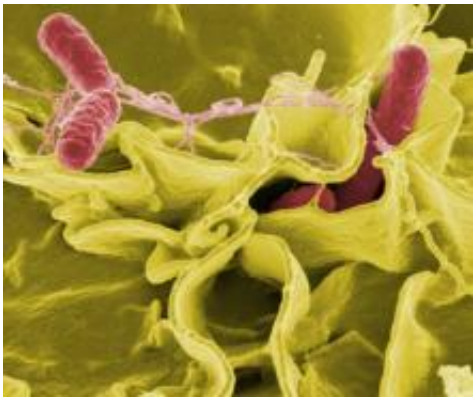
Antibiotics may help Salmonella spread in infected animals

October 20 2014



Salmonella Saintpaul bacteria (dark red)

Some people infected with pathogens spread their germs to others while remaining symptom-free themselves. Now, investigators at the Stanford University School of Medicine believe they may know why.

When the scientists gave oral antibiotics to mice infected with Salmonella typhimurium, a bacterial cause of food poisoning, a small minority—so called "superspreaders" that had been shedding high numbers of salmonella in their feces for weeks—remained healthy; they were unaffected by either the disease or the antibiotic. The rest of the mice got sicker instead of better and, oddly, started shedding like superspreaders. The findings point to a reason for superspreaders' ability to remain asymptomatic. They also pose ominous questions about the

widespread, routine use of sub-therapeutic doses of antibiotics in livestock.

About 80 percent of all antibiotics used in the United States are given to livestock—mainly cattle, pigs and chickens—because doing so increases the animals' growth rates. Experts have already voiced concerns about how this practice contributes to the rise of drug-resistant pathogens. But the new study, to be published online Oct. 20 in *Proceedings of the National Academy of Sciences*, highlights an entirely different concern.

"We've shown that the immune state of an infected mouse given antibiotics can dictate how sick that mouse gets and also carries implications for disease transmission," said Denise Monack, PhD, associate professor of microbiology and immunology and the study's senior author. "If this holds true for livestock as well—and I think it will—it would have obvious public health implications. We need to think about the possibility that we're not only selecting for antibiotic-resistant microbes, but also impairing the health of our livestock and increasing the spread of contagious pathogens among them and us."

Upon invading the gut, S. typhimurium produces a powerful inflammation-inducing endotoxin, which annually results in an estimated 1 million cases of food poisoning, 19,000 hospitalizations and nearly 400 deaths in the United States. Passed from one individual to the next via fecal-oral transmission, it is known to produce a curious pattern of pathology among infected individuals: Some 70-90 percent of those infected shed fairly light amounts of bacteria (and so are not very contagious). But the remaining 10-30 percent—superspreaders—remain symptom-free yet shed huge amounts of bacteria, causing the great bulk of the pathogen's spread through a population. The reasons for this dichotomy have not been understood.

Evading detection

From a public health standpoint, knowing how to easily and quickly identify superspreaders could help curtail or even prevent epidemics, Monack said. Yet superspreaders don't appear to be sick, so they evade treatment. At the moment, the only way to determine which category a person or beast belongs to is by inspecting each individual's stool, a procedure that would be inconvenient at best even with livestock.

But the Stanford team has discovered that the immune systems of superspreaders and non-superspreaders are in differing states, raising the possibility of a blood test that could make identifying superspreaders more practical.

Salmonella infection in mice is not uncommon, said Monack. "Mice in a barn can be infected with salmonella for a long time and not get sick. They run around perfectly healthy. They're happy little incubators for salmonella."

In Monack's lab, more than 1 in 5 salmonella-infected mice are superspreaders. "The mice we use are inbred," she noted. "So this difference in response to salmonella infection can't be just a simple matter of genetic mutations."

The Stanford investigators had previously published work showing that giving non-superspreader mice an oral antibiotic, which kills some of the friendly microbes that ordinarily inhabit mammals' intestines and provide protection against invading pathogens, led to a rapid increase in salmonella shed in their feces.

In the new study, the scientists gave streptomycin, an antibiotic, to salmonella-infected mice. They were surprised by the results. Overnight, the majority that had been shedding relatively low levels of salmonella in their feces now evidenced very high levels of the pathogen in both their gut and their feces. And within a few days, these antibiotic-treated,

formerly low-shedding mice became visibly ill. "They lost weight, had ruffled fur and hunched up the in corners of their cages," Monack said. "They also began to shed much larger quantities of bacteria." Several of them died. What was most surprising, though, was that superspreaders kept on shedding large amounts of bacteria while remaining blithely asymptomatic. Examination of the animals' intestines showed that gut concentrations of S. typhymurium in former non-superspreaders now rivaled those of superspreaders.

Giving the mice another antibiotic, neomycin, produced the same outcomes.

Symptom-free superspreaders

Postdoctoral scholar Smita Gopinath, PhD, the study's lead author, demonstrated that while all the animals harbored the pathogenic bacteria in their gut, the superspreaders—despite carrying even higher intestinal levels of salmonella and harboring more gut inflammation than the other mice—had a dampened immune response: Their overall systemic levels of several important pro-inflammatory signaling proteins, secreted by various types of immune cells to whip the immune system into an antimicrobial froth, were substantially lower than those of mice that had morphed from non-superspreaders to sickened superspreaders.

That explained the absence of symptoms in superspreaders, Monack said. Rather than mounting a heightened immune response to the pathogen, superspreaders appear to simply shrug off its presence. "Instead of jousting with the germ, they tolerate it," she said. "Their immune cells have been rewired and aren't responding to the inflammatory signals in the intestines the same way."

Antibiotics actually cause precisely the opposite of the intended effect in the salmonella-infected mouse population, Monack said. "The

superspreaders stay healthy and keep on shedding and transmitting disease. Somehow, in an as yet unknown manner, they're coping with S. typhimurium. The others temporarily shed more bacteria than before, although they're too sick to spread much disease."

The bacteria shed in bulk by former non-superspreader mice were every bit as infectious and virulent as those shed by bona fide superspreaders.

Could it happen in humans?

The phenomenon shown in mice hasn't yet been shown in humans, but should be checked out, said Monack. "We humans shouldn't take antibiotics lightly," she said. "We need to consider whether they're always beneficial when they're given to animals across the board, or when we take them ourselves."

On the positive side, she said, "if we can figure out what leads to this immune dampening in superspreaders, it could potentially be helpful in suppressing symptoms of people with chronic inflammatory intestinal disorders, such as Crohn's syndrome or inflammatory bowel disease."

More information: Role of disease-associated tolerance in infectious superspreaders, *PNAS*, 2014, www.pnas.org/cgi/doi/10.1073/pnas.1409968111

Provided by Stanford University Medical Center