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CHARACTERISTICS OF THE PLAGUE CULTURES ISOLATED FROM THE GORNO-ALTAYSKAYA AUTONOMOUR OBLAST IN 1961

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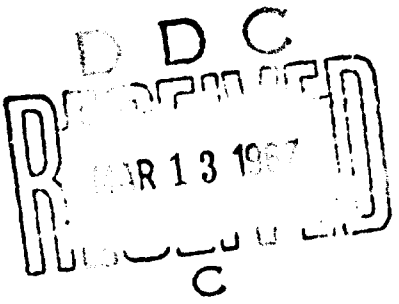

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CHARACTERISTICS OF THE PLAGUE CULTURES ISOLATED FROM THE GORNO-ALTAYSKAYA AUTONOMOUS OBLAST IN 1961

- USSR -

[Following is the translation of an article by A. M. Shamova in the Russian-language journal Izvestiya Irkutskogo gosudarstvennogo nauchno-issledevatel'skogo protivochumnogo instituta Sibiri i Dal'nego Vostoka (News of the Irkutsk State Scientific Research Anti-plague Institute of Siberia and the Far East), Vol 25, 1963, pages 25-33.]

We first isolated plague bacillus cultures from wild bees and their fleas [See Note] during the summer of 1961 in the Kosh-Agachskiy Rayon of the Gorno-Altayskaya Autonomous Oblast. A total of ten strains were obtained: 2 -- at the end of June and 8 -- at the end of August. Three strains (1571, 1476-1479, 357) were isolated from trapped animals: the Mongolian cony, the long-tailed sullik, and the steppe polecat; seven strains (64, 911, 912, 913, 905, 1122, 961) -- from the fleas of five species: Amphalius runatus, Paradoxopsyllus scorodumovi, Frontopsylla hetera, Amphipsylla primaris primaris, Chaetopsylla homoeus. The fleas of the first three species were collected from trapped Mongolian cony, and the remaining two -- from trapped steppe polecats.

([Note]: Epizootological and epidemiological information on the plague focus found has been presented in a study by A. K. Balabkin, A. M. Shamova, V. I. Sarzhinskiy, V. M. Lazarev, and N. Ya. Gorbacheva (in press).)

We conducted the bacteriological investigation of the wild animals following the methods proposed by L. A. Timofeyeva (1961).

Parenchymatose organs and lymph nodes of each rodent were ground in a porcelain mortar to which sterile sand and physiological saline solution had been added. The suspension of organs and lymph nodes was seeded on to Hottinger agar of pH = 7.2 with the addition of 0.01 - 0.1% dry hemolyzed blood. Using this method, plague bacillus cultures were isolated from steppe polecat and long-tailed susliks.

A culture of plague causative agent (1571) was isolated from Mongolian cony by seeding a suspension of organs and lymph nodes and by smearing the organs on to the agar surface.

All plague bacillus cultures, with the exception of one, were isolated by direct inoculation of the materials studied on to agar plates. Strain 64, from the flea Amphalius runatus, was obtained from a biopsy of a white mouse sacrificed with chloroform on the seventh day. Growth of plague bacillus was noted for seeding from liver (three colonies) and spleen (four colonies).

Pathologoanatomical changes occurred only in the Mongolian cony in the form of injections of the vessels of the subcutaneous cellular tissue and by swelling of the inguinal lymph nodes.

The fleas were investigated following the commonly adopted method without prior rinsing in physiological saline solution (Shiranovich and Treshchilin, 1959). Growth of plague bacillus on agar plates was profuse. Only one colony was raised from the flea Amphalius runatus (strain 911).

The study of the main properties of freshly prepared strain was undertaken in the laboratory of the epidemiological detachment of the Gorny-Altay Antiplague Division. Freshly isolated strains on plates of Hottinger agar in most cases produced growth in a day in the form of fine scalloped colonies; under the microscope formation of chromogenic weakly nodular centers were noted in many colonies, gradually intergrading into colorless dew-like periphery. Some of the colonies were well formed and had a convex chromogenic nodular center and colorless lacy fringes. Flat, colorless formations were also found in the form of "rosettes," "lacy doilies."

