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SUSCEPTIBILITY OF SOME SPECIES OF STEPPE RODENTS TO
ARTIFICIAL INFECTION WITH WEAKLY VIRULENT PLAGUE
STRAINS ISOLATED IN TRANSBAYKAL'YA

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SUSCEPTIBILITY OF SOME SPECIES OF STEPPE RODENTS TO
ARTIFICIAL INFECTION WITH WEAKLY VIRULENT PLAGUE
STRAINS ISOLATED IN TRANSBAYKAL'YA

- USSR -

[Following is the translation of an article by E. I. Klets, Z. S. Kudinova, V. S. Kolesnik, and G. P. Pletnikova in the Russian-language journal Izvestiya Irkutskogo gosudarstvennogo nauchno-issledovatel'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 34-46.]

In the summer of 1960, plague cultures were isolated from various rodents and fleas in the Transbaykalian enzootic focus, in the Gulzheng River valley (Kudinova, Larionova, Solodkaya, 1960), generally homogenous, typical as to bacterial morphology, growth characteristics on nutritive media, relationship to specific phage, and agglutinability of anti-plague serum.

Highly representative of these cultures was their innocuousness to guinea pigs and relatively weak virulence for white mice. This served as an incentive for more detailed study of the virulency of the isolated cultures, the more so in that they were detected on one of the areas of the enzootic focus after almost 15 years absence of bacteriological findings.

This report presents results obtained by the authors in studying the susceptibility of tarbagany, Dauriskiye susliks, Brandt voles, and Mongolian jerboas to artificial injection with these plague cultures.

Strain 580, isolated from fleas collected in a suslik burrow, strain 803 isolated from an adult Brandt vole, and strain 798 obtained from a trapped Daurskiy suslik underwent testing. A two-day old agar culture grown at 28° was selected for the infection. First strain 580 (flea) was tested. Tarbagany, Daurskiye susliks, Brandt voles, and white mice were infected subcutaneously with doses of 1,000, 100,000, 1 million and 1 billion bacilli -- three animals for each dose. All tarbagany and Brandt voles tolerated these dosages without visible harm to themselves and remained viable. Suslike did not succumb from the one billion bacilli dose. During the fifth-eighth one suslik perished from the 1,000 bacilli dosage, one from the 100,000 dosage, and one from the 10 million bacilli dose, but plague cultures were not isolated from the carcasses. The succumbed animals were autopsied and investigated according to the commonly adopted method. All surviving animals two weeks after infection were sacrificed with chloroform, excavated, and subjected to bacteriological and pathomorphological examination. Plague bacillus culture was isolated in the tarbagan group only from one animal, infected with a dose of 1 billion bacilli, and only from the site at which the bacterial suspension had been administered.

In the suslik group, plague bacillus cultures were isolated only at the site of administration of the bacterial suspension from all three animals receiving one billion bacilli, and from one of the three infected with 10 million bacilli. Plague bacillus culture was not isolated from a single Brandt vole. Culture also was not isolated from the surviving white mice, while from the succumbed mice plague bacillus growth was obtained from the organs. The inoculability of plague bacillus from the infected rodents is illustrated in Table 1.

When the animals were autopsied, a monotypical, but still very weakly pronounced pathologoanatomical picture was revealed almost independently of their biological species and infecting bacterial dose.

There were no changes as a rule detectable by the eye at the site where the bacterial suspension had been administered, and only among animals infected with the one billion bacterial cell dose (chiefly the susliks) was inflammatory focus encountered here, usually in the form of suppurative impregnation of tissues or organic intermuscular abscess.

