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DEPARTMENT OF THE ARMY
Fort Detrick
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ASPECTS OF THE ECOLOGY OF PLAGUE AND PSEUDOTUBERCULOSIS BACTERIA

Report 3

Fate of the Plague and Pseudotuberculosis Bacteria in Mixed Cultures

[Following is the translation of an article by V. A. Bibikova, L. N. Klassovskiy, L. M. Osadchaya, and V. S. Petrov, Central Asian Antiplague Institute, published in the Russian-language periodical ZhMEI (Journal of Microbiology, Epidemiology, and Immunobiology) No 5, 1967, pages 138-139. It was submitted on 15 Dec 1966. This article appeared in the section Authors' Abstracts.]

Studies were made of the cellular composition in mixed populations of the causative agents of plague and pseudotuberculosis on nutrient media, and also in the organism of warm-blooded animals and fleas.

In the work we used the highly virulent strain of plague causative agent No 100 (Dlm during subcutaneous infection of white mice and guinea pigs 10^2 microbial cells); pseudotuberculosis causative agent strain No 1421, virulent for white mice (Dlm 10^3 cells) and weakly virulent for guinea pigs; pseudotuberculosis causative agent strain No 4520, virulent for white mice in any method of infection (Dlm 10^2 cells) and guinea pigs (Dlm 10^3 cells); pseudotuberculosis microbe strain No 100-38 ("neogenic"), obtained from a population of virulent strain of plague microbe No 100 as a result of seeding of the latter on synthetic media without amino acids (by the start of the experiments the strain possessed weak virulence for white mice and guinea pigs). All three strains of the pseudotuberculosis causative agent were able to cause lethal infection in four species of jerboa (small, plumiped, Severtsov, and Zhitkova) during subcutaneous infection with relatively small doses of bacteria - 10^3 microbial cells (the high sensitivity of jerboas to infection makes these animals valuable models which are convenient for the experimental study of pseudotuberculosis).

All the tested strains of pseudotuberculosis causative agent (including "neogenic") were related to the first serological type according to Talya and possessed all the characteristic properties for this type of microorganism; the variant of strain No 4520 used in the experiment was characterized by a resistance to pseudotuberculosis bacteriophage.

For obtaining mixed populations we resorted to the mixing of titrated suspensions of the corresponding bacteria. The mixtures of cells of plague and pseudotuberculosis causative agents were inoculated in flasks with Hottinger broth (100 ml). The cultures were incubated for 12 days at 28° with a periodic determination of the ratio of causative agents of plague and pseudotuberculosis. The results of the experiments showed that the ratio of cells during cultivation in Hottinger broth changed rapidly in favor of the pseudotuberculosis bacteria. However, the complete elimination of the plague causative agent in the course of 12 days of cultivation did not take place, which testified to their ability to develop (though at a slowed down rate) in the presence of the pseudotuberculosis causative agent. During frequent reseedings (after every 2 days) on broth the dislodgment of plague microbes from the population took place more rapidly, but even after 5 reseedings they were still detected in mixed cultures.

During infection of white mice and guinea pigs with a mixture of cells of the plague causative agent strain No 100 and the more virulent (in our collection) pseudotuberculosis causative agent strain No 4520 a more intensive multiplication of the plague causative agent took place in the organism of susceptible animals. However, in 24% of the white mice and 60% of the guinea pigs a mixed infection developed. This was testified to by the simultaneous isolation of the two types of microorganisms from the organs of dead rodents. In such cases in seedings of organs we observed the profuse growth of plague and the less frequent isolated colonies of pseudotuberculosis bacteria.

In experiments with Xenopsylla cheopis fleas we used the same mixed cultures as in the experiments on animals. The fleas were infected by feeding on the tail hides of white mice which were filled with a mixture of defibrinated rabbit blood and bacteria. In the organism of fleas the ratio of cells in the population was changed in favor of the plague causative agent. This was testified to by direct counts of colonies in seedings from the contents of ventricles of fleas on agar plates. Lengthy periods of preservation in the organism of fleas were characteristic for the plague microbe. The bites of fleas, infected with a mixture of the plague and pseudotuberculosis causative agents, in some cases led to the emergence of mixed infection in susceptible animals. This took place in the presence of the pseudotuberculosis microbe in fleas, the gastroenteritic tract of which was obstructed by plague microbes. At the same time experiments on the infection of animals by the bites of fleas which had been preliminarily infected with pure cultures of pseudotuberculosis causative agent did not produce a positive result.