Disturbing new findings have provided a key link in the chain of evidence connecting antibiotics used on livestock to outbreaks of disease caused by antibiotic-resistant human pathogens

Superbugs on the Hoof?

When the severe diarrhea didn't stop after nine awful days, the 62-year-old Danish woman dragged herself to the emergency room at Bispebjerg Hospital in Copenhagen. The diagnosis was a cinch: food poisoning from *Salmonella*. Doctors rolled out their big gun, an antibiotic called ciprofloxacin that can vanquish the nastiest *Salmonella* strains in a few days. But as the hours passed, the infection worsened—becoming so bad that the *Salmonella* punched a hole in her colon, allowing it and other bacteria to invade the rest of her body. As the situation grew desperate, doctors blasted her with heavy doses of two more antibiotics and stitched up her damaged colon. The drugs knocked off the *Salmonella*, but other escapees from the gut sent her into septic shock; one by one, her organs failed. Four days after doctors realized the *Salmonella* was impervious to ciprofloxacin, she was dead.

The Danish woman was not the first person to succumb to a superbug resistant to antibiotics. But she and another *Salmonella* victim in the summer of 1998 put a human face on an alarming trend: pathogens rapidly acquiring resistance to drugs that are similar to antibiotics used for years to treat livestock. In a nice piece of detective work, a team led by microbiologist Henrik Wegener of the Danish Veterinary Laboratory in Copenhagen traced the drug-resistant strain of *Salmonella* to infected swine. To fight *Salmonella* outbreaks, some farmers had been dosing herds with enrofloxacin. It turns out that this drug and ciprofloxacin belong to a class of compounds called quinolones that gum up bacterial machinery for replicating DNA. The researchers traced the deadly strain to contaminated pork products from a single Danish herd. The findings, reported last November in *The New England Journal of Medicine (NEJM)*, are the strongest indictment yet implicating livestock antibiotics in human deaths. Says microbiologist Abigail Salyers of the University of Illinois, Urbana-Champaign: "It's the closest that anybody has come to a smoking gun." And just last week, researchers reported evidence linking a case involving a resistant *Salmonella* strain in the United States to the use of animal antibiotics.

For decades farmers have mostly had free rein in dosing livestock with antibiotics to treat illnesses, prevent infections, and fatten animals on less feed. With evidence mounting that this unfettered practice can spawn new superbugs, agencies worldwide are beginning to clamp down on antibiotic use in agriculture. The European Union has issued new rules limiting the use of several livestock antibiotics, while the U.S. Food and Drug Administration (FDA) has proposed similar regulations.

The moves have riled industry officials, who argue that antibiotics are essential to keeping animals healthy and the food supply safe. They contend that regulators and public health activists are blowing the problem out of proportion. The most serious threat, they point out, comes from indiscriminate use of antibiotics in people, not livestock. "We're not saying there isn't any concern," says Richard Carnevale of the Animal Health Institute, which represents U.S. animal-drug producers. "But in the whole scheme of things, we believe that it's relatively minor."

Wallowing in bacteria. After a vicious strain of drug-resistant *Salmonella* killed two people in Denmark in 1998, scientists traced the bug to a single herd of Danish pigs. The strain, they found, was resistant to a livestock antibiotic similar to the human drug that failed to cure the victims.

CREDIT: PETER OLSEN

A growing number of scientists, however, are taking the threat quite seriously, as is the British Royal Society of Medicine, which brought experts together in Washington, D.C., this week to brainstorm on the issue and to educate the public. Although drug use on the farm may have little to do with drug-resistant tuberculosis or other pathogens transmitted from person to person, it "has everything to do with bacteria acquired through the food supply," says medical epidemiologist David Bell of the U.S. Centers for Disease Control and Prevention (CDC).

Gut reaction

The case against antibiotic use in livestock rests largely on drug resistance observed in food-borne pathogens such as *Salmonella* or *Campylobacter*, which often infect animals without causing symptoms. First, microbial sleuths must link a livestock antibiotic to a drug-resistant strain. Next, they must show that the strain can survive the slaughterhouse. Finally, to cement the connection to human illness, they must prove that eating tainted meat leads to an infection that defies antibiotic treatment. The last link is the hardest to verify. "That's where the chain of evidence starts to get frayed," Salyers says.