Regional lymph nodes remained unchanged, less often became altered to some extent, but in general moderately,

Table 1

Inoculability of Plague Bacilli in Infection of Rodents with Strain 580 (flea)

Заражающая доза	Вид животного	Количество животных	Место заражения	Региональный лимф. узел	Селезенка	Печень	Кровь
1 млрд. (j)	Тарбаган (l)	2 (y)	-	-	-	-	-
	Тарбаган (l)	1 (y)	+	-	-	-	-
	Суслик даурский (m)	3 (y)	+	-	-	-	-
	Полевка Брандта (n)	3 (y)	-	-	-	-	-
	Белые мыши (o)	3 (a)	+	+	+	+	-
10 млн. (k)	Тарбаган (l)	3 (y)	-	-	-	-	-
	Суслик даурский (m)	1 (n)	-	-	-	-	-
	Суслик даурский (m)	1 (y)	-	-	-	-	-
	Суслик даурский (m)	1 (y)	+	-	-	-	-
	Полевка Брандта (n)	3 (y)	-	-	-	-	-
	Белые мыши (o)	1 (n)	+	+	+	+	-
	Белые мыши (o)	2 (y)	-	-	-	-	-

Symbols: Figure denote the number of animals of a given species; (y) -- surviving and then sacrificed with chloroform; (n) -- succumbing after infection; + -- culture isolated; - -- culture not isolated.

[Legend:] a) infective dose; b) species of animals; c) number of animals; d) site of infection; e) regional lymph node; f) spleen; g) lungs; h) liver; i) blood; j) one billion; k) 10 million; l) tarbagan; m) Daurskiy suslik; n) Brandt vole; o) white mice.

swelling, sometimes becoming mildly hyperemic. Other superficial lymph nodes became somewhat enlarged only in rare cases. The iliac lymph nodes in all species of animals, but less often in the tarbagan, on the side corresponding to where the culture had been administered also were somewhat swollen, more distinctly pronounced for a large infecting dose (1 billion bacilli).

The spleen was always of small size, with distinctly pronounced folliculi, more or less enlarged. The lungs looked completely normal. The liver, kidneys, and other organs also were without visible change.

We must especially note the pathologic-anatomical picture in white mice which were infected with a dose of 1 billion bacilli, succumbing from the disease, and from the organs of which plague bacillus cultures were isolated. At the infection site, hyperemia, gelatinous edema of cellular tissue, necrosis, and suppurative impregnation of tissues were noted. The inguinal and iliac lymph nodes were appreciably enlarged. The spleen was flaccid, the lungs hyperemic, liver in the state of parenchymatose (grainy-fatty) dystrophy.

Low susceptibility to infection of the animal species noted above was also established through a testing of two-day old broth cultures of the strains 580, 803, and 798. With one group of animals, the infection was subcutaneous, with another -- intraperitoneal. Tarbagany and susliks were infected with 1 ml, voles and mice -- with 0.5 ml per dose.

The animals, with the exception of white mice infected with the strains 580 and 798, did not succumb to the plague. The inoculability of plague bacilli in this experiment is shown in Table 2. It is characteristic that the growth of bacilli from surviving and then chloroform-sacrificed animals was not obtained.

When animals infected subcutaneously with broth culture were autopsied, no changes were found in the tarbagany and suslik at the site of injection. In the suslik group in only one case did cloudiness of tissues become evident, in another -- hyperemia, in a third (strain 803) in which the animal had succumbed on the third day, an abscess was found; in the remaining animals changes visible to the eye were absent. In white mice, both those sacrificed as well as those succumbing from the disease tissues were hyperemic and edemic, and in two of these an abscess was found, in one case lanced. In one mouse no changes at all were evident.

Regional lymph nodes in tarbagany were almost of normal size and appearance, in susliks and voles mildly or moderately enlarged, in white mice enlarged more appreciably and often hyperemic. Other lymph nodes in all species of animals in some cases appeared to be normal, in others swelling and fluid-laden appearance (hyperplasia) of the opposite inguinal lymph and iliac nodes was noted.

The spleen was either devoid of visible changes, or slightly enlarged, with well pronounced folliculi, sometimes giving the surface of the organ a granular appearance. Enlargement of the spleen was more distinctly pronounced in white mice, where in cases of murrain the organ appeared somewhat flaccid. The lungs were devoid of visible changes, and in only one of the succumbed mice was hyperemia noted. The liver in the tarbagany and susliks appeared completely normal. In the voles and white mice, sometimes (chiefly in the cases of animal murrain) were their signs of grainy-fatty dystrophy of the parenchyma; in one case a few grayish-white foci one-two mm in size were found in the autopsied mouse liver. Other organs appeared unchanged.