On the second day, the colonies became convex, circular, whitish in incident light; under the microscope they took on the form of chromogenic colonies with granular or nodular center and light lacy fringe or without this fringe.

The morphology of a strain isolated from the flea Frontopsylla hetera (905) showed a somewhat different morphology, namely: on the second day of growth, colonies of this strain were circular, slightly convex, whitish in incident light; under the microscope -- slightly convex, chromogenic, with even edges and smooth surface, without lacy fringe. Only a few colonies were gently nodular and had a narrow compact fringe. When researed the mosphology of the strain colonies became typical of plague bacillus. At more remote periods, the morphology of the colonies of most strains continued to remain typical, only in three strains did the colonies become completely smooth, with even edges, gray-brown in color, without a fringe; a dense, narrow fringe was noted only in a few colonies.

In the Hottinger broth, the colonies attained flocculent growth and a loose sediment on the day of culturing, on the surface -- a circular film on the test tube wall. In seven strains, the medium became transparent, three strains on the first day caused light turbidity of the medium.

The bacilli in smears taken from two-day old agar and broth cultures had the appearance of fine, polymorphous gram-negative bacilli with rounded ends. The bacilli were arranged singly and in clumps in the agar smears, and in the broth smears -- by pairs, short and long chains. Bipolarly stained specimens were found more often in broth smears.

All isolated cultures after a day of growth on color differential medium (Timofeyeva, Aparin, Golovacheva, 1961) altered the color of the tapered surface of the medium to blue, and that of the column -- to reddish-orange and light brown.

All strains were lyzed by Irkutsk polyvalent plague and pseudotubercular bacteriophages (series 1, titer 10^{-9}) on solid and liquid nutritive media, fermenting in 1-2 days glucose, maltose, galactose, dextrin, xylose, with acid formation; glycerine was fermented on the third-fourth days; 7 strains cleaved ramnose on the first day, three strains -- on the third day; lactose, arabinose, dulcite, inulin, inosite, and sorbite were not fermented by the strains during the 14 days of observation.

In the epidemiological detachment, the virulence of six strains of plague causative agent was studied in 10-15 days after their isolation [See Note]. White mice and guinea pigs were chosen as the experimental animals, which were

