical, pathogenic and geographic. The latter trait is apparently attributable to evolution of *F. tularensis* as a pathogen with strictly obligate localization in sites corresponding to different landforms of different continents [8]. We were impressed by the absence of transitional (intermediate) forms (strains) between subspecies in their distribution over the same territories, for example, Nearctic and Holarctic subspecies in the United States. This could serve as an indication of a high, genetically fixed level of differences in traits (biological isolation) between subspecies, which comes close to the species level. We cannot rule out the possibility that additional traits will be found in the future, which distinguish the subspecies, or that new subspecies will be discovered. We cannot concur with the above-cited opinion of the Subcommittee for Taxonomy that demonstration of biochemical, pathogenic and geographic differences is not enough for intraspecific taxonomy of *F. tularensis*.

and that this species should be considered homogeneous. The absence of serological differences between subspecies, which is apparently what confuses the members of the Subcommittee, cannot serve as an obstacle to determination of subspecies taxons. In this case, the other traits are sufficient and use thereof, as stated above, is quite valid from the standpoint of modern biosystematics. An analogous situation prevails in intraspecific taxonomy of *Y. pestis*, in which taxons of the subspecies rank, which are quite real according to a number of properties, cannot be differentiated serologically.

As for *F. tularensis* biovars, for the time being they have been found only in the population of the Holarctic subspecies and singled out on the basis of a few distinguishing traits--Japanese biovar according to fermentation of glycerin and range, biovars I and II according to sensitivity to erythromycin, other macrolides and, in part, ranges. We cannot rule out the possibility of new biovars being discovered in the future.

Conclusions

1. The results of a study of 174 strains of *F. tularensis* isolated in a number of countries of the Old and New World, including 123 strains in the USSR, make it possible to distinguish intraspecific taxons of this pathogen referable to 3 subspecies: Holarctic, Nearctic and Central Asian, and within the Holarctic species 3 biovars: Japanese, I ery^S and II ery^R.
2. The subspecies differ in several stable traits, including biochemical and pathogenic properties, as well as ranges, whereas biovars differ in some single trait and, in part, range.
3. The North American strains of the Holarctic subspecies do not differ from biovar I of Eurasian Holarctic strains.
4. The absence of serological differences between different taxons is not an obstacle to intraspecific taxonomy of *F. tularensis*.

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AN ENDEMIC TULAREMIA SITE ON KERCH PENINSULA (CRIMEA)

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[Article by G. M. Golkovskiy, G. F. Mitsevich, A. B. Khaytovich, Ye. V. Alekseyev, P. G. Korchevskiy, V. A. Korchevskaya, M. I. Mikheykin, M. K. Petrova, A. P. Kolotygina, R. S. Ayzendorf, O. P. Zhmurova, Ye. S. Limonov and S. K. Andreyeva, Crimean Plague-Control Station, USSR Ministry of Health, and Crimean Oblast Sanitary and Epidemiological Station, Simferopol]

[Text] An outbreak of tularemia was recorded for the first time on Kerch Peninsula in the Fall-winter period of 1951-1952, and it involved primarily the rural population [7]. The diagnosis was based on clinical manifestations, positive serological reactions and allergy tests. Bacteriological confirmation could not be obtained. In an epizootiological survey, which was made during the period of the outbreak, as well as subsequent studies over the next 20 years, which included bacteriological examination using biological tests (on white mice) with infected suspensions prepared previously from organs of small mammals and their ectoparasites, failed to demonstrate *F. tularensis*. Sporadic cases of tularemia were observed up to 1955.

In 1974, the method of detection of antigen of *F. tularensis* in predatory bird pellets by means of erythrocytic diagnosticums, using the passive hemagglutination reaction (PHAR) and antibody neutralization (ANR), was first used in Crimea. Of the 1495 pellets examined, which had been collected in 1974 (spring) to 1978 in the southern part of the spit of the Arabat point [projection] and adjacent regions of Kerch Peninsula, 9.2% were positive. Annual findings served as grounds to assume that *F. tularensis* circulated on the territory of Kerch Peninsula.

Several researchers have noted the uniqueness of soil, climate, geobotanical and entomological features of Kerch Peninsula, distinguishing it as an independent geographic rank--Eastern Crimea. The mammals encountered there include the European hare, social and common vole, house and common field-mouse, common white-toothed shrew and others. It has been reported that this territory is inhabited by 23 species of *Ixodes* ticks [4] and 49 species of gamasid ticks, 39 flea species [5], 15 species of bloodsucking mosquitoes [2] and 5 species of gadflies [3]. Consequently, conditions exist on the territory of this peninsula for circulation of the pathogen of tularemia infection.

Our objective here was to establish the existence of an endemic site for tularemia in the territory of Kerch Peninsula in Crimea, by means of isolating the pathogen, and the expediency of preventive measures.

