Plague bacteria take refuge in amoebae

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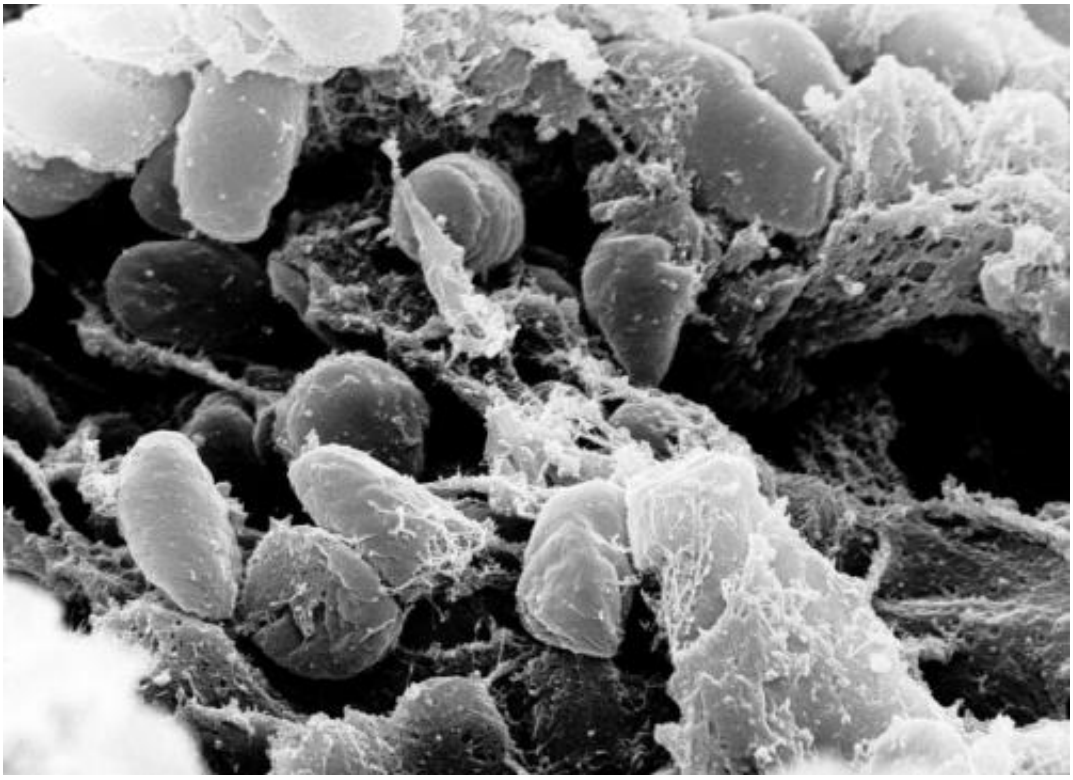

A scanning electron microscope micrograph depicting a mass of *Yersinia pestis* bacteria in the foregut of an infected flea. Credit: Wikipedia

Yersinia pestis, the bacterium that causes bubonic plague, can survive within the ubiquitous soil protozoan, the amoeba, by producing proteins that protect against the latter microbe's digestion. The research is published April 28th in *Applied and Environmental Microbiology*, a journal of the American Society for Microbiology.

The research is important because plague is a re-emerging disease, according to the Centers for Disease Control and Prevention, with 95 percent of cases occurring in sub-Saharan Africa and Madagascar. Modern antibiotics are effective, but without prompt treatment, plague can cause serious illness, or death.

Y. pestis spreads from rodent to rodent, and sometimes to human, often via fleas It uses the protective niche of the amoeba to abide when conditions are unfavorable to its spread, that is, when rodents are scarce, said Viveka Vadyvaloo, PhD, Assistant Professor, Paul G. Allen School for Global Animal Health, Washington State University, Pullman.

Amoebae are similar to certain human immune cells, the macrophages, in their ability to engulf bacteria, or other nourishing items of similar size.These are taken up within special compartments called vacuoles, which in both amoebae and humans are capable of digestion. (image: scanning electron micrograph of a mass of Yersinia pestis.)

"With this in mind, graduate student Javier Benavides-Montaño separately cultured three distinct *Y. pestis* strains that have been associated with human epidemics, with a common laboratory strain of the free-living soil amoeba, Acanthamoeba castellanii, in a medium that supports the latter's growth," said Vadyvaloo.

Benavides-Montaño then tested *Y. pestis*' ability to enter and survive within the amoeba. To do so, he killed any bacteria that were outside of the amoebae, and then gently lysed the latter, and then placed the lysed content on a medium that encourages *Y. pestis* to grow. They were able to culture *Y. pestis* only after the amoebae had been lysed.

The investigators also used electron microscopy to peer inside intact amoebae, and found *Y. pestis* within the vacuoles.

"To understand more about how *Y. pestis* might be surviving within amoebae we considered how *Y. pestis* survive in human macrophages," said Vadyvaloo. "Macrophages usually engulf bacterial pathogens and destroy them, but some bacterial pathogens are able to avoid being killed therein by producing proteins that block the digestion." Indeed, that is the key strategy for a number of human pathogens. Some such proteins are known. So the investigators used mutant *Y. pestis* that doesn't produce one of these proteins. Those mutants failed to survive within the amoebae.

Vadyvaloo said that amoebae's longstanding reputation as Trojan horses for human pathogens led her to investigate the possibility that plague bacteria could abide within their vacuoles. The best known example of this phenomenon had been Legionnaires' Disease, a respiratory disease that was discovered in 1976 after an outbreak among attendees at a convention of the American Legion in Philadelphia.

"This study serves as a proof of principle that amoebae can support prolonged survival of *Y. pestis* in the environment," said Vadyvaloo. It may encourage a search for this interaction within areas of Colorado and New Mexico where plague is endemic. And that, she said, could enable prediction of potential disease re-emergence, thereby reducing spread to humans.

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