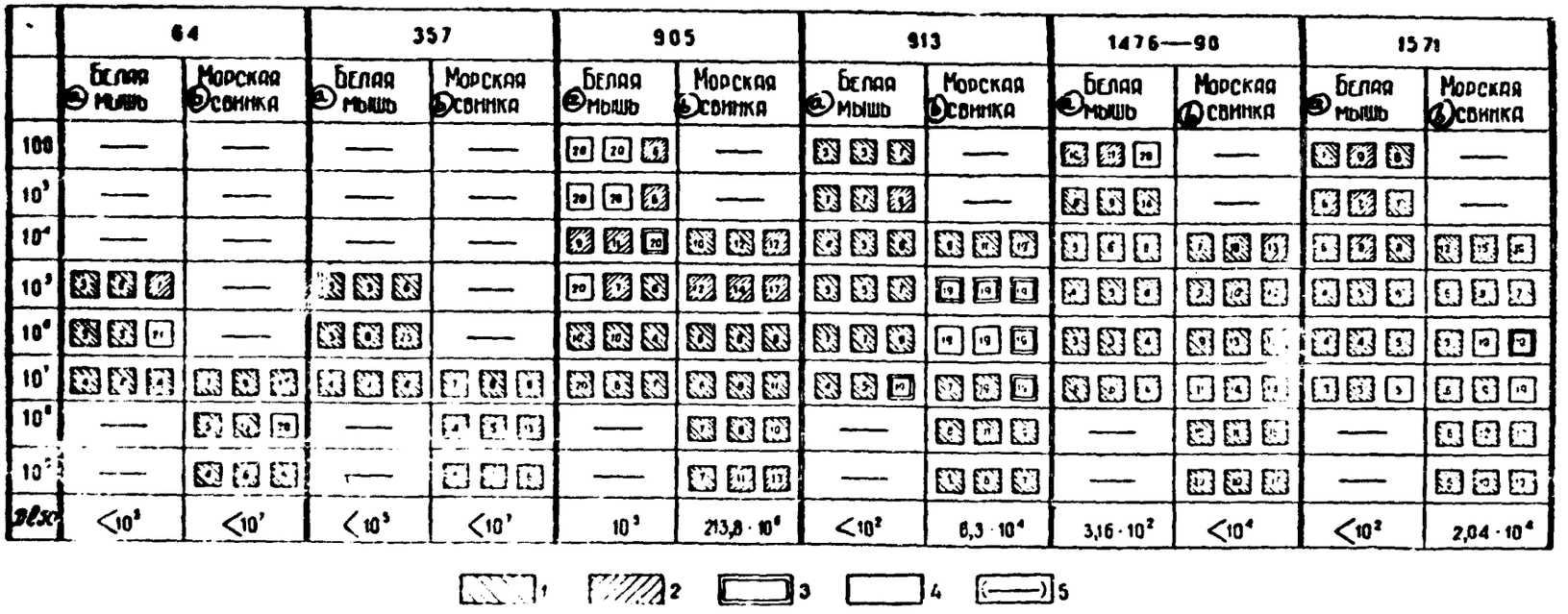

Fig. 1. Virulence of plague bacillus strains for white mice and guinea pigs in the initial study. 1 -- animal perished, plague bacillus culture isolated in bactero logical investigation; 2 -- animal perished plague bacillus culture not isolated in bacteriological investigation; 3 -- animal sacrificed with chloroform, plague bacillus culture isolated in bacteriological investigation; 4 -- animal sacrificed with chloroform, plague bacillus culture isolated: 5 -- animals were not infected. Numbers in squares indicate the time in days when the animal had perished or had been sacrified with chloroform.

[Legend:] a) white mouse; b) guinea pig.

infected subcutaneously with various doses of two-day old agar culture. Each dose was used to infect three animals. The mean lethal doses were chosen as indices of virulence for each strain according to the Reid and Mench method (Figure 1).

([Note]: The virulency of two stains was studied by V. P. Smirnov and A. K. Balabkin.)

In calculating the LD_{50} those animals which perished during the first 10 days and from which no culture was isolated, were excluded from the experiment, but those which succumbed later than 10 days, not yielding culture, were regarded as having survived.

Three strains (913, 1476-90, 1571) proved to be highly virulent for white mice and relatively weakly virulent for guinea pigs. The virulence of strain 905 was considerably less both for white mice and for guinea pigs.

Detailed study of isolated cultures was conducted by the authors in the microbiological division of the Irkutsk Antiplague Institute at the end of 1961 in 2.5 months after their isolation.

The properties of the isolated strains were compared with the properties of control plague B. pestis strains (EB and 1435) and the strain of the causative agent of rodent pseudotuberculosis (1).

All plague bacillus cultures on sheets of Hottinger agar yielded the plague-characteristic growth in a day in the form of "cracked glass," "lacy fringes," "rosettes," and colonies with incipient weakly chromogenic nodular center. In two days, the colonies were in most cases convex, granular, or nodular, chromogenic, with colorless lacy zone or without such a zone; formations were also found in the form of "rosettes," "lacy fringes."

In the Hottinger broth, the growth was the same as in the initial study; flocculent wall growth, loose sediment at the bottom; only sometimes did the strains give rise to mild turbidity of the broth during the first three days, generally, however the medium remained transparent; formation of a film on the broth surface was observed on the second-fourth day.

Morphology of the bacilli in smears of two-day old agar and broth cultures corresponded to its counterpart in

the initial study.

All strains when seeded on 0.3% semiliquid agar grew only along the site of injection, that is, were immobile, yielding a distinct capsule when raised on tapered 1% blood agar in the dessicator with increased carbon dioxide content (temperature 37°, staining according to the Giss method).

Enzymatic activity of the strain was studied on the Giss media with 1% carbohydrate and alcohol content (with the exception of ramnose -- 0.5%). The results of the study are presented in the table.