Material and Methods

Special studies were conducted to confirm the foregoing, and they included bacteriological examination of small vertebrate animals and their ectoparasites, water from superficial reservoirs, together with serological testing of clots of blood from the heart. The main studies were pursued directly at the sites of collection of field material and they were completed at a stationary laboratory base. In some cases, animal carcasses and organs were transported for a long time at a temperature of about 0°C, which preserved the pathogen [1].

Results and Discussion

In the fall 1978 period of our work, we examined 624 mammals (189 social voles, 130 house mice, 225 common field-mice, 4 gray hamsters, 76 common white-toothed shrews), 468 ectoparasites collected from small vertebrates (186 fleas, 128 Ixodes and 154 gamasid ticks) and 114 water samples taken from reservoirs. We isolated 28 strains of *F. tularensis* from the organs of mammals by means of the biological test, including 10 from organs of social voles, 6 each from house mice and common field-mice, 4 from common shrews and 2 from gray hamsters. Nine cultures were isolated from ectoparasites (5 from Ixodes ticks, 2 each from fleas and gamasid ticks). In addition, four strains were isolated from specimens collected from environmental objects.

In all cases, the cultures of *F. tularensis* were obtained as a result of biological tests on white mice. The animals died of tularemia at different times: on the 2d day (1 case), 6th (2), 7th (5), 8th (2), 9th (1), 11th (3), 12th (1) and 15th (1) days. In addition, cultures were isolated through biotests on animals sacrificed on the 6th day--1, 12th--21, 15th--2 and 16th--1. Most cultures (25) were isolated in the first passage and the others in the 2d-3d passage. In some cases, numerous passages were required due to the presence of mixed infection (the mammals were stricken with listeriosis, pasteurellosis and salmonellosis). The isolated cultures were identified according to cell morphology, agglutination with tularemia serum in a reactive titer, absence of growth on ordinary nutrient media and presence thereof on McCoy and Antsiferov media, specific fluorescence in immunofluorescence tests and positive agglutination reaction in the antibody neutralization test.

Examination of washings from 624 blood clots using the antibody neutralization reaction revealed positive results in 0.6% of the cases, which is indicative of possible survival of pathogens. However, it is not expedient to examine clots for diagnostic purposes, since the possibility of circulation of the pathogen is minimal.

Thus, the results of these studies confirmed the assumption that the territory of Kerch Peninsula is enzootic, and *F. tularensis* was isolated there for the first time. A total of 14 enzootic sites were found located over a territory with an area of 1620 m². In view of the environmental distinctions of that locality, the described tularemia site should be identified as the steppe type in the classification of Olsuf'yev [6].

Evidently, the failure to isolate the pathogen during the epizootiological inspections in previous years is related to the fact that the collected material was stored at ambient temperature in a preservative based on glycerin. Probably, the preservative and ambient temperature did not assure viability and retention of virulence by *F. tularensis* in the climate of Crimea.

It should be noted that the rural population groups of Kerch Peninsula for whom there is a risk of tularemia are inoculated against this disease. This apparently explains the absence of the disease in humans.

Conclusions

1. It was proven that there is an enzootic tularemia site of the steppe type on the territory of Kerch Peninsula.
2. One should use as a preservative factor a temperature of about 0°C or infect animals at the site of collection of specimens to preserve *F. tularensis* when material (trapped mammals and collected ectoparasites) is transported.

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RESULTS OF MANY YEARS OF STUDIES OF ENDEMIC BACKWATER-SWAMP TYPE TULAREMIA SITES, AND PREVENTION THEREOF ON THE EXAMPLE OF LENINGRAD OBLAST

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[Text] A many-year comprehensive study of endemic tularemia sites in Leningrad Oblast enabled us to demonstrate a number of their typical distinctions. On the territory of this oblast, we have identified endemic sites of the meadow-field, backwater-swamp and forest types [1]. The largest number of tularemia cases (about 1000) was recorded during the epidemic period between 1948 and 1955, when there were mainly threshing-related and natural [household?] outbreaks of tularemia. Minor outbreaks and sporadic cases, which were more often transmissible, were also recorded later, up to 1963. Not a single case of human tularemia has been recorded since 1964, in spite of the activity of enzootic sites. Their intensity and, consequently, the potential epidemic hazard have been consistently confirmed by detection of *F. tularensis*, mainly in sites of the backwater-swamp type.

By virtue of landform and geographic characteristics, backwater-swamp type sites are widespread in Leningrad Oblast and they present several distinctions: they are localized in swampy, heavily shrubbed small floodplains of numerous rivers, streams and brooks with adjacent limited meadow sections of forests. The central part of this oblast, south of Leningrad (Gatchinskiy, Tosnenskiy, Lomonosovskiy, Volkhovskiy Rayons) and part of the territory where new rayons of Leningrad are presently situated have the worst tularemia problem. In this territory, which had been severely demolished during the war, there are endemic tularemia sites where cases of tularemia among people were recorded previously, starting in 1941-1943 and up to 1963. Frequent local epizootics of tularemia were observed there (for almost 40 years) among common voles, water rats, harvest mice and, not infrequently, *F. tularensis* was also found in the water of streams and brooks. Human cases were often linked to drinking water infected with *F. tularensis*. Thus, in 1962-1963, it was determined that the waters of a considerable number of various reservoirs (19 out of 53 inspected) were infected in Tosnenskiy and Volkhovskiy Rayons, and cases of tularemia among people were recorded [2].