Table 2

Inoculability of Plague Bacilli from White Mice when Infected with Broth Culture of Strains Tested*

а) Штамм	б) Способ заражения	в) Количес- во живот- ных	г) Место вве- дения	д) Регионар- ные лим- фоузлы	е) Селезенка	ж) Легкие	з) Печень	и) Кровь
580	Подкожный Д	1 (п)	+	+	+	-	+	-
	.	1 (у)	-	-	-	-	-	-
	Внутрибрюшинный К	1 (п)	+	+	+	-	+	-
	.	1 (у)	-	-	-	-	-	-
798	Подкожный Д	2 (п)	+	+	+	+	+	-
	Внутрибрюшинный К	2 (п)	+	+	+	+	+	-
8С3	Подкожный Д	2 (у)	-	-	-	-	-	-
	Внутрибрюшинный К	1 (п)	-	-	-	-	-	-
	.	1 (у)	-	-	-	-	-	-

*Symbols are the same as in Table 1, page 3.

[Legend:] a) strain; b) method of infection; c) number of animals; d) site of administration; e) regional lymph nodes; f) spleen; g) lungs; h) liver; i) blood; j) subcutaneous; k) intraparenteral.

In intraparenteral infection, changes in general were as weakly pronounced as for subcutaneous, and were limited to mild hyperplasia of surface lymph nodes and spleen (chiefly its folliculi) with moderately pronounced grainy-fatty dystrophy of the parenchymatose organs in the succumbed animals (white mice).

After it was established that doses of 1,000, 100,000, 1 million, and 10 million bacilli did not result in the death of tarbagany, voles, and susliks, larger doses were selected for infection -- 3, 5, 10, and 15 billion bacilli. Four tarbagany and four susliks were infected with each dose, six Brandt voles, six white mice, and two guinea pigs. The results of the experiment are partially represented in Table 3. As we can see from this table, tabargany, daurskiye susliks and guinea pigs subcutaneously infected (strain 580 -- flea, and strain 803 -- vole) tolerated even doses of 15 billion bacilli without harm to themselves.

White mice always succumbed both from these large doses as well as from smaller (1 billion bacilli). The susceptibility of the Brandt vole could vary as a function of the strain chosen for infection. Strain 580 (flea) was found to be almost innocuous to the animal; thus, at dosages of 1, 3, 4, and 5 billion, all the animals survived, but at dosages of 10 and 15 billion only one vole out of six selected succumbed for each dosage (a total of two out of 18 perished). Strain 803 (vole), found to be incapable of killing voles at a dosage of 3 billion bacilli, indoses of 10 and 15 billion bacilli resulted in the death of a considerable fraction of these animals (a total of 9 out of 18 perished).

When material from succumbed rodents underwent bacteriological investigation (most of these perished on the third-7th day) quite satisfactory inoculability of the organs was established, which can be seen in Tables 4 and 5.

When the animals were autopsied swelling was noted at the infection site, and upon sectioning -- moderate hyperemia and hemorrhagic edema (chiefly after administration of strain 803 bacilli), disseminated suppurative impregnation of tissues, their inflammation, or the presence here of a limited abscess.

Regional lymph nodes were moderately enlarged (up to 5-6 mm), often hyperemic and edemic, sometimes adhered to the cellular mass, and in some cases hemorrhagic. Of the removed lymph nodes, the iliac and inguinal were slightly enlarged.

Table 3

Relative Susceptibility of Several Species of Field and Laboratory Rodents to Plague Bacillus Strains Tested

Испытуе- мый штамм	Вид животного	Заражающая доза				
		15 млрд.	10 млрд.	5 млрд.	3 млрд.	1 млрд.
800 (блошинный)	Тарбаган	0,4	0,4	0,4	1/4	0/3
	Суслик даурский	0,4	0,4	0,4	1/4	0/3
	Полевка Брандта	1,6**	1/6	0,6	0/6	0/3
	Белая мышь	6,6	6/6	6,6	5,6	3/3
	Морская свинка	0/2	0/2	0,2	0,2	—
803 (полевоч- ный)	Тарбаган	0,4	0,4	0,4	0,4	—
	Суслик даурский	1/4	0/4	1/4	0/4	—
	Полевка Брандта	3/3***	4/6	2,6	0/6	—
	Белая мышь	6,6	6/6	6/6	6/6	—
	Морская свинка	0,2	0,2	0,2	0/2	—

* Denominator denotes the number of infected animals, numerator -- the number of succumbed animals.