As we can see from the table, all strains actively fermented in one-two days, with acid formation, glucose, maltose, mannite, dextrin, more weakly -- galactose and xylose, and glycerine on the third-fourth day. Ramnose was fermented by all strains, beginning from the first days, distinct acid formation was noted on the second day. Six strains cleaved arabinose on the 15-20th day. Not one strain decomposed lactose, saccharose, raffinose, dulcite, and sorbite during the 20 days of observation. The cultures grew in litmus infusion with acid formation on the first day; milk was not curdled; indole was not formed; hydrogen sulfide was liberated on the second day in Hottinger broth; gelatin was not diluted.

Comparing data on fermentive activity of strains with respect to carbohydrates and alcohols in the initial and second study, we can say that the strain activity remained unchanged, if we do not consider the fermentation of ramnose.

In the first study, three strains (64, 357, 912) fermented ramnose on the third-fourth day, and in the second -- all strains fermented ramnose on the first-second day.

The fact of ramnose fermentation by plague bacillus cultures isolated from the territory of the Gorno-Altayskaya autonomous oblast, according to the data of R. V. Kovaleva (1958), V. P. Babenyshev, etc. (1960), Yu. V. Kanatov, et al (1961) on ramnose-positive strains isolated chiefly from various species of voles (Brandta, common, social). We have collected similar data (Shamova, 1959). In an article by Z. I. Shchekunova and L. V. Vasyukhina (1961) it is reported that 51 ramnose-positive strains have been isolated from Mongolian conies and their fleas, Altay marmot badger, Daurskaya cony, and Dzhungarskiy hamster in the Mongolian plague focus, near the boundary of the Gorno-Altayskaya Autonomous Oblast. The

Cultural-biochemical Properties of Strains

(a) Номера штаммов	(b) Капсулообразование	(c) Подвижность	(d) Ферментация углеводов и спиртов: (e) глюкоза	(f) лактоза	(g) мальтоза	(h) маннит	(i) сахароза	(j) арабиноза	(k) галактоза	(l) дульцит	(m) декстрин	(n) глицерин	(o) сорбит	(p) рафиноза	(q) целлоза
61	-	—	K_1		K_1	K_1	—	K 10–	K_2	—	K_2	K_3	—	—	K_3
317	+	—	K_1	-	K_1	K_2	—	—	K_2	—	K_1	K_3	—	—	K_3
345	-	-	K_1	—	K_1	K_1	—	—	CK_1		K_2	K_4	—	—	K_3
911		-	K_1		K_2	K_2		CK 15 - 20	CK_1		K_2	K_1		—	CK_2
912	+	—	K_1	—	K_2	K_2	--	K 15 20	CK_1		K_2	K_4	—	—	CK_2
913	+	—	K_1	—	K_2	K_2	—	—	CK_2	—	K_2	K_4	—	—	CK_2
961		—	K_1	—	K_1	K_2	—	K 15 20	K_2	—	K_2	K_4	—		K_3
1122		—	K_1	—	K_2	K_2	—	—	CK_1	—	K_2	K_4	—	—	K_3
1476 90		—	K_1	—	K_2	K_2	—	CK 15 20	CK_1	—	K_2	K_4	—	—	CK_2
1571	-	—	K_1	—	K_1	K_2	-	CK 15 – 20	CK_1	—	K_2	K_4	—	—	CK_2
1435	+	-	K_1	—	K_2?	K_2	—	K_2	K_3	—	K_1	K_3	—	—	K_2
Ell		—	K_1		K_1	K_1		K_2	K_3	—	K_1	—	—	—	K_2
B рис		+	K_1	—	K_1	K_1	—	K_2	K_3	—	K_2	CK_2	—	—	K_2

[table continued]