In some districts (presently part of Leningrad), where there were intensive epizootics during the early postwar years and there were tularemia cases among humans, radical changes in landform occurred: all useful land was filled and developed (and still being developed) and large areas have been improved, which are now occupied by fruit orchards, residential complexes and modern livestock complexes. But there are still persistent enzootic tularemia sites in this oblast. We have had this territory under constant observation for many years, and implemented the necessary preventive measures.

Water rats, common voles, common shrew and others are the main sources of tularemia in sites of the backwater-swamp type. Water rats inhabit the banks of rivers, streams and ditches extensively. Their number is low in all areas, ranging from 0.5 to 5 specimens per 100 trap-days. Water rats come in close contact with small mammals (common voles and common shrews) that inhabit the same region in small meadow sections adjacent to the bank strip. Water rats are trapped in stacks, ricks and regions near houses [or farms]. Muskrats live along the banks of these small bodies of water. Only isolated specimens can be trapped, and in one case *F. tularensis* was isolated from them. In essence, the number of small mammals is small. In some years (1965, 1967, 1973, 1974, 1975, 1978) there have been increases in their number in meadow regions to 21 specimens per 100 trap-days. During the same years, an increase in number of common redbacked voles and common shrews was determined, with maximum of 26% trapped; the concentration of rodents in stacks and ricks reached 35 specimens per 100 trap-days.

On the whole, there are not many *Ixodes* ticks (*Ixodes ricinus* and *Ixodes persulcatus*) there, a maximum of 60 specimens per flag-h. As a result of man's industrious activities (agrotechnical measures, development of cultivated pasturage) there has been an appreciable decline in number of *Ixodes* ticks in recent years.

One of the rather typical distinctions of sites of the backwater-swamp type is periodic detection of *F. tularensis* in infected water of small streams. The water was examined by means of collecting numerous samples and injecting them subcutaneously to white mice. We had occasion repeatedly (1962-1963, 1967-1968, 1975-1976, 1978-1979) to observe prolonged and constant infection of water in some streams from December to May and even to June. Contamination of water ceased with elevation of water temperature and onset of spring floods (faster current).

We demonstrated the persistence of some sites and reinfection of water. Thus, in all the years of our study, *F. tularensis* was detected in 5 seasons in Slavyanka River (Gatchinskiy Rayon), 4 times in Sablinka River, 3 times in Tigoda River (Tosnenskiy Rayon), 3 times in Chazhenka River (Volkhovskiy Rayon), twice each in the Gryadka and Ravan Rivers (Tosnenskiy Rayon), etc., out of 20 bodies of water (about 70 points where water samples were collected). In some years, one could clearly relate infection of water to a local tularemia epizootic among rodents and insectivores: common voles, water rats, muskrats, common shrews, etc. Their carcasses, which got into reservoirs, served as the source of long-term infection of water in the wintertime (the reservoirs were frozen to the bottom, there was insignificant flow of water and a thick snow cover, up to 1 cm in height). In some cases, we were unable to detect

epizootics among small mammals (1966-1967, 1975), which must be related to the insufficient number of animals examined, difficulty of detecting epizootics in the fall and spring among amphibian species, as well as their low number.

Results of bacteriological testing for tularemia (1962-1979)

Object	Quantity (specimens, samples) tested	Quantity of cultures isolated																		
		in all	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979
Small mammals	51 674	86	1	49	10	—	—	—	—	18	—	1	2	—	—	—	2	—	3	—
Water	3 579	346	4	99	—	—	10	20	31	16	—	—	—	—	13	36	—	29	88	—
Bits of nests, etc.	1 770	9	—	4	4	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
Ixodes ticks	177 920	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
Other ectoparasites	62 346	3	—	1	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Totals		445	5	153	14	—	10	23	31	35	—	1	2	—	—	13	38	—	32	88

As shown by our data (see Table), from 1962 to 1979 (in 18 years) we isolated 445 cultures of *F. tularensis*, including 86 strains from small mammals and 346 from water. Isolated bacteria were demonstrable per milliliter of tested water, but occasionally their number reached 100, as determined by titration in white mice. The strains were isolated by means of biological tests on white mice and, in rare cases, direct cultures when examining organs of small mammals.

Isolation of many cultures from water confirms the significant role of the water factor in circulation of the pathogen of tularemia in endemic sites of Leningrad Oblast, as well as the possibility of broad spread of the pathogen through water beyond the limits of local sites.

