In Search of *Salmonella*'s Smoking Gun

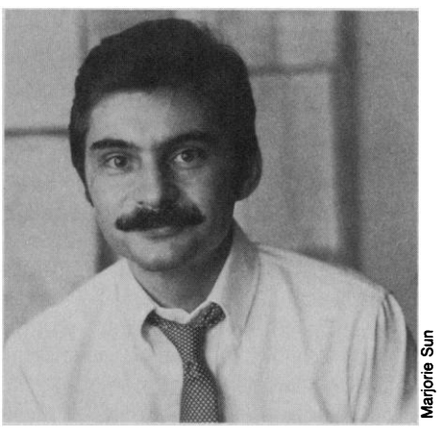
Epidemiologists trace the circuitous path of Salmonella newport, *directly linking for the first time human illness to animals fed low doses of antibiotics*

On Saturday, 19 February 1983, Scott A. Holmberg, a young epidemiologist at the Centers for Disease Control in Atlanta, got a phone call from his boss, Mitchell Cohen, with some interesting news. Cohen told him that Minnesota state epidemiologist Michael Osterholm had reported 11 recent cases of *Salmonella* poisoning. While salmonellosis is a relatively common disease in the United States—40,000 cases are reported every year—two things made these particular cases stand out. All the infections were caused by *Salmonella newport*, a bacterial strain not normally found in the northern part of the United States. And most of the patients had taken antibiotics 1 to 2 days before they became sick. Furthermore, they had been very sick, Osterholm noted. Although a majority of people with a *Salmonella* infection develop mild symptoms, these Minnesota patients had been hospitalized with severe diarrhea, abdominal cramps, nausea, and vomiting.

Osterholm and Cohen discussed possible causes. One was that use of the antibiotics in conjunction with a second unknown factor had made these people ill. Or, though it seemed unlikely, the antibiotics themselves were contaminated. If either were the case, health officials potentially had a national problem on their hands. Would CDC be interested in investigating, Osterholm asked Cohen. The next morning, Sunday, Holmberg was on a plane to Minneapolis.

Eventually, after months of painstaking research, Holmberg with the help of Osterholm and South Dakota epidemiologist Kenneth A. Senger, pinpointed the source of the infection. Piecing together a complex set of events, they showed that the *Salmonella* came from beef and thereby demonstrated for the first time a clear link between the use of antibiotics in animal feed and human illness. Although previous studies have offered strong evidence that a direct association existed, researchers have not been able to connect all the dots in the path between the animals and an outbreak of human illness. The study by Holmberg and colleagues, published last month in the *New England Journal of Medicine*,* provides the smoking gun, according to many scientists, and it has renewed calls for a federal ban on the use of penicillin and tetracycline in animal feed, which farmers used to promote growth in livestock.

Osterholm picked up Holmberg at the airport and on the way downtown, the two chatted briefly about toxic shock syndrome. Osterholm had played a leading role in linking tampon use to toxic shock. But the conversation then turned to matters at hand. "At first blush, it seemed impossible that we had a contaminated antibiotic," recalls Holmberg, a short, trim fellow with a heavy brown mustache. "How could bacteria grow in the nice, dry environment of a drug capsule?" Preliminary findings showed that the first four patients interviewed had all taken amoxicillin, a widely used penicillin derivative. But it turned out that their medications had been manufactured by two different companies. As a precaution, Osterholm ordered amoxicillin from the same drug lots pulled off local pharmacy shelves.



Marjorie Sun

Scott A. Holmberg, CDC epidemiologist

Sunday afternoon and evening, state and federal officials worked with urgency to determine whether the antibiotics were the culprit. They turned up a few additional bits of information, some reassuring, some not. They discovered, for example, that the amoxicillins had a common source after all. The second drug firm had purchased amoxicillin from the first company and then packaged the drug itself. Furthermore, stool samples from the victims showed that they were infected with the same strain of *S. newport*, one that was resistant to common antibiotics, such as penicillin and tetracycline.

On the other hand, Holmberg and another colleague learned after additional phone calls that some of the other victims had taken penicillin, not amoxicillin, before they became ill. That weakened the case against contamination. On top of this, they discovered that the two antibiotics had been bought at different pharmacies and three patients had not taken antibiotics at all. So it seemed highly improbable that the drugs were tainted, Holmberg says.