** Culture of plague bacillus obtained only from site of infection and regional lymph node.

*** Plague bacillus culture isolated only from one vole.

[Legend:] a) strain tested; b) species of animal; c) infecting dosage; d) 15 billion; e) 10 billion; f) 5 billion; g) 3 billion; h) 1 billion; i) (flea); j) (vole); k) tarbagan; l) Daurskiy suslik; m) Brandt vole; n) white mouse; o) guinea pig.

the submuscular almost unchanged. In some cases the spleen appeared to be unchanged, in others slightly swollen, and as a rule, it was either of ordinary size (0.5-1.5-2 cm) or even reduced; in addition, it was flaccid, pale red incolor, with well-pronounced folliculi; in two mice (of the number receiving 3 billion strain 803 bacilli) the spleen was unevenly permeated with disseminated and coursing yellowish gray small

Table 4

Inoculability of Plague Bacilli from Rodents Succumbing
After Subcutaneous Administration of a Suspension
of Two-Day Old Culture (strain 580 -- flea)

Инфицирующая доза в микробах (а)	Вид животного (б)	Место введения (в)	Региональный лимфоузел (д)	Селезенка (е)	Легкие (ф)	Печень (г)	Кровь (h)
15 млрд. (i)	Полевка Брандта (м)	+	+	-	-	-	-
.	Белая мышь (н)	+	-	+	-	-	-
.	.	-	-	+	+	+	+
.	.	+	-	-	-	+	-
10 млрд. (j)	Белая мышь (н)	-	-	+	+	-	+
.	.	+	+	+	+	+	-
.	.	+	-	+	+	+	+
5 млрд. (k)	Белая мышь (н)	+	+	+	+	+	-
.	.	+	+	-	-	-	-
.	.	+	+	-	-	-	-
.	.	-	-	+	+	+	+
3 млрд. (l)	Сусляк даурский (о)	+	-	+	+	+	+
.	Белая мышь (н)	-	-	+	+	+	-
.	.	+	+	-	-	-	-
.	.	-	-	+	-	+	-
.	.	-	-	+	-	-	-
.	.	-	-	+	-	+	-

Symbols: + = growth of plague bacilli was obtained; - -- growth of plague bacilli was not obtained.

[Legend:] a) infecting dose and numbers of bacilli; b) species of animals; c) site of administration; d) regional lymph node; e) spleen; f) lungs; g) liver; h) blood; i) 15 billion; j) 10 billion; k) 5 billion; l) 3 billion; m) Brandt vole; n) white mouse; o) Daurskiy suslik.

foci, possibly hyperplastic folliculi. The lungs in some animals remained unchanged (strain 580), in others (chiefly

strain 803) hyperemic, somewhat flattened; in one mouse (strain 803) the lungs were permeated by single reddish-brown foci, which as our personal experience has indicated, are found in guinea pigs after being administered live plague vaccines. The liver often was devoid of visible changes. Sometimes it was enlarged, flaccid, and appeared in cross section to be slightly dull; in addition, in several animals of the number infected with strain 803 culture (1 vole and 3 white mice) the liver was permeated with single small grayish white foci. Other organs lacked any peculiarities.

A picture of sepsis very similar to the situation in plague was found in histological examination of several mice; in particular, noteworthy is the abundance of bacilli at the injection site, in regional lymph nodes, and in the spleen, where they form characteristic clodlike clusters. In one vole an abundance of liver-permeating necrotic foci was found, infiltrating the stellate and epitheloid cells. Instances of decided granular dystrophy of the parenchyma cells of the liver and kidneys were observed in the absence of inflammatory changes in these organs.