Luckily for the Danish team, the deadly bug did not take them by surprise. It's a variant of *Salmonella typhimurium* DT104, a strain that resists five common antibiotics and had flared up in many European countries—but rarely in Denmark. Hoping to keep it at bay, Danish officials set up in 1997 what Wegener calls the world's most aggressive surveillance system for resistant *Salmonella*. They test for drug resistance in every Danish patient who sees a doctor for a *Salmonella* infection, about 3200 people a year; in roughly 1 million samples of meat shipped each year to grocery stores; and in nearly every flock of chickens and herd of pigs—the usual sources of *Salmonella* that infect people—raised for the market.

When word came on 18 June 1998 that a quinolone-resistant strain had shown up in a hog slaughterhouse on the island of Zealand, Kåre Mølbak of Copenhagen's Statens Serum Institute leaped to action. By coincidence, earlier that day his team had identified samples of a vicious DT104 strain in five Danish patients. This strain, and the one in the slaughterhouse, beat back the same

seven drugs—two more than other DT104 strains. Mølbak's team started phoning patients, slaughterhouse workers, and meat wholesalers. By nightfall they learned that all the patients had bought pork from shops supplied by the Zealand slaughterhouse.

"That made the hypothesis simple," says Wegener, who was consulting with Mølbak throughout the day: The pork was the source of the super DT104. Records later showed that just one of the 37 herds slaughtered for that shipment was infected by the resistant strain. Although the herd had not been treated with quinolones that year, others on nearby farms had, and *Salmonella* can easily jump from herd to herd, Wegener says. What's more, DNA fingerprinting showed that the drug-resistance genes in the patients were identical to those in the pigs.

Now, another case, reported in last week's *NEJM*, suggests that a second new drug against *Salmonella* has been compromised by a livestock antibiotic. Bacteriologist Paul Fey of the University of Nebraska Medical Center in Omaha and his colleagues described how a 12-year-old Nebraska boy became infected by the same *Salmonella* strain as had cows on his father's ranch. Apparently, says Fey, the cows had contracted the resistant *Salmonella* from a ceftiofur-treated herd on another ranch. When the boy became ill, doctors treated him with ceftriaxone, an antibiotic similar to ceftiofur. The boy recovered—but not thanks to the ceftriaxone, which hardly dented the *Salmonella*. The strain was resistant. That worries Fey, as doctors already have their hands tied when treating childhood *Salmonella*: Front-line quinolones can't be used because they impede bone development. That's why Fey predicts "dire consequences" for children's health if the ceftriaxone-resistant strains spread.

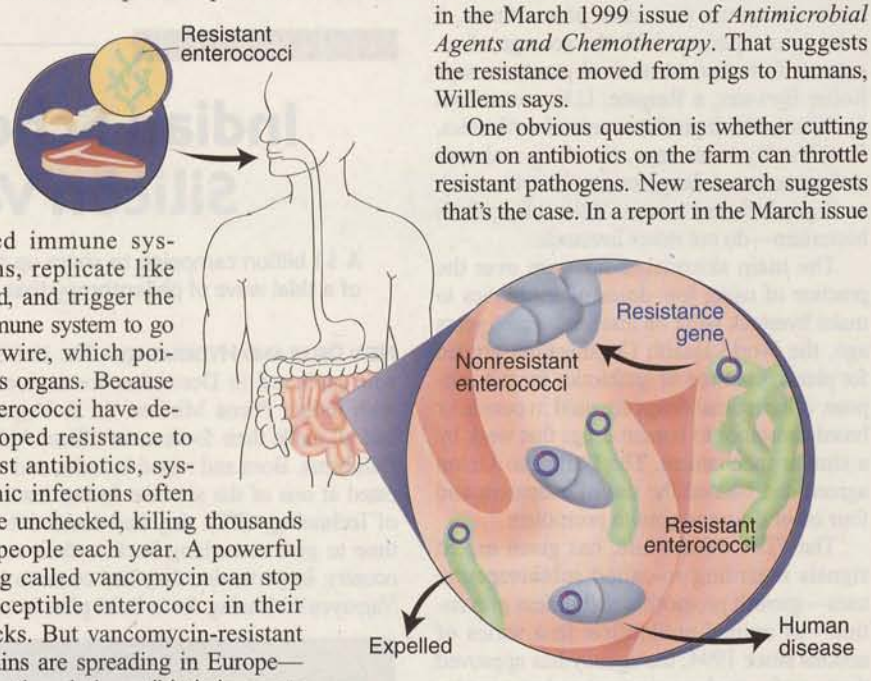
Even in the absence of a direct link between farm and table, circumstantial evidence can be damning. Spurning the CDC's advice, the FDA in 1994 approved the use of quinolones for preventing infections of an intestinal bacterium, *Campylobacter jejuni,* in poultry. Since then, the percentage of quinolone-resistant *Campylobacter* cultures in routine sampling in people has skyrocketed from 1% to 17% in just 7 years, Kirk Smith of the Minnesota Department of Public Health and his colleagues revealed last year in *NEJM*. *Campylobacter* very rarely kills people, Smith says, "but it's a severe enough illness that you're just hating life." And it shows that *Salmonella* isn't the only bug to worry about.

