

Protozoa: An Animal-Research First

Protozoa Bolsters Bacterial Virulence *Inside* Animals

ARS scientists have found that an antibiotic-resistant strain of *Salmonella* becomes especially virulent when tucked inside protozoa in the rumen, or first stomach, of cattle. Protozoa are one-celled predatory organisms that engulf and destroy most bacteria.

This breakthrough finding at ARS's National Animal Disease Center (NADC) in Ames, Iowa, represents the first time that disease-causing bacteria have been found to gain strength from interaction with protozoa inside animals. It suggests that naturally occurring digestive tract protozoa may be a place where dangerous bacteria can also lurk and develop.

The Ames scientists also found a way to combat this virulent *Salmonella* strain by use of a cleansing process, called "defaunation," which rids the rumen of protozoa.

"We've known for a long time that protozoa exist in cattle," says NADC microbiologist Mark Rasmussen, who teamed with veterinary medical officer Steven Carlson to lead this study. "Indeed, protozoa benefit the cow. They assist it with digestion.

"We'd never thought of protozoa as being reservoirs of disease in animals. But we found that there's something about being inside the protozoa that turns on certain defense mechanisms that the bacteria later use to cause infection."

Carlson and Rasmussen conducted this research with postdoctoral molecular biologist Zoe McCuddin and microbiologist Sharon Franklin. All are in NADC's Pre-Harvest Food Safety and Enteric Diseases Research Unit.

Salmonella is one of the leading causes of foodborne illness in people. The bacteria can be traced to poultry, beef, pork, and other foods of animal—and plant—origin. In animals, salmonellosis is usually a self-limiting diarrheal disease not requiring antimicrobial therapy. But antibiotics are needed when severe diarrhea or systemic infections occur. "Unfortunately, many *Salmonella* strains have become resistant to many antibiotics," says Carlson.

A Sickening Relationship

The Ames scientists studied the relationship between rumen protozoa and *Salmonella's* virulence and resistance to antibiotics.

Carlson explains that some bacterial pathogens, including *Salmonella*, appear to resist destruction while inside protozoa. "Not only may the protozoa be a holding place for certain pathogens," he says, "but they seem to create a 'survival of the fittest' scenario for the *Salmonella* that leads to more virulent pathogens."

According to Rasmussen, the strengthening of disease-causing bacteria while they occupy protozoa is a phenomenon that's been known since independent studies revealed a probable role of protozoa in strengthening *Legionella*. That bacterium causes Legionnaires' disease, an airborne pulmonary affliction that got its name when an outbreak in 1976 killed 34 people at an American Legion convention in Philadelphia.

"The process has been shown with free-living protozoa in places such as water-cooling towers and ponds," says Rasmussen. "Now, here at NADC, we've seen this process for the first time at work inside an animal."

Disease-causing bacteria have been found to gain strength from interaction with protozoa inside the digestive tract of cows.

JAMES FOSSE (D384-1)



Microbiologists Mark Rasmussen and Sharon Franklin examine a culture of rumen protozoa.

Electron micrographs on this page provided by Sharon Franklin, NADC. Colorization by Stephen Ausmus.

A Particularly Virulent Strain

The scientists focused on an *S. enterica* strain named DT104. “That’s a multiresistant, hyperpathogenic foodborne pathogen believed to be more virulent than its antibiotic-sensitive counterparts,” says Carlson. “Calves infected with antibiotic-resistant DT104 are 13 times more likely to die when compared to calves infected with antibiotic-sensitive versions of this strain.”

He says that, while factors causing DT104’s hyperpathogenicity have not been completely identified, the strain seems to owe its resistance to multiple antibiotics to

an integron—or gene cluster—structure designated as “*Salmonella* genomic island 1” (SGI1). Its genes encode resistance to five different antibiotics.

The Ames researchers examined 38 *Salmonella* strains. Only those possessing SGI1 were found to be more virulent, as measured by a dramatic increase in their ability to invade intestinal cells after exposure to protozoa.

“We now believe that DT104’s enhanced invasive ability is the result of an overactivation of invasion genes while within the challenging environment inside the protozoa,” says Carlson. “The hyperinvasive DT104 is then released from the rumen protozoa after normal digestion and travels into the intestine.”

The defaunation technique came about from efforts by the researchers to counter this troublesome sequence. “It’s a chemical process in the rumen that’s similar to deworming,” says Rasmussen. “Potentially, we can clean the animals out and eliminate the hidden bacteria on a regular basis.”

He says that fewer DT104 cells were recovered from tissues of infected animals when protozoa were destroyed through defaunation.

“This shows that if rumen protozoa are a significant reservoir of resistance and virulence, periodic defaunation of the bovine rumen may be warranted. And while protozoa are a normal part of rumen microbiota, their temporary removal doesn’t appear to harm the animals.”

Rasmussen says this study likely represents an initial step toward discoveries with wider implications.

“There are many different organisms known as intracellular pathogens, and we want to see whether they go through the same process,” he says. “The *Salmonella* study was our first look into this phenomenon inside animals.”

“What we’ve done is expand the view of how *Salmonella* works and devise a possible strategy against it,” McCuddin adds. “Defaunation has stoked interest in the livestock and dairy industries as a preventive measure. But the overall study may have opened the door to understanding other pathogens.”—By **Luis Pons, ARS.**

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

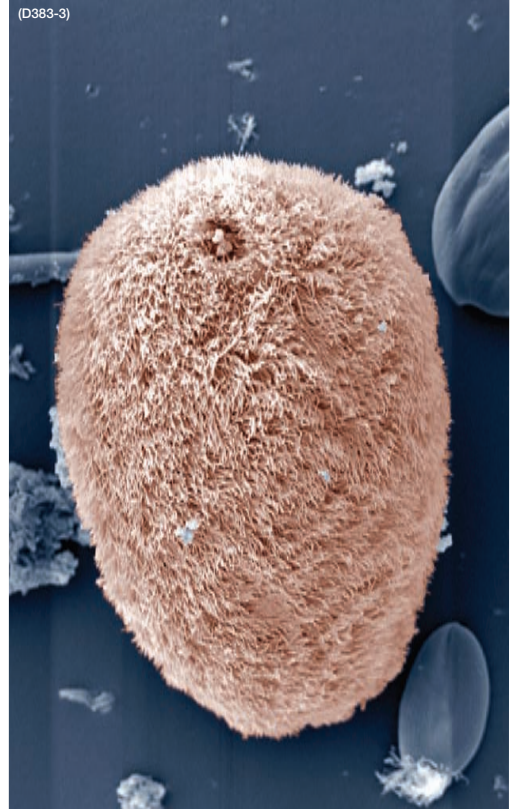
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The protozoan *Ophryoscolex* spp. has a unique and complex cell structure. With an average length of 185 micrometers, it is one of the largest in the cow’s rumen.

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Isotricha intestinalis, a protozoan distinguished by its mouth position. This protozoan can be up to 200 micrometers long, making it the largest in the rumen.

Entodinium protozoan undergoing early stage division. Ranging in length from 35 micrometers to 95 micrometers, they are the smallest protozoa in the rumen.

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