We cannot relate maintenance of backwater-swamp sites of tularemia to *Ixodes ricinus* and *Ixodes persulcatus* ticks inhabiting that region, since numerous examinations thereof yielded negative results in a considerable number of cases. In the entire time of our study, which covered about 180,000 specimens of *Ixodes* ticks, we isolated only 1 culture of *F. tularensis*. We cannot rule out the role of *Ixodes trianguliceps* and *Ixodes apronophorus* burrow ticks, which could serve as the chain of preservation of infection in a site, but this question requires investigation.

All of the strains of *F. tularensis* isolated in all these years from water, small mammals and *Ixodes* ticks were typical and highly virulent to white mice and guinea pigs. The strains were referable to the Holarctic race. At the recommendation of N. G. Olsuf'yev, we tested the sensitivity of the isolated cultures to erythromycin. Out of 101 strains, 39 were sensitive and 62 were resistant to erythromycin. It is of great interest that we found (in 8 experiments) resistant and sensitive strains in the same bodies of water, in double tests (from the same collection point) and even (in 6 cases) in one test in different variants in two white mice that died. As indicated by Kudelina and

Olsuf'yev [3, 4], this is attributable to the fact that the epizootic among small mammals infected by stream water was mixed, being caused by two variants of strains.

As we have already mentioned, the last cases of human tularemia occurred in 1962-1963 (15 cases in 8 parts of Tosnenskiy Rayon). These were referable to individuals who had not been immunized against tularemia, came from other oblasts, as well as local inhabitants. Infection occurred as a result of consuming infected water from rivers and streams, as well as using it for household purposes (washing, bathing). Not a single case has been recorded since 1964, although in some areas of these active endemic sites the risk of human infection was high. We have to relate the absence of tularemia cases primarily to the constantly implemented preventive measures and, first of all, to highly effective immunization and reimmunization against tularemia. The stratum of inhabitants immune to tularemia constitutes 90-95%.

Conclusions

1. Endemic tularemia sites of the backwater-swamp type in Leningrad Oblast are localized in the swampy floodplains of small flowing reservoirs (rivers, streams, brooks).
2. The water of small streams, which plays a considerable role in circulation of *F. tularensis* in endemic sites, is often an indicator of an epizootic process.
3. In the wintertime, water is infected by small mammals (water rats, common voles, common shrews and others) that inhabit the banks of waterways.

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ACTIVATION OF ENDEMIC TULAREMIA SITES OF THE MEADOW-FIELD AND STEPPE TYPES
IN TULA OBLAST IN 1977-1978

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[Text] The entire territory of Tula Oblast is enzootic for tularemia. There is prevalence of sites of the meadow-field type, where the common vole is the main source of infection, less often the house mouse and European hare. Infection circulates with the involvement of *D. pictus* and, probably, *I. trianguliceps* Ixodes ticks, particularly in the southeastern regions, where it is the only species of Ixodes ticks [1, 3, 4].

Morbidity was the most intensive in 1938-1952, when the morbidity index reached 5000.0 per 100,000 population in several regions in different years. More than 12,000 cases were recorded in all in these years, and they were referable to 1114 settlements. For the next 20 years, up to 1973, against the background of mass scale immunization, only 35 people in 33 settlements contracted tularemia. In these years, epizootics were local in nature.

While mass reproduction of common voles occurred at a cycle of 3-4 years up to 1952, and the fall population size of these animals reached 20,000-30,000 entrances [to tunnels or burrows?] per hectare, in the period from 1953 to 1962 the number of common voles exceeded 1000 entrances per ha only in some places. In these 10 years, only 3 cases of tularemia were recorded [3, 6]. Such a situation, which also developed in other central oblasts, warranted the assumption that the territory of tularemia sites of the meadow-field type had improved, from the standpoint of health conditions. The results of a special investigation of this matter [5], in which we also participated, revealed that endemic sites of tularemia of the meadow-field type continue to exist in central regions of RSFSR. After an interval of many years, there was a rise in number of voles in 1963, when the number of entrances per ha of clover fields reached 11,000 in some places; however, examination of more

than 8000 rodents failed to demonstrate cultures of *F. tularensis*. In the winter of 1964, 1 case of the disease was recorded. In 1966, there were many voles in the northern part of Tula Oblast; up to 5000 entrances per ha. That year, the tularemia epizootic was focal (12 cultures were isolated in the territory of 3 rural soviets of 2 rayons) and two people were stricken with tularemia. The next local rise in number of voles occurred in 1968 (5000 to 15,000 entrances per ha, 9 cultures isolated, no cases recorded), and in 1974 there was a full-fledged tularemia epizootic (37 cultures of the pathogen were isolated; average of 52% positive bird pellets for tularemia in the 1974-1975 season, the maximum being 76%; number of voles in the fall reached 6000-9000 entrances per ha in some fields). A total of 12 people in 7 rayons were stricken with tularemia.