When material from other rodents was subject to partial histological examination incapsulated and organized foci of leucocytaric infiltration (abscesses) were found at the site of culture injection (usually with inflammatory proliferation of polycellular giant cells the Langgans type) among other elements; moderate lymphoid hyperplasia and a serious-catarrrhal state of the cerebral sinuses were observed in the regional lymph nodes; in the removed lymph nodes as a rule there were no pronounced changes, or else they were limited to insignificant lymphoid hyperplasia; disseminated granulomatosis in the form of compact foci of reticular and epitheloid cells was established in the spleen of one tarbagan, while in other animals more or less distinct follicular hyperplasia was observed; cellular infiltration of interalveolar membranes was noted in the lungs, often with incipient epitheloid-cellular nodules; changes were completely absent in the liver of several animals, while in others bounded diffuse clusters of elements of reticulo-endothelium near blood vessels could be seen, which in one case (in a tarbagan with granulomatosis) was combined with necrosis of the parenchyma cells in the center of the foci; similar foci of "endothelial" element proliferation appeared infrequently in the kidneys.

The pathohistological picture described is characterized, in contrast to the picture in typical plague, by slightness and benign character of changes; in contrast not only

Table 5

Inoculability of Plague Bacilli from Rodents Succumbing After Subcutaneous Administration of a Suspension of Two-Day Old Culture (strain 803 -- vole)*

Заражающая доза (в микробах) ^(a)	Вид животного ^(b)	Место введения ^(c)	Региональный лимфоузел ^(d)	Селезенка ^(e)	Легкие ^(f)	Печень ^(g)	Кровь ^(h)
15 млрд. ⁽ⁱ⁾	Полевка Брандта ^(b)	+	+	+	-	+	-
.	Белая мышь ^(b)	+	-	-	-	-	-
.	.	+	-	+	-	-	-
.	.	-	-	+	+	+	-
.	.	+	-	+	+	+	-
.	.	-	-	+	+	+	+
10 млрд. ⁽ⁱ⁾	Полевка Брандта ^(b)	-	-	+	+	+	-
.	.	-	-	+	-	-	-
.	Белая мышь ^(b)	+	+	+	-	+	-
.	.	+	+	+	-	+	-
.	.	+	+	-	+	-	-
.	.	-	-	+	+	-	-
5 млрд. ⁽ⁱ⁾	Суслик даурский ^(b)	-	+	-	-	-	-
.	Полевка Брандта ^(b)	+	+	-	+	-	-
.	Белая мышь ^(b)	+	+	-	-	-	-
.	.	-	+	+	-	+	-
.	.	+	-	+	+	+	+
.	.	-	-	+	+	+	-
.	.	-	-	+	+	+	-
3 млрд. ⁽ⁱ⁾	Белая мышь ^(b)	+	+	+	-	+	-
.	.	+	+	-	-	-	-
.	.	+	-	-	-	-	-
.	.	+	+	-	-	-	-
.	.	+	-	+	-	+	-

*Symbols are same as in Table 4.

[Legend:] a) infecting dose and numbers of bacilli; b) species of animals; c) site of administration; d) regional lymph node; e)

spleen; f) lungs; g) liver; h) blood;
 i) 15 billion; j) 10 billion; k) 5 billion;
 l) 3 billion; m) Brandt vole; n) white mouse;
 o) Daurskiy suslik.

to experimental plague, but also to the vaccinal process induced by cultures of the plague strain 17 or EB 229, noteworthy here was granulomatosis, as a rule, with the formation in the nodules of polynuclear giant cells. This fact relates to some extent the established changes with those obtaining in pseudotuberculosis, although the biological uniqueness of the plague strains themselves is not precluded. The low susceptibility to infection by strains isolated in Transbaykal'ya was also revealed in white mice and guinea pigs in several parallel experiments. Composite data from the results of these experiments is presented in Table 6.

