**AD NUMBER**

AD815215

**NEW LIMITATION CHANGE**

**TO**

Approved for public release, distribution unlimited

**FROM**

Distribution authorized to U.S. Gov’t. agencies and their contractors; Critical Technology; DEC 1965. Other requests shall be referred to Army Biological Laboratories, Fort Detrick, Frederick, MD.

**AUTHORITY**

SMUFD D/A ltr, 15 Feb 1972
CONCERNING THE MAIN PROPERTIES OF STRAINS OF P. PESTIS ISOLATED IN 1962 IN ARMENIA

Translation No. 1713

DECEMBER 1965

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of

U.S. ARMY

BIOLOGICAL LABORATORIES

FORT Detrick, Frederick, Maryland
DISCLAIMER NOTICE

THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.
DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

This publication has been translated from the open literature and is available to the general public. Non-DOD agencies may purchase this publication from Clearinghouse for Federal Scientific and Technical Information, U.S. Department of Commerce, Springfield, Va.

Technical Library Branch
Technical Information Division
CONCERNING THE MAIN PROPERTIES OF STRAINS OF P. PESTIS
ISOLATED IN 1962 IN ARMENIA


Translation performed by Sp/7 Charles T. Oertel, Jr.

From July through August 1962 in the territory of the northwest outskirts of Zangezur in the Armenian SSR, a plague epizootic was observed among common voles. During the stated period the laboratory of the Sisian Epidotryad investigated 2347 specimens of common voles, 484 social voles, 197 snow voles, 22 water voles, 13 bush voles, 435 forest mice, 209 house mice, 75 Zakavkasiki hamsters, 62 forest dormouses, 4 Persian gerbils, and 1 slepushonka (meaning unknown, probably related to SLEPSHES - Spalacidae (a family of rodents).)

All told during the period of observation 47 strains of P. pestis were isolated; of these 14 were from common voles and 33 from fleas collected in the nests of these voles (Ctenophthalmus vladimiri - 27 strains, Ceratophyllus caspius - 3, Frontopsylla elata caucasica - 2, Amphipsylla rossica - 1).

The investigation of rodents of other species, captured in the zone of the epizootic and adlinging regions, produced a negative result in all cases.

In the process of the investigation on the territory where the epizootic had taken place, not one rodent was detected which had died from plague. The cultures of the plague microbe were isolated only from voles which had been captured alive. Apparently the infectious process in common voles took place mainly in a benign manner. This assumption is supported to a certain degree by the data of laboratory investigations. Thus, for example, 6 strains were obtained from animals, in whose organs upon autopsy there were noted only the necrotic changes which are characteristic for a lingering course of the disease. Besides this, in 5 rodents from which cultures of the plague microbe were isolated, apparent pathologoanatomical changes were generally absent.
It is necessary to note that in a whole number of cases the
direct inoculation of the organs of rodents and fleas on nutrient media
did not expose the presence of the causative agent in them and cultures
of the plague microbe were obtained only due to a biological test animal.

In the present report the materials are presented which character-
ize the main properties of the 44 strains of P. pestis which were
isolated during the epizootic.

At the time of isolation all the strains were found in the R-form.
During the course of a 2-3 week period of observation following iso-
lolation, the phenomena of dissociation and the signs of affection by
bacteriophage were not noted in the cultures (an exception was strain
367, obtained from a white mouse, infected by a group of Ct. wladimiri
fleas, in which the growth of the structure of the colonies became very
untypical on the second day). In the majority of the strains the form
of the colonies was the same and was characterized by a dark brown,
raised, fine grained center with a smooth, pale, irregular edge, and a
lacy peripheral zone. Upon aging the colonies lost the peripheral zone,
the center became more compact and the intensity of pigmentation increased.

Growth on broth was typical — the broth was clear, there was a
porous precipitate and a parietal granular or flaky suspension. While
the broth cultures were maintained in the incubator, a delicate film,
which split easily upon shaking, developed. During the study of the
strains in the first generations, turbidity of the broth was not noted.

In smears from the organs of voles and from biotest animals the
microbe was in the form of a polymorphic gram-negative bacillus with
bipolar staining. Mobility of the bacterial cells was absent (checking
was done on semiliquid agar).

For studying the biochemical, serological and other properties of
the isolated cultures, in all cases we used the third generation in
nutrient media.