Active reproduction of the common vole was again noted in the fall of 1976. In some straw stacks, up to 46 voles were caught per 100 trap-nights in the winter. In the spring of 1977, there were local tularemia epizootics with isolation of a few cultures of the pathogen in two adjacent rayons (Venevskiy and Kireyevskiy). There also, three cases of tularemia among people were recorded in April and May. After the voles migrated from the stacks, their number in some places reached 600-1000 entrances per ha. During the summer, there was an increase in field species of rodents throughout this oblast. In October, there were up to 16,000-20,000 entrances per ha in some fields of perennial grasses and grain crops, in November up to 70,000-80,000 and in some places 150,000. In late autumn and winter, 48% of the animals trapped in settlements were common voles. In spite of rodent extermination that had been performed, there were an average of 32 animals per 100 trap-nights in the grain, fruit and vegetable warehouses of some farms in January 1978. Such a high number of murid rodents had not been observed in this oblast since the Great Patriotic War, and it was attributable to the good feed base and other living conditions due to incomplete harvesting of grain and vegetable crops because of weather conditions involving considerable areas for 2 consecutive years (1976, 1977). There remained much unstacked straw in the fields, and fall plowing was not performed completely.

The extensive tularemia epizootic recorded in November 1977 continued to June 1978. By this time, there was a drastic decline in number of voles, and the population thereof subsequently underwent a profound depression.

In the described epidemic season of 1977-1978, 2044 animals were examined (including 351 carcasses), as well as 13,550 ticks and 1127 environmental objects (rodent excrements, nests, fodder samples, produce, water); 2098 biological tests were performed, 166 cultures of *F. tularensis* were isolated from 77 points in 21 rayons and Tula. One culture of *F. tularensis* was isolated from each of the following: common field-mouse (3.0 per 1000), harvest mouse (43.5 per 1000) and black rat (10 per 1000). The concurrent isolation of *Listeria*, *Erysipelothrix* and *Yersinia* was indicative of the fact that, like in prior years, the epizootic was mixed.

F. tularensis was isolated all winter long, the first one being isolated on 4 November 1977 from common vole carcasses and the last on 20 June 1978 from house mice in Plavsk.

Table 1. Quantity of cultures of *F. tularensis* and other pathogenic bacteria isolated during the 1977-1978 epizootic

Specimen examined	Quantity of specimens	Number of biol. tests	Quantity of isolated cultures				<i>F. tularensis</i> cultures/1000 specimens
			<i>F. tularensis</i>	<i>Erysipelothrix</i>	<i>Listeria</i>	<i>Yersinia</i>	
Common voles	1 124	430	96	19	11	2	85
House mice	264	98	8	—	—	—	30
Ixodes ticks	12 241	348	3	—	—	—	0.25
Gamasid ticks	1 309	8	2	—	—	—	1.25
Environmental objects	1 127	1014	54	10	4	—	50

At the recommendation of the tularemia laboratory of the Institute of Epidemiology and Microbiology imeni Gamaleya, we used the Kudelina method [2] to determine erythromycin sensitivity of 110 strains of *F. tularensis* isolated during that epizootic. We found that 42 (38%) strains were sensitive and 68 (62%) resistant to erythromycin, which coincided with the results of Kudelina. We isolated either both biotypes of *F. tularensis* or one of them in different parts of the oblast. We failed to determine a correlation between type of strain and material from which it was isolated.

Examination of pellets of predatory birds gathered in August and September 1977 yielded negative results. The first positive antigen findings in pellets were made in specimens collected in November. In the spring of 1978, pellets were collected from 19 out of 23 rayons, and positive results were obtained in a mean of 23.4% of the cases, ranging from 4 to 40% in different rayons. Inspection of 64 rural soviet territories, some villages and cities revealed tularemia antigen in 42 localities. Up to 10% of the positive results were recorded in 4 localities, 11-20% in 6, 21-30% in 12 and 31-40% in 6. Positive results in 40-70% of the cases were obtained only in 4 rural soviets in Bogoroditskiy Rayon. Tularemia antigen was found in 1/3d of the 32 sectors where less than 40 pellets were examined. These data are indicative of irregular distribution of epizootics over the territory of this oblast.

The entire rural population of this oblast over 14 years of age received inoculations against tularemia every 5 years, and the size of the immune stratum is checked annually. According to data recorded on form No 86, at the start of the full-fledged epizootic coverage by immunization constituted 81.4% of the rural population. Actual immune stratum, according to screening by the tularin test of 19,293 people, constituted a mean of 85.3, 82.5 and 86.3% in this oblast for 1974, 1975 and 1976, respectively (Table 2). The immune stratum was small in medical sectors with vacancies in feldsher positions or frequent turnover thereof.

Tularemia cases started in October 1977 and continued to June 1978; 1 to 20 cases were recorded in each of the administrative rayons of the oblast. In all there were 235 diagnosed cases (12.3/100,000) confirmed by elevation of agglutinin titer.