Table 6

Susceptibility of Guinea Pigs and White Rats to Plague Bacilli Strains Tested (Composite Data for Five Strains: 420, 580, 798, 803, 805)

a) Вид животных	b) Доза заражения в млн. об.б.					
	c) 100 тмс.	d) 100 млн.	e) 500 млн.	f) 5 млрд.	g) 10 млрд.	h) 15 млрд.
Морская свинка i) . . .	0/8	0/8	—	0/15	0/4	0/4
Белые крысы j) . . .	—	—	2/30	4/30	—	—

Remark. Denominator denotes number of infected animals; numerator -- number of succumbed animals.

[Legend:] a) species of animal; b) infective dose in number of bacilli; c) 100,000; d) 100,000,000; e) 500,000,000; f) 5,000,000,000; g) 10,000,000,000; h) 15,000,000,000; i) guinea pig; j) white rats.

As we can see from this table, guinea pigs did not succumb from any of the infective dosages used (from 100,000 to 15,000,000,000 bacilli). Of the 30 rats infected with 5,000,000,000 bacilli, only four succumbed, and out of 30

infected with the 500,000,000 bacilli dosage -- only two.

Separately investigated was the susceptibility of the Mongolian jerboa (trapped outside the enzootic zone). White mice and guinea pigs were selected as controls. Experimentation revealed (Table 7) that upon subcutaneous infection with strain 803 culture all the animals perished from a dosage of 15,000,000,000 bacilli; only some of the infected animals succumbed from the 5,000,000,000 bacilli dosage. Dosages from 5,000,000 and below did not prove fatal.

In one of our experiments (Table 3) animals surviving after infection were divided into two groups. The animals of one group, receiving 15,000,000,000 bacilli, were sacrificed with chloroform two weeks after infection, autopsied, and subjected to bacteriological and pathomorphological investigation.

Below is presented data found in the autopsy of tarbagany, susliks, voles, and guinea pigs.

No changes were established at the site of culture administration in tarbagany; a more or less large bounded abscess usually lying within the femoral muscles was found in susliks, especially in those receiving strain 803 culture; extensive tissue necrosis was observed in Brandt voles, which once more was commonest in animals given strain 803 culture. In the guinea pig group a lanced abscess (strain 803) was found in one case out of four at the site of inoculation, while in the remaining animals distinct changes either were not found, or moderate hyperemia was recorded. Lymph nodes, chiefly regional and iliac, were more or less appreciably hyperplastic, which was more clearly pronounced in voles and guinea pigs. The spleen in some cases remained unchanged independently of the animal species involved, while in others appeared slightly swollen, sometimes even apparently flaccid. Whitish spots (about 1 mm in size) were found in the liver of one tarbagan, one vole, one guinea pig, the spots appearing very numerous in the vole; in the remaining animals the liver as well as other organs appeared to be unchanged. Upon bacteriological investigation of this group of rodents a plague bacillus culture could be isolated only from one suslik at the inoculation site.

The second group of animals surviving after being administered 3, 5, and 10 billion bacilli, were newly inoculated one month after this infection (conventionally regarded as immunization), with a virulent strain of plague bacillus, the lethal dose of which is 10 bacilli for guinea pigs and

Table 7

Susceptibility of Mongolian Jerboa to One of the Strains Tested (No 803)

а) Вид грызунов	б) Заражающая доза в микробах									
	50	5 тыс.	50 тыс.	500 тыс.	5 млн.	50 млн.	500 млн.	5 млрд.	10 млрд.	15 млрд.
Песчанка монгольская ①	0/3	0/3	-	-	0/3	-	-	6/9	4/6	6/6
Белая мышь ②	1/3	2/3	2/6	1,6	2/9	2/6	5/6	9/9	-	-
Морская свинка ③	-	-	-	-	0/3	-	-	0/3	-	0/3

Remark. Denominator denotes the number of infected animals; numerator -- the number succumbing after inoculation.

[Legend:] a) rodent species; b) infective dose in number of bacilli; c) 5,000; d) 50,000; e) 500,000; f) 5,000,000; g) 50,000,000; h) 500,000,000; i) 5,000,000,000; j) 10,000,000,000; k) 15,000,000,000; l) Mongolian jerboa; m) white mouse; o) guinea pig.

white mice. This was done in order to discover whether the isolated strains exhibited immunogenic properties and whether they could be used as vaccine strains, since they satisfied one of the requirements placed on the vaccine strain -- innocuousness for guinea pigs in a dose of 10-15 billion bacilli when subcutaneously administered.