(r) Рамноза	(s) Молоко	(t) Лакмусовое молоко	(u) Желатина	(v) Образование индола	(w) Образование сероводорода	(x) Нитрификация	(y) Денитрификация	(z) Ферментация мочевины, по Ленской	(a') Редукция метиленовой синьки	(b') Рост на голодно-кислом агаре	(c') Рост на бепептонном агаре из разведений	(d') Отношение при титрации по Аппельману к фагам: (e') чумному	(f') псевдотуберкулезному
СК; $К_2$	—	$К_1$	—	—	+2	—	—	—	+	—	1–2	10^{-9}	10^{-9}
СК; $К_2$	—	$К_1$	—	—	+2	—	—	—	+	—	1–3	10^{-9}	10^{-9}
СК; $К_2$	—	$К_1$	—	—	+2	—	—	—	+	—	1	10^{-9}	10^{-9}
СК; $К_2$	—	$К_1$		—	+2		—	—	+	—	1	10^{-9}	10^{-9}
СК; $К_2$	—	$К_1$	—	—	+2	—	—	—	+	—	1	10^{-9}	10^{-9}
СК; $К_2$	—	$К_1$	—	—	2	—	—	—	+	—	1–3	10—	10^{-9}
СК; $К_2$	—	$К_1$	—	—	+2	—	—	—	+	—	1–5	10^{-9}	10^{-9}
СК; $К_2$	—	$К_1$	—	—	2	—	—	—	+	—	1	10^{-9}	10^{-9}
СК; $К_2$	—	$К_1$	—	—	+2	—	—	—	+	—	1	10^{-9}	10^{-9}
СК; $К_2$	—	$К_1$		—	+2	—	—	—	+	—	1–2	10^{-9}	10^{-9}
		$К_1$			+3	—	·		+	—	1–5	10^{-9}	10^{-5}
—	—	$К_1$	—	—	+2	—	+++	—	+	—	1–5	10^{-9}	10^{-9}
$К_1$	—	[illegible]		—	+3	—	++++	+++	++++	+4	1–15	—	10^{-5}

[Legends on following page]

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Symbols: K - acid formation; CK - weak formation of acid; - alkaline media; (+) -- formed, motile, reduced by 10-15%; (-) - did not form, nonlabile, not diluted, not coagulated, does not grow, not lyzed, not fermented; numbers -- time in days.

[Legend:] a) strain number; b) capsule formation; c) motility; d) fermentation of carbohydrates and alcohols; e) glucose; f) lactose; g) maltose; h) mannite; i) saccharose; j) arabinose; k) galactose; l) dulcite; m) dextrin; n) glycerin; o) sorbite; p) raffinose; q) xylose; r) rhamnose; s) milk; t) litmus infusion; u) gelatin; v) indole formation; x) nitrification; y) denitrification; z) fermentation of urea, according to Lenskaya; a') reduction of methylene glue; b') growth on acid-deficient agar; c') growth on peptone-deficient agar from dilution; d') ratio when titrated according to Appleman to phages; e') plague; f') pseudotuberculosis.

plague bacillus cultures we isolated behaved typically on the differential-diagnostic media. On the Bessonova nonpeptone agar, the strains grew weakly from 1-3 dilutions; they did not grow on acid-deficient agar; methylene blue was 10-15% reduced in 1-3 days; no strain cleaved urea during the three days of observation (according to the Lensk method. The strains did not possess nitrifying and denitrifying properties. All the strains were lyzed by polyvalent plague and pseudotuberculosis bacteriophages prepared by the production affiliate of the Irkutsk Antiplague Institute in the city of Khabarovsk (series 1, titer 10) on solid and liquid nutritive media. All strains were lyzed up to titer when titrated according to Appleman. All the strains were agglutinated with anti-plague serum (series 62, titer 1:2000) made by the Saratov Antiplague Institute, in the titer of 1:400-1:1600.

Thus, the strains we isolated correspond to controls B. Pestis strains EB and 1435 in general in cultural and biochemical properties and differed in differential-diagnostic characteristics from the causative agent of pseudotuberculosis (with the exception of rhamnose fermentation).