The rural population constituted a mean of 60.9% of the tularemia cases. The diseases were scattered all over the oblast. One case was recorded in each of 113 rural settlements, 2 in each of 12 and 3 in each of 2 others. The

absence of group morbidity against the background of an extremely intensive tularemia epizootic was attributable to the large immune stratum in most settlements, but in such a situation it was not sufficient to prevent isolated cases of the disease.

Table 2. Results of checking quality of vaccination against tularemia using the tularin test

Year of inspection	Quantity of checked medical sectors	Quantity of sectors with immune stratum			
		to 50%	51-75%	76-90%	91% & over
1974	67	$\frac{3}{4,5}$	$\frac{10}{14,9}$	$\frac{25}{37,3}$	$\frac{29}{43,3}$
1975	96	$\frac{10}{10,5}$	$\frac{14}{14,5}$	$\frac{27}{28,1}$	$\frac{45}{46,8}$
1976	61	$\frac{2}{3,3}$	$\frac{10}{16,4}$	$\frac{25}{40,9}$	$\frac{24}{39,4}$
Totals	224	$\frac{15}{6,7}$	$\frac{34}{15,1}$	$\frac{77}{34,4}$	$\frac{98}{43,8}$

Note: Absolute indicators are given in numerator and relative ones (%) in denominator.

The longest interval between the last manifestation of infection in a settlement was 36 years, and in 92 settlements the infection had been recorded for the first time.

Morbidity increased with age: 1 case per 100,000 preschool children, 9 at 7-14 years of age, 8 at 15-19 years, 10 at 20-49 years, 18 at 50-59 years and 20 cases at 60 or more years of age. Men constituted 49.3% of all the cases.

Agricultural workers constituted 32.8%, industrial workers 20.8%, housewives and retired people 15.7%, white-collar workers 13.2%, school children and students 11.5%, workers in enterprises dealing with storage and processing of agricultural products 3.8%, preschool children 0.8% and others 1.3%.

In 73 cases (31.0%), the agricultural type of infection was determined, in 133 (56.6%) the domestic, in 9 (3.8%) industrial (while working at granaries and produce warehouses, alcohol plants, mill combines); 2 cases were related to hare hunting and 2 to muskrat hunting. In the vast majority of cases (71.5%), the aspiration mechanism of transmission of infection was involved (inhaling dust when working with infected fodder, grain, etc.), whereas contact (17.4%) or alimentary (11.1%) routes of infection were encountered much less often.

In 70.2% of the cases the pulmonary form of the disease was found, in 19.5% the bubonic form was diagnosed without visible portal of entry of infection; sore throat and bubonic form constituted 7.2%, the oculobubonic form 0.8% and abdominal 2.1%. Of those stricken with tularemia, 114 people (48.5%) were not in a category that needed to be immunized (urban residents, children up to 14 years of age, people with medical contraindications, etc.); 78 patients had not been immunized at the proper time for different reasons (new arrivals,

individuals refused to be immunized); 52 cases were recorded among people who were immunized, 8 of whom were immunized in 1973, 12 in 1974, 17 in 1975, 4 in 1976, 7 in 1977 and 4 in 1978. In the last 4 cases, the disease occurred 1-1.5 months after immunization. It was not determined that there had been any flaws in the technique for giving the vaccination. The diagnosis was confirmed by elevation of agglutinin titer from 1:400 to 1:3200. We had not observed the disease in such a number of immunized people previously, and this can be attributed essentially to use of a poor quality of vaccine.

The existing situation made it necessary to take a number of additional steps, in addition to planned work, to eradicate tularemia and prevent outbreaks where there was maximum activation of infection. By decision of the oblistpolkom, a list was approved of immediate steps for the control of tularemia. Progress in implementing this decision was checked and discussed at meetings of the oblast and rayon ispolkoms. A total of 18 oblast administrations, associations and trusts were involved in organizing epidemic-control and preventive measures, and they implemented a set of technical, rodent-exterminating and agrotechnical measures referable to material [objects] under their jurisdiction. The oblast interagency committee for rodent extermination, which was founded in 1975, coordinated this work in 11 rayon committees, whereas there was a staff working to exterminate rodents in the 3 most stricken regions. Indications were broadened for giving tularemia vaccines to the urban population that had cattle in their private farm, as well as those sent to do farm work in the spring, and in student construction detachments. All of the rural population working in urban enterprises were immunized. In 1977-1978, 296,800 people were immunized, including 132,000 urban residents. In 1978, the immune stratum in the rural population was brought up to an average of 89.3% in this oblast. There were no recorded cases of tularemia among urban residents involved in farm work in the spring of 1978 (overhaul of equipment, sorting grain, potato pits). Nor were there any cases of tularemia among children and personnel located in summer health-improvement institutions in the oblast.