The experiment was performed on a limited number of animals and its preliminary results can be characterized by the following data (of Tables 8 and 9). Strains 580 (flea) and 803 (vole) produced in susliks, voles, and guinea pigs some degree of resistance to inoculation with 50, 20, and 2000 times the lethal dose of the virulent plague bacillus. It was possible to attain the same degree of resistance in guinea pigs after they had been previously immunized with a relatively low dose -- 100,000,000 or 100,000 bacilli of each of the weakly virulent strains.

Table 8
Results of Testing Immunogenic Properties of Strains
580 (Flea) and 803 (Vole)

a) Группы животных	b) Вид грызуна		
	в) суслики даурские	г) полевки Брандта	д) морские свинки
Иммунизированные (ф) штаммом 580	0/12 (250 тыс.) (i)	4/15 (10 млн.) (k)	1/6 (20 тыс.) (m)
Иммунизированные (ф) штаммом 803	0/11 (250 тыс.) (i)	3/10 (10 млн.) (k)	1/6 (20 тыс.) (m)
Контрольные (неимму- низированные) (h)	2/4 (5 тыс.) (j)	3/4 (50 тыс.) (l)	4/4 (10 тыс.) (n)

Remark. Denominator denotes the number of inoculated animals; numerator -- the number of these succumbing following inoculation, including those from which plague bacillus cultures were isolated; parenthesized is the number of bacilli in the dosage of virulent strain 1435 chosen for inoculation; animals previously receiving different doses of the strains tested were grouped together by species.

[Legend:] a) group of animals; b) rodent species; c) Daurskiye susliks; d) Brandt voles; e) guinea pigs; f) immunized with strain 580;

g) immunized with strain 803; h) control (non-immunized); i) 250,000; j) 5,000; k) 10,000,000; l) 500,000; m) 20,000; n) 10,000.

We must, however, remember that although the development of acute infectious process is prevented in guinea pigs, still after 22-30 days following inoculation these animals began to succumb with symptoms of severe emaciation and in the absence of any appreciable specific or general-infective changes, in which case no plague bacillus culture could be isolated. We are prone to regard this phenomenon as a form of the disease corresponding to cachexia observed in the lingering course of plague.

Table 9

Results of Inoculating Guinea Pigs with Plague Fifty Days After the Animals were Immunized with Cultures of the Strains Tested

Иммунизирующая доза (в микробах) ①	Заражающая доза ②	③ Количество животных	
		Всего заражено ④	из них пало ⑤
100 тыс. ⑥	500 Dcal (10 тыс. микробов штамма 1435, Dcal которого равно 20 микробам) ⑧	8	1
100 млн. ⑦		8	1

[Legend:] a) immunizing dose (in numbers of bacilli); b) infective dose; c) number of animals; d) total inoculated; e) subtotal succumbing; f) 100,000; g) 100,000,000; h) 500 Dcal (10,000 bacilli of strain 1435, the Dcal of which is 20 bacilli).

The low susceptibility of laboratory animals to plague strains with attenuated virulence, and the difficulties arising as a result of this in the bacteriological investigation of field material has long necessitated turning to attempts to find agents capable of reducing the resistance of the animal organism to artificial inoculation. Agents suggested for this purpose (Shtel'man, 1960; Kozakevich, Minkov, Sidorova,

1960; Akimovich, Dobrotavetova, 1960; Bratkova, 1960) include cortisone, histamine, Trypan blue, yolk of hens egg, etc., which are recommended to be subcutaneously administered to the animals before inoculation or simultaneously with inoculation.

We administered hen yolk simultaneously with inoculation in a separate experiment to reduce the resistance of the rodent organism to the weakly virulent strains under study. The experiment was conducted with white mice (since they are the generally accepted biopsy animals when working under field conditions). The results of the experiment shown in Table 10 demonstrate that the simultaneous administration of a bacterial suspension containing hens egg yolk increases the susceptibility of the animals to inoculation by weakly virulent or vaccine strains.