Some provocative news from Osterholm also cast doubt on the contamination theory and instead lent credence to the hypothesis that a second factor was involved. On 26 January, one of the ten victims, a woman, developed pharyngitis and took amoxicillin. The next day, she was in the hospital with salmonellosis. For 5 days, her husband "dutifully came to see her," Osterholm notes. On the sixth day, the husband went home with "a scratch in his throat" and took some of his wife's antibiotic. Two days later, he too became very sick and was hospitalized as well. Osterholm retrieved an amoxicillin capsule from the couple and brought it back to the laboratory to be cultured, but by then there was strong agreement, according to Holmberg, that "these two histories pointed up the strong possibility that the couple were infected with the bacteria, but were asymptomatic, and that their illness was triggered by antibiotic use." But the source of infection was still a complete mystery.

"From here on out, it was classic epidemiology," Holmberg says. He spent Monday on the phone, asking patients what they had eaten and where they had traveled. "There was nothing unusual. If you were to pick a normal American diet, they had eaten it. They had all eaten milk, eggs, chicken, beef, and pork." Nor were their diets different from those of the controls selected—patients who, in the previous year, had become ill from a strain of *S. newport* that was sensitive to antibiotics.

With no leads, Holmberg called all the patients and asked if he could drop by to chat. He rented a car and drove out to the patients' homes, hoping that personal interviews might turn up a clue. "Do you use i.v. drugs or pot," he asked them, recalling that a recent outbreak of salmonellosis in Steubenville, Ohio, had

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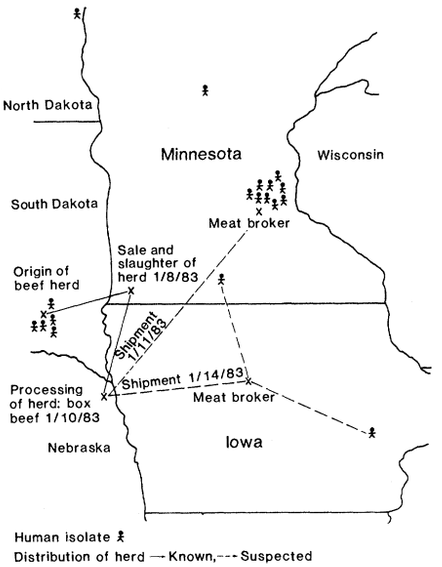
been traced to contaminated marijuana. He rummaged through the patients' kitchens, inspecting the contents of their cupboards and refrigerators. "This was my first time looking in people's kitchens," he says. But he came away empty-handed. "I thought it might be pickles or condiments. There were cans of beef stew common to two homes and that's all," Holmberg says.

Then came startling news. The laboratory results were back from the analysis of the amoxicillin capsule. The culture analyzing the capsule's surface was negative. But on the petri dish with the antibiotic was a single colony of *S. newport*. Osterholm says, "You've never seen so much excitement over a single bacterial colony."

"There were two alternatives," Holmberg says. "One, it was a mistake. But these were superb technicians who had performed the test. Two, there was the unfortunate prospect that the antibiotic was indeed contaminated, but it was hardly strong evidence," he explains. "Here was a real dilemma. If we went public with the news, we were worried about unnecessarily scaring patients and their doctors across the country. But if we did not warn the public, then we were morally culpable. In this situation, you really have to play the odds. After an agonizing half-day, we decided not to sound the alarm, given such little evidence to go on. We should have seen many more colonies if the antibiotic was contaminated." (Holmberg says he can only speculate about the origin of the lone colony. "How do you take pills from a bottle?" Holmberg asks. "You usually shake a few out at a time, take one, and put the rest back. We suspect that this is what the wife and the husband did, and their hands were contaminated with the *Salmonella*. Then during the lab procedures, the amoxicillin powder somehow touched the outside of the capsule before it was cultured.")

After 12 days of investigation in the Twin Cities, Holmberg flew back to Atlanta frustrated. "I was disappointed. I had only one piece of information. These people had been ill from an antimicrobial resistant strain of *S. newport*."

Holmberg went over his findings with Cohen, who is chief of CDC's enteric diseases branch. Cohen's own background contributed subtly but significantly to the investigation. In the late 1970's, Cohen worked with molecular biologist Stanley Falkow of Stanford, a leading researcher in the study of transferrable drug resistance among organisms. Studies by Falkow and others have shown strong evidence that bacteria engage in genetic trading to transfer drug resistance. In the presence of antibiotics such as penicillin, this exchange intensifies because the drugs exert selective pressure on the bacteria. These findings by Falkow and others have led a majority of leading scientists to believe that subtherapeutic levels of antibiotics mixed with animal feed are in the long term dangerous to human health.