In studying the sensitivity of the isolated plague bacillus cultures to streptomycin on agar media using the

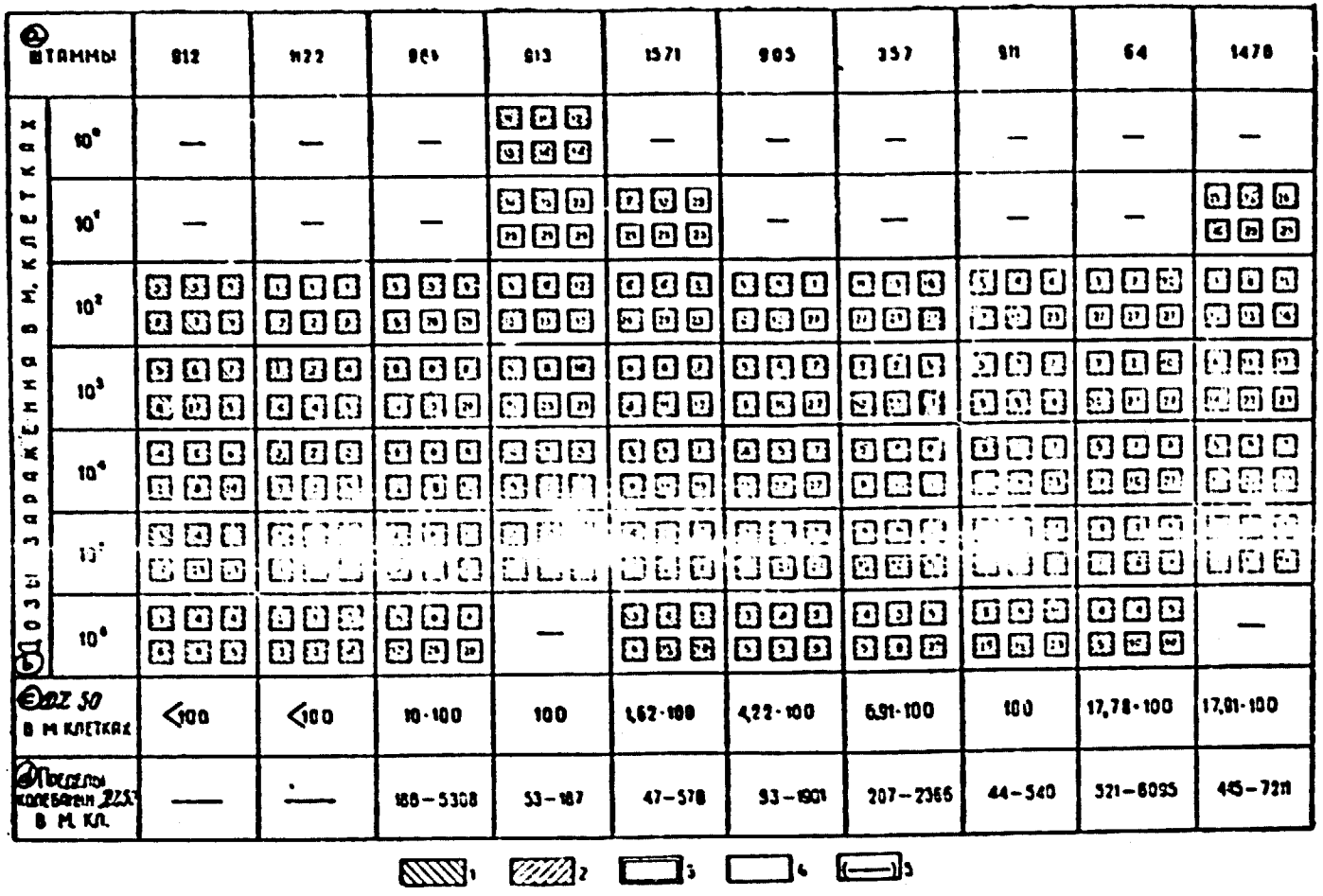

Fig. 2. Virulence of plague bacillus strains for white mice [Remainder of caption same as in Figure 1, page 4]

[Legend:] a) strains; b) inhibitory dosages in bacterial cells indicated; c) LD_{50} in bacterial cells; d) limits of fluctuations in LD_{50} in bacterial cells.