Thus, in testing the strains 580, 798, 803, and 17 (the last one usually proves fatal to white mice in a dose of 500,000,000) it was ascertained that a bacterial suspension in physiological saline solution in a dose of 1,000,000 bacilli does not result in the death of white mice; of 24 animals infected subcutaneously, only one mouse succumbed with negative bacteriological data. At the same time, a suspension of bacilli of this same strain in yolk induced the death on the 4-8 day of 19 mice out of 24 inoculated, where plague bacillus culture was isolated from 10 mice.

Table 10
Effect of Hens Egg Yolk on the Susceptibility of White Mice to Experimental Inoculation with Weakly Virulent Plague Bacillus Strains

а) Материал для заражения	б) Испытуемый штамм			
	580	803	798	17
Взвесь микробов в физиологическом растворе а)	1/6	0/6	0/6	0/6
Взвесь микробов в курином желтке б)	4/6	6/6	6/6	3/6

Remark: Denominator denotes the number of inoculated animals; numerator -- the number of those succumbing.

[Legend following page]

[Legend:] a) material for inoculation; b) strain tested; c) suspension of bacilli in physiological solution; d) suspension of bacilli in hens egg yolk.

Conclusions

Preliminary data obtained in testing susceptibility of several steppe rodents and laboratory animals to artificial inoculation by plague bacillus strains isolated in Transbaykal'ya in the summer of 1960 has shown that all these strains are weakly virulent for most of the animals tested and behave as avirulent with respect to guinea pigs and tarbagany. White mice showed the highest sensitivity to inoculation, where even from relatively low doses lethally progressing infection with impregnation of tissues and organs by bacilli developed. It was possible to observe the same kind of acute process in individual cases of Brandt voles, in Daurskiye susliks, and Mongolian jerboas when these animals were infected with doses of from 3,000,000,000 bacilli and higher. Bascillary impregnation of organs and tissues of the inoculated animals succumbing upon the development of infectious process proved as a rule to be significant and easily detected not only in the culturings, but also in the impression-smears made upon autopsy of the succumbed rodents, and also in histological sections.

But still, it was not possible to discover bacilli either bacterioscopically or bacteriologically even when an infective dose of 15,000,000,000 bacilli was used in surviving animals (tarbagan, suslik, Brandt vole, etc.) when the animals were autopsied two weeks after inoculation. And only in a few cases was bacterial growth observed in the culture taken from the site at which the infective material had been introduced (Daurskiye suslik). The susceptibility of rodents, in particular white mice, could be considerably increased if along with the bacterial suspension hens egg yolk was administered. In these cases the inoculated mice succumbed in fact from low doses, which when introduced without the yolk did not result in the death of the animals. The same regularity prevails when white mice were administered plague bacillus 17 culture in combination with yolk. Here the animals succumbed from a dose of 1,000,000 bacilli, while when the culture was given without yolk doses of 500 million -- 300 million bacilli were necessary.

The necessary susceptibility of animals to inoculation recorded obviously stems from the inhibiting effect of the protective functions of the organism. It can be assumed that both in the natural environment when conditions unfavorable to the rodents prevail the same increase in sensitivity of rodent organism to weakly virulent plague strains can develop. The infectious process of these cases can be accompanied by the entry of the bacillus into the blood stream, thus making inoculation of the fleas possible. The latter evidently occurs also when the plague bacillus is isolated (strain 580, flea) from a suspension of fleas collected at the entry to the suslik burrow.

The assumed inhibitory action of parenterally administered yolk on the resistance of the organism to infections must be used in investigating the enzootic focus. The biopsy animal (white mice) must be administered the material under study in a mixture with hens egg yolk. In addition, the yolk can be given to trapped rodents (voles, susliks), then observing them for 7-10 days in order to detect latently occurring infectional process.

Testing of the immunogenic properties of the strains (580, 803) revealed their pronounced ability to protect animals against subsequent inoculation by a culture of virulent plague strain. However, it still remains unclear whether this ability would be evidenced at lower dosages.

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