SOURCE: A. SALYERS; ILLUSTRATION: C. CAIN

Fomenting resistance

A more insidious threat comes from pathogens passing their genetic know-how to bacteria in our gut. Resistance develops like this: Under an antibiotic blitz, a tiny fraction of any population of otherwise-susceptible bacteria can survive, because they possess mutations—acquired randomly or from other bacteria that slip them rings of DNA called plasmids—that counter an antibiotic's effects. Because different strains swap genes routinely, and even different species exchange genes from time to time, one bug can gird itself with another's resistance genes.

In a particularly troubling example, gene transfer appears to have turned gut-dwelling bacteria called enterococci into a public health threat. Most of the time, people and their intestinal bacteria get along fine, but enterococci can infect hospitalized patients with weakened immune systems, replicate like mad, and trigger the immune system to go haywire, which poisons organs. Because enterococci have developed resistance to most antibiotics, systemic infections often rage unchecked, killing thousands of people each year. A powerful drug called vancomycin can stop susceptible enterococci in their tracks. But vancomycin-resistant strains are spreading in Europe—even though the antibiotic is new to the European scene.



Deadly pas de deux. Usually a kind of bacteria called enterococci dwell peaceably in our gut. But new findings suggest that drug-resistant bacteria in contaminated food or water can slip enterococci the genes conferring resistance. For people with weakened immune systems who are susceptible to enterococci infection, the drug-resistant enterococci pose a grave threat.

Vancomycin resistance already is a huge problem in the United States. The drug has been used for years in hospitals, allowing bugs to develop resistance. But that doesn't explain why resistance is cropping up in healthy people in Europe, says microbiologist Wolfgang Witte of the Robert Koch Institute in Wernigerude, Germany. The evidence suggests another reason: In 1974, the European Union approved the use of avoparcin, an antibiotic that, by an unknown mechanism, makes livestock grow fatter on less feed. Avoparcin and vancomycin kill bacteria by blocking an enzyme essential for building the cell wall. Not surprisingly, enterococci in livestock that resist avoparcin also can withstand vancomycin. Despite strict procedures, enterococci from the gut on occasion infect meat during slaughter. If a person eats undercooked meat tainted with resistant enterococci, the livestock strain can transfer to the human strain the genes conferring resistance to vancomycin. Alarmed by the potential link between avoparcin and vancomycin resistance, the European Union banned avoparcin in 1997.

A team led by molecular microbiologist Rob Willems of the Dutch National Institute of Public Health and the Environment in Bilthoven has evidence that such gene transfers are occurring. The researchers found identical sequences of transposons—DNA snippets that can jump from one bacterium to another—with identical resistance genes in enterococci from people and from pigs. These transposons are different from ones found in resistant enterococci from cows, chickens, and turkeys, the researchers report in the March 1999 issue of *Antimicrobial Agents and Chemotherapy*. That suggests the resistance moved from pigs to humans, Willems says.

One obvious question is whether cutting down on antibiotics on the farm can throttle resistant pathogens. New research suggests that's the case. In a report in the March issue of the *Journal of Antimicrobial Chemotherapy,* a group led by veterinary microbiologist Anthony van den Bogaard of the University of Maastricht in the Netherlands showed that by 1999, 2 years after avoparcin was taken off the market in Europe, the prevalence of vancomycin-resistant strains in pigs, chickens, and people in the Netherlands had dropped to half the 1997 levels.

Culling the antibiotic herd

To guard against the nightmare of animal enterococci or other bugs planting the seeds of resistance in more dangerous pathogens, governments worldwide are cracking down on the use of drugs in livestock. The first moves came after a British panel in 1969 recommended banning growth-promoting

antibiotics that spur resistance to drugs used in human medicine. The panel's advice was partly heeded in Europe, where key antibiotics like penicillin and tetracycline were taken out of agricultural use in the 1970s.

But it has been mostly ignored in the United States, where industry officials insist that antibiotics keep animals healthy and thus safeguard the food supply. "While there's a theoretical link [between resistant strains in livestock and people], we think that there's so many things that need to happen that the risk is diminishingly small," says the Animal Health Institute's Carnevale. Even if antibiotic use on the farm does pose a threat, it pales in comparison with the scourge of resistance from human medical practices, says Robin Bywater, a Reigate, U.K., consultant for Pfizer, which produces animal antibiotics. Besides, Bywater says, the drug-resistant pathogens most dangerous to people—such as *Staphylococcus aureus* or the tuberculosis bacterium—do not infect livestock.