а) штаммы	912	1122	911	913	1571	905	357	861	64	1476
б) дозы заражения в м. клетках 10^2	—	—	—	—	—	—	—	—	—	
10^3										
10^4										
10^5										
10^6										
10^7										
10^8										—
10^9				—						—
в) LD_{50} в м. клетках	$21,63 \cdot 10^6$	$<10^3$	$10 \cdot 10^6$	$22,96 \cdot 10^1$	$4,55 \cdot 10^3$	$100 \cdot 10^6$	$145,5 \cdot 10^6$	$66,97 \cdot 10^3$	$218,6 \cdot 10^4$	10^2
г) Пределы колебаний LD_{50} в м. кл.	$4,64 \cdot 10^6 - 98,4 \cdot 10^6$	—	$2,54 \cdot 10^6 - 39,36 \cdot 10^6$	$2,59 \cdot 10^1 - 196 \cdot 10^1$	$0,785 \cdot 10^3 - 26,3 \cdot 10^3$	$39,36 \cdot 10^6 - 314 \cdot 10^6$	$27,3 \cdot 10^6 - 781,6 \cdot 10^6$	$14,45 \cdot 10^3 - 302 \cdot 10^3$	$30,6 \cdot 10^4 - 1563 \cdot 10^4$	$0,43 \cdot 10^2 - 8,95 \cdot 10^2$

1 2 3 4 5

Fig. 3. Virulence of plague bacillus strains for guinea pigs [Remainder of caption same as in Figure 1, page 4]

[Legend:] a) strains; b) inhibitory dosages in bacterial cells indicated; c) LD_{50} in bacterial cells; d) limits of fluctuations in LD_{50} in bacterial cells.

method of series solution of an antibiotic (Antibiotiki [Antibiotics], 1956, No 2) all strains proved highly sensitive: 1.5-3.8 micrograms of streptomycin inhibited the growth of plague bacillus. The control streptomycin-resistant B. pestis strain 491 grew in the presence of 250 micrograms of streptomycin.

The virulence of the strains was studied on guinea pigs and white mice. The method of the study was the same as that adopted in the initial investigation, only five-six animals were chosen for each dosage. The average lethal dosages were calculated according to Reid and Meuch method, and the limits of their fluctuations according to the Pezzi formula (Figure 2).

All the strains proved to be relatively highly virulent for white mice, and the most virulent proved to be the strains 911, 912, 913, and 1122 isolated from the fleas Amphalius runatus, Paradoxopsyllus scorodumovi, Amphipsylla primaris primaris (mean lethal dosage -- 100 bacterial cells, and less). The virulency of the remaining strains ranged from 162 to 1791 bacterial cells.

The virulence of the strains of guinea pigs was considerably less than for white mice. Only the strains 1122 and 1476-90 proved to be highly virulent for guinea pigs: the average lethal dosage was 1000 bacterial cells and lower. The LD_{50} of other strains fluctuated from $4.5 \cdot 10^3$ to $218 \cdot 10^6$ bacterial cells.

The results of the study of strain virulency for guinea pigs are given in Figure 3.

Isolation of a large number of plague bacillus cultures from guinea pigs sacrificed with chloroform on the 23-27th day can point to the capacity of these strains to induce lingering plague forms. This is supported by data of the average longevity of guinea pigs after infection -- 12-15 days, and only when infected with the strain 1122 did the lifespan equal 3.5 days.

Plague strains which are weakly virulent for guinea pigs and highly virulent for white mice have been described by many authors (Kovaleva, 1958; Kanatova, Kanatov, Bezhkova, 1961; Shchelkunova, Vasyukhina, 1960, etc.).

Comparing the virulency of strains for white mice guinea pigs noted in the initial and second study, we can

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see that during the first five months it does not change significantly.

CONCLUSIONS

1. For the first time in the territory of the Gorno-Altayskaya Autonomous Oblast, plague bacillus cultures have been isolated from wild beasts and their fleas.

2. Plague bacillus culture has been isolated from the predatory mammal flea Chaetopsylla homoeus, which up to now have not been found to be spontaneously infected.

3. The isolated cultures are typical in their cultural-biochemical properties and pathogenicity of plague bacillus, but have several peculiarities, namely: they ferment ramnose on the first day; in most cases they are weakly virulent for guinea pigs and relatively highly virulent for white mice.

4. The virulency of strains for guinea pigs and white mice during the first five months after isolation remained essentially unchanged.

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