This map traces the movement of S. newport. [*Source:* New England Journal of Medicine]

Farmers use antibiotics in animal feed to promote growth, the same antibiotics—primarily penicillin and tetracycline—that are used to treat human disease. (How antibiotics promote growth is controversial.) Many scientists believe that this use of antibiotics causes livestock to "select for" drug-resistant microbes. These bacteria are then passed on to humans, who are more vulnerable to infection because they use the same medications. But past efforts by researchers to pinpoint a direct causal relationship has been thwarted because of the complexity of the food processing and distribution system in the United States.

From the day Osterholm had called, Cohen had a hunch that they might be looking at a drug-resistant bacteria passed through contaminated food source, but it was only a possibility. Unlike previous investigations, however, federal officials now have a new tool to help trace the origin of bacterial contamination. The technique, developed by Thomas O'Brien at Harvard Medical School, analyzes the plasmids of an organism using electrophoresis gels. Each microbe has its own plasmid profile, in effect, a fingerprint, that makes one organism distinct from even similar strains. Using this method, CDC determined that ten Minnesota patients had the same exact strain of drug-resistant *S. newport*.

At Cohen's suggestion, Holmberg wrote state health departments across the nation asking whether they had come across other cases of drug-resistant *S. newport*. He waited. Almost 1 month later, on Wednesday, 30 March, South Dakota state epidemiologist Kenneth Senger called Holmberg and told him that between November and late February, he had reports of four cases of drug-resistant *S. newport*. All had taken antibiotics and all had been hospitalized. They were all from the same southeastern part of the state. Three of the patients, Senger noted, had the same last names. They recovered. The fourth patient, an elderly man, died.

This was the break in the case that Holmberg had been hoping for. For the next several days, Holmberg made numerous phone calls to South Dakota in search of a common link among the South Dakota patients and the Minnesota cases. He learned some fascinating and also some tragic information.

The three patients with the same last name were relatives. One of them was a dairy herder. His cousin lived with her 3-year-old daughter on a farm several miles away. But they had little contact with one another. "They had seen each other at church and waved to one another, and that's all," Holmberg says.

He asked the dairy farmer, who had won local awards for the cleanliness of his farm, whether he had had any problems with his cows. The farmer remembered that in November, an epidemic of diarrhea had spread through his herd and several calves died. Holmberg inquired whether the animal feed could have been contaminated. No, the farmer said. He and his uncle grew their own feed and his uncle had had no problems. Oh, and by the way, the farmer mentioned, he thought that agriculture officials had recovered a *Salmonella* isolate from one of the dead calves. Holmberg immediately asked whether he supplied milk to his cousin and her family. No, the farmer said. Holmberg says, "Again, I thought I had hit another dead end."

At the same time, he was finding out more information about the 69-year-old man who had died. Hospital authorities said that the man had been admitted in mid-December with abdominal injuries sustained in a farm accident. He underwent a sigmoidoscopy, a colon exam, in preparation for surgery and 8 days later developed diarrhea. He was given several types of antibiotics, but responded to none. Twenty days after the onset of diarrhea, the man died. Drug-resistant *S.*

newport was isolated from his blood, sputum, and stools before he died.

Hospital personnel said that the dairy farmer's cousin had been admitted around the same time and that she also had undergone a sigmoidoscopy, but they dismissed the suggestion that the man had contracted the *S. newport* from her. They had followed the manufacturer's directions to disinfect the equipment used in the colon exam. Furthermore, the elderly man was examined a day after the woman. In reviewing the hospital records, however, Holmberg discovered that the woman had been the last patient to undergo a sigmoidoscopy and that the man was the first the next day to be examined with the same equipment.

Holmberg was still stumped about the connection between the three relatives and their infections. "They had done nothing together and the man had a dairy herd. That's all I had." Then Holmberg remembered that the dairy farmer had an uncle. He called the farmer back, Holmberg learned that the uncle owned a herd of beef cattle and that their two farms were adjacent. The uncle provided his family with beef, the dairy farmer said. "The uncle would pick a particularly good animal, slaughter it, and provide trim hamburger meat and good steaks to his nephew," Holmberg says. "I called his cousin and, lo and behold, she had gotten beef from the same herd. "I went back to my files on the Minnesota cases and what did they all eat? Ground beef." Holmberg tracked down the isolate of the dead calf that had been sent to a U.S. Department of Agriculture laboratory in Ames, Iowa. It had the same plasmid profile as the bacteria found in the Minnesota patients. On 4 April, Holmberg hopped on a plane and headed to Sioux Falls to meet up with state epidemiologist Senger.