On the first day of observation the strains under study fermented,
with the formation of acid without gas, glucose, mannitol, maltose, and
incompletely rhamnose. On the second to the fourth days, and later in
some strains, acid formation was recorded in litmus milk, glycerin, and
lactose. Some of the strains caused a weak splitting up of saccharose
(16 strains out of 44). Upon checking the biochemical properties on the
medium suggested by L. A. Timofeyeva, in the first two days there was
noted a change of coloration which is characteristic for P. pestis:
Column of the medium was orange, and the slanted part — blue. Starting
with the third day a change was recorded in the color of the tapered
surface, which indicated the fermentation of lactose by the strains. It
must be noted that when studying the fermentation activity of the strains on carbohydrates the appropriate controls were set up -- with the P. 
pestis EV vaccine strain and two strains of E. coli. One of the strains 
of E. coli -- No 11 (reference number), intensively fermented the entire 
collection of carbohydrates, with the exception of saccharose.

Not one of the strains formed indole, some gave off hydrogen 
sulfide. The test with urea was negative in all cases (the observations 
were carried out for three days). The reducing capability in respect 
to methylene blue was checked in 22 strains, also with negative results. 
All the strains reduced nitrites to nitrites, that is, possessed a 
denitrifying capability.

It must be noted that when studying later generations of the 
strains, results were obtained which testified to the heterogeneity of 
their cellular composition. In particular the appearance was recorded 
of a different type of colonies -- chromogenic and achromogenic with 
smoothened out contours. There was a marked division of the population 
of two strains into rhamnose-positive and rhamnose-negative variants 
(the populations of the remaining strains were not investigated in this 
respect). In the strains which fermented saccharose a tendency was 
ascertained toward the loss of this feature.

The ability for growth on hungry media was tested in 27 strains. 
The investigation showed that on deficient-acid agar the stated strains 
do not grow following the standard seeding. On peptone deficient agar 
in the first two days, growth was noted from $1-4$ dilutions, and in 
later periods (5 days) in 12 strains the growth of individual colonies 
was recorded up to the 10th dilution.

All the strains turned out to be sensitive to polyvalent plague 
and pseudotuberculosis bacteriophage. During titration according to 
Appelman the plague caused lysis in $10^{-7}$ and $10^{-8}$ power of dilution with 
a phage titer of $10^{-9}$. The sensitivity to pseudotuberculosis phage was 
approximately the same in the strains studied ($10^{-6}, 10^{-7}$). 

Serological properties were studied in 20 strains -- they were all 
agglutinated by plague serum up to a titer (1:1600).

By the method of diffusion in agar with the help of discs, a check 
was made of the sensitivity of 21 strains to antibiotics: Streptomycin, 
biomycin, levomycetin, and penicillin. All the strains turned out to be 
sensitive to streptomycin. Different results were obtained in respect 
to biomycin and levomycetin. Out of the 21 strains checked, 13 turned 
out to be highly sensitive to levomycetin, 6 were mildly sensitive, and 
2 were resistant. Biomycin acted on 13 strains, and 8 strains were 
resistant. It is interesting to note that almost all the strains (19 out 
of 21) were sensitive to penicillin.

The virulence of the isolated cultures was checked on white mice, 
guinea pigs, and white rats.
On white mice 23 strains were titrated. In the majority of these, doses of 10 and 100 microbial bodies (m.t.) caused the death of part of the infected animals. With an increase of the infecting dose there was an increase in the percentage of mice which died. Absolutely lethal doses were: 10 m.t. in one strain, 100 m.t. in 5 strains, 1,000 m.t. in 13 strains, 10,000 m.t. in 3 strains, and 100,000 m.t. in one strain.

The virulence of 9 strains was studied on guinea pigs. The infections were carried out subcutaneously. Infection doses up to 1 billion m.t. were used. Of the entire number of test animals only one pig died from one billion m.t. This was in 11 days following infection, and *P. pestis* was seeded out from the regional lymph node. The rest of the animals remained healthy and were destroyed in 30 days. A bacteriological investigation of them produced a negative result.

The white rats turned out to be sensitive to the isolated strains. For these animals the Dcl equaled one billion m.t., but a significant number of them died from the administration of 10,000 and even 1,000 m.t.

In preliminary tests the immunogenic properties of 4 strains were studied. It was established that a single subcutaneous administration of 100 m.t., and larger doses of these strains protected guinea pigs from subsequent infection with 200 Dcl of the highly virulent *P. pestis* strain 261.

In this manner, the cultures of 1962, isolated in Armenia from common voles and their fleas, based on their main biological properties (relatively weak virulence, fermentation of rhamnose and glycerin) are identical on the whole to the strains isolated during the epizootic of 1958--1959 in north-western Armenia.

**Literature**

