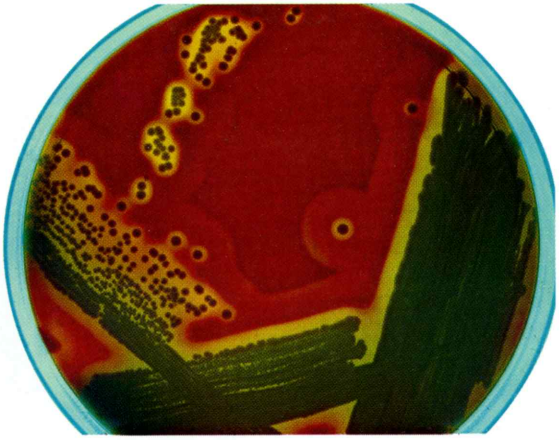
Clostridium perfringens

New ways to type strains of a deadly bacteria



Clostridium perfingens *bacteria on a blood agar plate. Double zones of hemolysis (breaking down of red blood cells) surrounding the bacteria indicate toxin production.*

It has killed minks on a ranch in Montana, sheep in Australia, hundreds of pigs and cattle across the U.S. and even a rare shoebill stork at the San Diego Zoo. Exhibiting frighteningly violent and rapidly progressing symptoms, *Clostridium perfringens*-induced intestinal diseases have confounded livestock producers and veterinarians around the world, along with medical doctors who at times have been stunned to diagnose in humans what is more commonly considered an animal disease.

"The organism is so promiscuous in terms of its hosts that it's found wherever there are domestic animals," says Glenn Songer, a veterinary scientist in the College of Agriculture at The University of Arizona. "It makes a lot of toxins, and it's almost always lethal."

Many years ago scientists developed a scheme where they divided the bacterium into five types based on production of four toxins. These four main toxins — known as alpha, beta, epsilon and iota toxins — combined with many other toxic substances created by the bacteria, produce nearly 25 different diseases.

Diagnosing a clostridial disease requires isolating the pathogen and determining which toxins are involved. The three traditional means have included:

1. growing the organism in liquid culture, getting rid of the bacteria and looking for the toxins
2. searching for toxins in the gut of an animal already dead from the disease
3. injecting the toxins into mice or guinea pigs and neutralizing them with specific antibodies to see whether the toxic effect is from that particular strain.

All of these methods have drawbacks, including the tendency to yield false negatives by not being sensitive enough; false positives from a lack of specificity; and a humanitarian concern with the guinea pig and mouse assays. (They are no longer commonly used.)

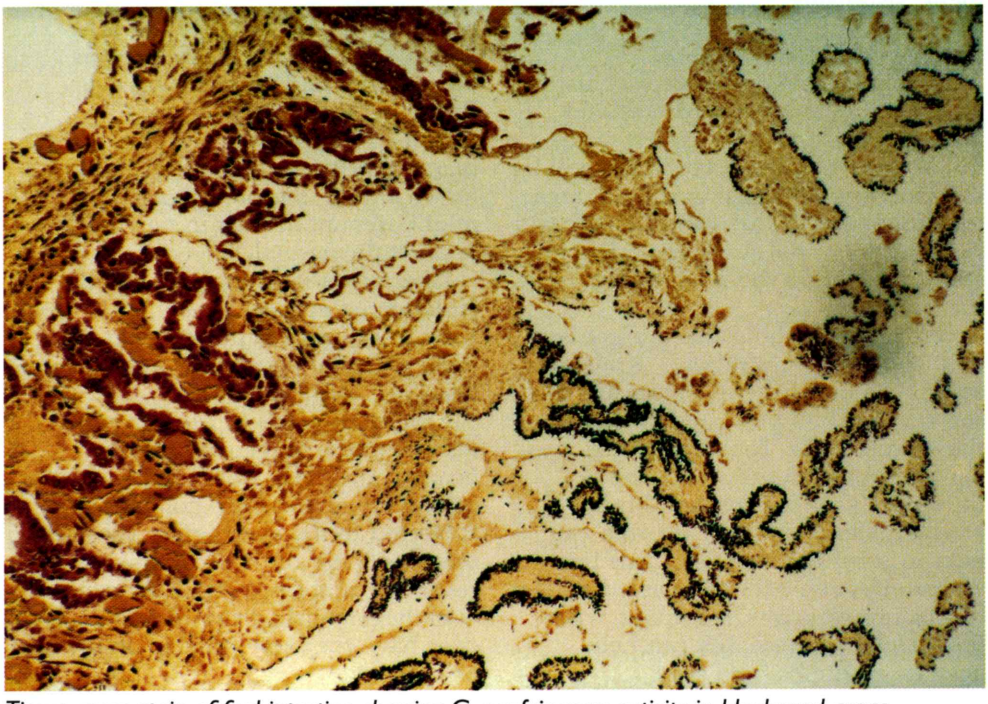
Songer has been working since 1990 with graduate student Ralph Meer, now a UA nutritional scientist, with Dawn Bueschel, a research technician in the Department of Veterinary Science and Microbiology, and with graduate students to find a practical way to assay isolates of the disease. They have developed what is called a multiplex polymerase chain reaction (PCR) assay that allows simultaneous detection of the four major toxin genes plus the gene for enterotoxin, for *C. perfringens* in one test.