The two men drove out to the farms and immediately uncovered more clues. They noticed that the two farms shared a common fence and that a dairy calf had strayed onto the beef farm property. They interviewed the uncle. His beef herd had had no health problems, he told them. All 105 head of cattle went to slaughter in mid-January. He also said he bought tetracycline by the bag and added a handful or so to a ton of feed to promote growth and prevent disease in his livestock. An isolate from the dairy herd owner was recovered. It too matched the plasmid profile of the dead calf.

"I knew what the story was" or at least a good part of it, Holmberg says, "I knew that between this point here in South Dakota and that point there in Minnesota, there was a connection. Nature does not hand you coincidences like this. I was pretty sure that the beef herd was the source. If you look at the timing, the slaughter took place just before the outbreak in Minnesota, and it was not that far away from the Twin Cities. Now all I really wanted to do was connect these two points with a plausible sequence."

Senger suggested that they pay a visit to the slaughterhouse, which was located in southern Minnesota. Perhaps plant officials could help trace the movement of the beef. Thanks to a new computerized system at the plant, plant officials easily found the relevant records. On 8 January, the beef were slaughtered and

> "Nature does not hand you coincidences like this," Holmberg says.

59 carcasses were shipped 2 days later to a meat processor in Nebraska. Holmberg contacted the meat processor. There the beef from the carcasses was butchered and some of the cheaper cuts of meat were "boxed." This boxed meat is eventually sold to supermarkets, which then grind it into hamburger. At this point, the beef from the uncle's herd may have been mixed with meat from other herds, Holmberg says. In any event, the meat processor shipped 40,000 pounds of boxed beef to a meat broker near the Twin Cities and about 30,000 pounds to another broker in Iowa. The Minnesota meat broker also had computerized records and told Holmberg and Senger which supermarkets the beef had been delivered to in Minneapolis and St. Paul. Eight of the *Salmonella* victims had shopped for their ground beef at six of these stores. The Iowa meat broker had also supplied stores in Minnesota and Iowa where two additional patients had purchased ground beef.

Holmberg was almost done with the investigation. Holmberg never could obtain a plasmid profile from the suspect beef. That would have clinched the investigation, he says. But the isolate taken from the sample from the dead dairy calf was persuasive. To make sure that the plasmid profile taken from the calf was unique, he obtained from a federal laboratory 91 samples of *S. newport* isolates collected in 1982 and 1983 from infected animals located across the country. Only the fingerprint from the calf sample identically matched those of the patients.

The ultimate source of the beef herd's suspected infection will probably never be known. Holmberg notes that in his analysis of the 91 isolates, he noticed that some of the plasmid profiles of animals in Texas were similar to the calf's and that animals from other states in between Texas and South Dakota were increasingly similar, but not identical. Holmberg speculates that the bacteria may have traveled north with the movement of livestock, changing its genetic makeup ever so slightly.

The accumulated evidence of the investigation almost conclusively shows that the uncle's cattle became biological factories of drug-resistant *S. newport* because they were fed low doses of antibiotic, says Holmberg, who, like others, sees this as part of an extremely worrisome trend. In a study published recently in *Science* (24 Aug., p. 833), Holmberg and CDC colleagues conclude that the fatality rate for people infected with drug-resistant *Salmonella* is 21 times greater than for individuals ill from *Salmonella* that is not antibiotic resistant. In addition, the study says that food animals are usually the source of the drug-resistant bacteria that infect people. Based on a review of CDC records of all outbreaks of drug-resistant *Salmonella* poisoning between 1971 and 1983, 69 percent or 11 of the 16 strains of drug-resistant *Salmonella* that made people sick were traced to food animals.

By the end of the Midwest investigation, Holmberg tallied up 18 people in four states, who had became infected with the organism and were hospitalized. An untold number of other less severe cases went unreported. CDC estimates that only 1 percent of all *Salmonella* infections are reported in the United States. There's one more lesson, Cohen of CDC notes. Twelve of the 18 had taken penicillin or amoxicillin. This created an environment in which the drug-resistant bacteria had a selective survival advantage. Three of these 12 had taken antibiotics without doctor's directions. "Self-administration of antibiotics is not prudent," Cohen says. Widespread use of broad spectrum antibiotics by people, as in animals, can also promote the development of drug-resistant bacteria.

Says Holmberg, "This investigation represents bad news for the meat industry and for humans if indiscriminate antibiotic use continues."—**MARJORIE SUN**

This is the first of two articles on the use of antibiotics in animal feed. The next article will examine efforts to ban their use in the United States.