Extermination of rodents was organized everywhere. In 2 years, a total of 85,543,600 m² in settlements and 1106.02 ha of fields were treated. In the early spring of 1978, after the flood tides, 3000 wells (52.4%) and 37,000 summer houses in collective gardens were disinfected in rural areas. The scope of extermination work in the fields was not adequate, and it did not have an appreciable effect on reduction in number of Muridae, particularly in regions where their density was the highest. Some work was done with regard to putting epidemiologically important targets into the proper sanitary and technical condition.

Conclusions

1. In 1977-1978, the tularemia epizootic involved the entire territory of Tula Oblast, but its distribution was uneven. During this period, infection of common voles constituted 8.5%. In several regions, *Listeria*, *Erysipelothrix* and *Yersinia* were isolated, in addition to *F. tularensis*.
2. Morbidity was diffuse; in most rural settlements (89%), 1 case per location was recorded and chiefly people who were not immunized were stricken.
3. However, in 52 cases the disease developed in immunized individuals.

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EXPERIMENTAL LATENT TULAREMIA IN COMMON VOLES

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[Article by K. N. Shlygina and N. G. Olsuf'yev, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The possibility of nonlethal infection in highly sensitive animal species was demonstrated in our previous studies [2, 3] using a highly virulent Holarctic *F. tularensis* strain, No 503. In these experiments we used one of the natural and most widespread routes of infection among common voles--the alimentary route (feeding them carcasses of animals that died of acute tularemia). Necrophagia and cannibalism are widespread among this rodent species in its natural habitat. During a tularemia epizootic, animals can consume tens to hundreds of billion of bacteria. It was proven experimentally that the presence of many dead bacteria in the consumed substrate, along with live ones, could cause development of nonlethal infection in highly sensitive species. No study had been made of the significance of such animals to epizootiology of tularemia. For this reason, we decided to investigate this question.

Materials and Methods

In experiments with a highly virulent strain, the number of sick animals was low and did not exceed 1% of those that survived. We assumed that use of a strain with somewhat attenuated (in nature) virulence would enable us to produce many cases of tularemia. Indeed, in this study, there were 6 times more such animals. We used Holarctic strain No 165 of *F. tularensis*, which was isolated from a common field-mouse in 1964 [1] and stored in the laboratory in lyophilized form. Some attenuation of virulence of this strain was demonstrable only in experiments on guinea pigs, when doses of 1 to 1000 bacterial cells injected subcutaneously elicited death of some animals. But this dosage was found to be completely lethal to white mice and common voles. We again tested the strain before starting the experiments, and its virulence was the same as initially.

The experiments were conducted on 433 *Microtus subarvalis* voles, which had been raised in the laboratory. The animals were infected by feeding them on carcasses of animals that died of acute tularemia. The method of feeding the animals was described before [3].

Results and Discussion

After contact or ingestion of carcasses, 170 voles died of acute tularemia, 53 died of extraneous causes and 210 survived. We tested for immunity to tularemia to determine whether any of these animals had contracted the disease. For this purpose, 26-33 days after feeding them the carcasses we submitted them to hypodermic test infection with 10 DCLm of highly virulent strain No 503. Some of the surviving animals were reinfected with 100 DCLm of the same strain. We found that 14 voles (6.6% of the survivals) were immune. We conducted our further studies on 13 animals.

We tested 11 voles in vivo for demonstration of carriers (more precisely, bacteriuria). We analyzed urine from each animal by the biological test on white mice. In all, we collected 98 specimens of urine, in 46 of which that were taken from 8 voles we demonstrated *F. tularensis*. Urine was collected in a special cage, usually many times, starting on the 12th-20th day after testing immunity (see Table). There were 2 animals that were of special interest (Nos 8 and 9), in whom each of 10 specimens collected between the 12th and 63d day contained *F. tularensis*. Bacteria were demonstrable for 1 to 1.5 months (observation period) in 4 animals (Nos 1, 4, 11, 12). Bacteria were demonstrable for short periods of time in 2 voles (Nos 10, 13).

Subsequently, we tried to provoke exacerbation of infection in 9 animals (Nos 1-9, 4 of which presented bacteriuria). We used the following as provocative factors: hydrocortisone (for 3 days, 5 mg intramuscularly) for animal No 1; cooling (bathing in ice water for 3 days) for Nos 2 and 3; radiation (800 rad) for animals Nos 4-7; *Salmonella* infection ($1 \cdot 10^5$ bacterial cells per os) for animals Nos 8 and 9. As a result, five animals died shortly and the others were sacrificed soon after no more deaths occurred. The animals were submitted to bacteriological and serological examination (see Table). Significant contamination by *F. tularensis*, at least 10 million per gram, was found in only one (No 4) of the animals that died, which was apparently due to exacerbation of the infectious process under the influence of radiation. In the organs of the other animals that died, there were either no *F. tularensis*, or else contamination was moderate and did not differ appreciably from the findings in organs of sacrificed animals. Consequently, it can be assumed that death occurred as a direct result of the provocative factor, rather than exacerbation of the infectious process. No bacteria were found in the spleen of animal No 8, which had died, whereas they had been demonstrable in urine virtually up to the day that it died of salmonellosis. Evidently, the focus of infection was localized only in the kidneys in this animal.