The main skirmishes now are over the practice of using low doses of antibiotics to make livestock fatter on less feed. Three years ago, the World Health Organization argued for phasing out use of antibiotics for this purpose, if the animal drugs are used in people or breed resistance to human drugs that work by a similar mechanism. The European Union agreed and banned the use of avoparcin and four other drugs as growth promoters.

The FDA, meanwhile, has given mixed signals regarding so-called subtherapeutic uses—growth promotion and illness prevention—of animal antibiotics. In a series of actions since 1994, the agency has approved the use of quinolones to treat and prevent infections in poultry and beef cattle. But in 1998 it floated draft regulations that would raise the bar for all uses of new animal antibiotics. The regulations would require companies to carry out resistance studies before and after a drug's approval, and to pull any drug from the market if the target bacteria develop resistance to human antibiotics. "We're most concerned about those pathogens for which the disease is serious in humans and for which the drug we're considering may be the drug of last resort," such as quinolone-resistant *Salmonella* and vancomycin-resistant enterococci, says Stephen Sundlof, director of the FDA's Center for Veterinary Medicine. "The only scientific way we have to do it is to look at it on a case-by-case basis."

Congress, however, may prod the FDA into a more aggressive stance. A bill introduced last year by Representative Sherrod Brown (D–OH) would order companies to discontinue using seven antibiotics for any reason other than to treat illness in animals—unless the industry proves that the drugs won't harm human health. Brown hopes the bill, opposed by the agriculture industry, will pass in 2 or 3 years. "The burden should be on the drug industry to prove that they are safe, not on the FDA to prove 100% that they are unsafe," he told *Science*.

Researchers are also trying to provide industry with alternatives to antibiotics that can keep livestock healthy. These include probiotics, in which healthy gut bacteria are infused into animals before they are weaned to crowd out pathogens; vaccines; and animal husbandry practices that prevent infections from spreading from farm to farm. Although these possibilities hold promise, "there is no one magic bullet" in the pipeline, says microbiologist Paula Fedorka-Cray of the U.S. Department of Agriculture's Russell Research Center in Athens, Georgia.

Wegener and others believe that U.S. regulators must follow the lead of their European counterparts and act quickly to get livestock antibiotics off the market for uses other than treating sick animals. Otherwise more outbreaks like the one in Denmark could occur, he says, adding, "I have difficulty understanding why we should take that risk."

–DAN FERBER

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PHILANTHROPY

Indian Schools Cash In on Silicon Valley Wealth

A $1 billion campaign to shore up the elite Indian Institutes of Technology is part of a tidal wave of philanthropy that hopes to raise up Indian higher education

NEW DELHI AND HYDERABAD—The 13 CEOs who gathered in December for a meeting with Indian Prime Minister A. B. Vajpayee had all made their fortunes in Silicon Valley, California. Born and raised in India, and educated at one of the six elite Indian Institutes of Technology (IIT), they had decided it was time to give something back to their native country. In particular, they had come to seek Vajpayee's blessing for a bold plan to raise $500 million from wealthy IIT alumni like themselves for new buildings, equipment, and programs at their alma maters.

The prime minister was delighted, but urged them to think even bigger: "He said, why not pick up the entire tab?" recalls Kanwal S. Rekhi, the retired technology chief at Novell Inc., who now runs a small California company, IndUS Entrepreneurs, that fosters collaborations between the two democracies. The startled executives ran the numbers and calculated the cost to modernize the institutes at $1 billion. Unfazed by the sum, the self-made multimillionaires said OK, but under two conditions: The money, to be raised over 6 years, would go directly to the IITs, and an independent board of trustees, made up of the country's business and academic elite, would replace the government as overseer. Vajpayee said he'd look into it.

These high-tech tycoons are part of a startling new trend in India. Even as they begin passing the hat for the IITs, another group of Indian-born, U.S.-based software engineers led by Purnendu Chatterjee, managing director of the New York–based Chatterjee Group, wants to raise the same amount—$1 billion—to set up a series of world-class centers of higher learning in India (*Science*, 31 March, p. 2389). And individual expatriate Indians are donating to other high-tech causes back

Deep pockets. Kanwal Rekhi, right, and others hope to raise $1 billion for the six Indian Institutes of Technology, including the Delhi campus, inset, headed by V. S. Raju, left.

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