Anthrax can grow and reproduce in soil, researchers find

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(Phys.org)—Anthrax has the unexpected ability to grow and reproduce while lurking in soil – increasing the deadly bacteria's chances to infect cattle and other mammals, researchers at the University of Virginia School of Medicine have discovered.

Until now, experts have widely believed that anthrax spores remain dormant in soil until eaten by cattle, then germinate and cause the deadly disease. But U.Va. researchers have found that the spores can attack a common soil and water amoeba, Acanthamoeba castellanii, turning these single-celled organisms into anthrax incubators.

"These amoeba normally eat bacteria and kill them, but Bacillus anthracis has figured out some way to manipulate that amoeba so that it can actually grow inside the amoeba and increase its numbers," Ian J. Glomski, an assistant professor of microbiology, explained.

The process, he notes, gives the anthrax a selective advantage. "The interactions with the amoeba, essentially, are making certain that the anthrax has the tools to kill the amoeba, and those same tools are potentially being used to infect animals and humans," he said.

The discovery helps answer longstanding questions about the bacteria and may lead to new techniques to control anthrax and prevent infection around the world.

Anthrax outbreaks typically occur after rainy weather in warm months,

usually striking animals that graze in depressions where the grass is greenest. Scientists have commonly thought that rainwater runoff was concentrating spores in low-lying areas. The conclusion was logical, based on what science knew about anthrax.

"If you put Bacillus anthracis into soil with basically any other common soil bacteria, it will be out-competed. The other bacteria will eat up all the nutrients before Bacillus anthracis can do significant growth," Glomski said. "So for all intents and purposes, it has been thought that the spores sit in the ground and do nothing until they go into an animal and cause disease."

But the U.Va. researchers, inspired by postdoctoral fellow Rafik Dey, wondered if something else was happening in the warm, moist, alkaline earth where the spores are most common.

"There's a rich history of amoeba being associated with diseases," said researcher Paul S. Hoffman, a professor of infectious diseases. "We tried to make that connection with the anthrax by asking, 'Could the amoeba have a role in the environment?'"

To find the answer, they set out to recreate the warm, wet conditions in the lab. When they placed anthrax spores in sterile creek water, there was no sign of germination. But when they combined spores and Acanthamoeba castellanii, a type of amoeba active in warm weather, the result was a nearly fiftyfold increase in spores in the creek water within 72 hours. Under optimal conditions of approximately 37 degrees Celsius (about 99 degrees Fahrenheit), the spores increased nearly a hundredfold.

The U.Va. researchers believe anthrax preys upon Acanthamoeba castellanii and other amoeba because the bacteria contains two plasmids – a type of DNA molecule – that the anthrax needs for growth. Lab tests

using an anthrax strain without plasmids did not generate additional spores. That information could help scientists begin to determine which genes allow anthrax to reproduce in these amoeba (and potentially other protozoa).

"We may find other species of amoeba that are even better at this than what we were using in the lab," Hoffman said. "We may be at the tip of the iceberg."

While the availability of a veterinary vaccine helps prevent anthrax outbreaks in the United States, U.Va.'s discovery could benefit the many areas of the world that struggle with the persistent and pestilent bacteria. Unvaccinated cattle can't graze without dying in some fields in Europe and elsewhere, Hoffman said.

"Just the knowledge gives you a general sense of where not to put your animals if you do have problems," Hoffman said. "In developing countries that don't have a lot of resources, you can strategize how to avoid certain areas because you know that will be problematic at a particular period of the year."

Glomski noted that the discovery offers new targets for researchers seeking to prevent the spread of anthrax. "If we can figure out any way to disrupt the cycle, that would effectively eliminate the problem. It could be doing something to the bacteria, doing something to the amoeba, doing something to prevent their interaction …" he said. "If we really understand those interactions, we'll have more and more points of intervention to think about."

The findings have been published online by *Applied and Environmental Microbiology*, a journal of the American Society for Microbiology.

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