Antibodies to *F. tularensis* were demonstrated both in the animals that died and those that were sacrificed (No 6 was not tested).

Three voles (nos 10, 11, 12), which died without any intervention on the 41st, 178th and 304th days after testing for immunity and 1 vole (No 13), which was sacrificed on the 313d day, were of special interest.

F. tularensis was isolated from 2 of the voles that died, in one of which (No 10) we demonstrated considerable contamination of organs, while we did not assay bacteria in the other (No 11). We were unable to isolate bacteria from the vole that died on the 304th day of an unknown cause.

Results of bacteriological and serological examination of voles

Vole No	Day of urinalysis for demonstration of <i>F. tularensis</i>		Outcome, day after immunity test		Test for carriers		Agglutination with blood serum, titer
	positive result	negative result	died	sac-rif.	organs examined	quantity of bacter. per gram tissue	
1	45	44	46		Mixture of spleen, liver and lung	$1 \cdot 10^8$	1:20*
2	Not tested			33	Spleen	$6 \cdot 10^2$	1:320
3	"			33	Spleen	$7 \cdot 10^3$	1:320
4	26-30, 35-38, 42	19, 22, 41	43		Spleen	$> 1 \cdot 10^7$	1:40*
					Liver	$> 1 \cdot 10^7$	
5		19, 26-30, 34-37, 40	44		Lung	$1 \cdot 10^5$	
					Spleen	$1 \cdot 10^4$	
6		26-30, 34-37, 40, 41	42		Liver	$1 \cdot 10^3$	1:40*
					Spleen	None	Not tested
					Liver		
					Lung		
7		26-30, 35-38, 42-46		49	Spleen	None	1:320
					Liver		
8	12-15, 54-56, 61-63		65		Lung	None	1:10*
					Spleen		
9	12-15, 54-56, 61-63			70	Spleen	"	1:640
10	12-15		41		Spleen	$1 \cdot 10^5$	1:40*
					Liver	$1 \cdot 10^4$	
					Lung	$1 \cdot 10^2$	
11	13-15, 32	18, 19, 33, 34	178		Spleen	Bacteria found, but not counted	0*
					Lung		
12	19, 25, 26, 32, 36	35, 37	304		Spleen	None	0*
					Liver		
					Lung		
13	20, 21	15, 17, 22, 23, 70, 71		313	Spleen	$1 \cdot 10^4$	1:160
					Liver	$1 \cdot 10^2$	

*Washings from thoracic organs were examined.

F. tularensis was isolated from vole No 13, which was sacrificed on the 313d day, by direct culture on coagulated yolk medium (1 colony grew). A test of the virulence of this culture revealed that it corresponded to strain No 165, which had been used to feed the animals. The results of titration of organs of this vole revealed that the spleen contained a considerable quantity of bacteria for this remote time ($1 \cdot 10^4$ /g). Consequently, this vole was a carrier for over 11 months.

Thus, the results of these studies confirmed once more the possibility of non-lethal tularemia in highly sensitive species. According to our observations, the carrier state developed in the vast majority of cases (11 out of 13), with minimum duration of 1-1.5 months and maximum of 4 months. Foci of infection could persist not only in the spleen, liver and lungs, but the kidneys. Most of the vole carriers (8 out of 11) eliminated bacteria in urine at the tested times (12-63 days). Tularemic nephritis with bacteriuria had also

been observed in the Pennsylvania vole, guinea pig and Canadian beaver [5] which were vaccinated and then infected. The long duration of carrier state in animals (6-11 months) and bacteriuria (2 months) warrant the assumption that a chronic, latent process was formed. It is believed that long-term carriers are the result of rapid depletion of some elements of the immune system [4], serving as an indication of inadequate immunity. The attempts we made to provoke exacerbation of a latent process by diverse deleterious factors failed to yield clearcut results. At the same time, there were instances of spontaneous death among carrier animals at different times, including the long term. All of the foregoing warrants the assumption that such animals possibly play a role as one of the harborers and sources of infection in nature.

Conclusions

1. Alimentary infection of *Microtus subarvalis* common voles with an *F. tularensis* strain with somewhat attenuated virulence under experimental conditions that are close to natural ones led to nonlethal infection in 14 out of 433 experimental animals (6.6% of those that survived).
2. A carrier state developed in most (11 out of 13) of the infected animals, as well as bacteriuria (8 out of 11), with maximum duration of up to 2 months (observation time).
3. Persistence of *F. tularensis* in voles that had been infected can last for 6-11 months (duration of observation period).
4. All of the foregoing warrants the assumption that such animals may play a role as one of the harborers and sources of infection in nature.

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