This represents a major breakthrough, since individual tests were previously needed for each toxin. It also eliminates the "silent gene" problem inherent in the other assay techniques, where certain toxin genes went undetected. Because vaccines are only effective if they target the exact toxin responsible for the illness in an individual animal, this method will help speed diagnosis of *C. perfringens* in disease outbreaks and assist the development of specific vaccines.

> ***"We thought maybe we could replace toxin detection with detecting the gene for the toxin."***

"We hypothesized that if the toxin is being produced, then the gene has to be there," Songer says. "We thought maybe we could replace toxin detection with detecting the *gene* for the toxin. We developed a method based on polymerase chain reaction, where a small number of DNA sequences are amplified. We determined the sequence of the gene to make the primers and then developed the assays."

Songer and Meer took field isolates from animal, human and feed sources and compared the genotypes they had found with the phenotype, or actual physical attributes of the bacterial toxin production already established through traditional assays. They obtained 100 percent agreement, first using an assay in which they ran separate reactions for each of the four major toxin genes. Then they adapted

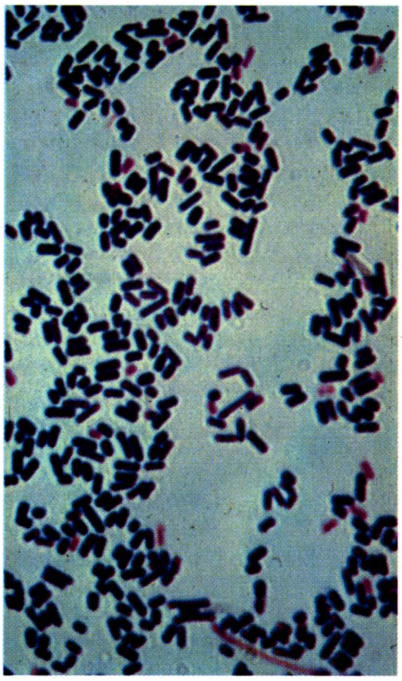


Tissue gram stain of foal intestine showing C. perfringens *activity in blackened areas.*

A Potentially Lethal Pathogen

Once an animal contracts a disease caused by *Clostridium perfringens* it's often too late to do anything about it, according to Glenn Songer, UA veterinary scientist. Death comes quickly and violently. This type of bacteria, occurring in five different strains identified by toxin type, produces a host of toxic proteins; nearly twenty have been described scientifically and there may be more. These toxins can act rapidly in the body, causing severe diarrhea, dysentery, gangrene, muscle infections and various other forms of enteric (gut) disease. The symptoms vary in intensity and variety depending on the individual toxin and its host.

C. perfringens can cause disease in most domestic animals and some wildlife, including horses, poultry, sheep, birds, rabbits, goats, hogs, cattle, mink, ostrich, emu, dogs, cats, and others. Humans have also become infected, although cases of enteritis have been localized, most notably in the highlands of Papua New Guinea where it occurs as a severe, usually fatal form of food poisoning that kills the small intestine.



Grain-stain of equine intestinal tissue showing gram-positive rods of Clostridium perfingens *(stained dark).*

In spite of its potential danger as an infectious agent, the avirulent forms of bacillus are commonly found in the intestinal tracts of warm-blooded animals, and it also inhabits terrestrial, marine and aquatic environments. The trouble starts when the balance of bacteria in the gut is disrupted, giving *C. perfringens* a chance to proliferate unchecked. It may contaminate soil, animal feed and litter, or be transmitted directly from infected to healthy animals.

C. perfringens related livestock infections have been reported in every state in the nation and in most parts of the world. Although surgery can save human victims, it is often not feasible to perform it on domestic animals. The most practical way to handle *perfringens*-related illnesses in animals is to prevent them in the first place.

this to a multiplex test to detect all four, plus the enterotoxin gene, simultaneously. The test was up and running by 1994, and Songer says the UA lab has used it to diagnose thousands of *C. perfringens*-related illnesses at the request of community and scientific professionals.

"We get five to ten calls a week from people who want information," he says. "We've typed 3,000 isolates on request, from all across the North America." The lab has served practicing veterinarians, livestock producers, and professionals from biologics companies. The tests usually focus on clostridial enteritis diseases in traditional food animals: dairy and beef cattle, sheep, goats, pigs; plus some cases with ostriches, llamas, alpacas, mink and horses.

When a very valuable thoroughbred foal died in California, Songer and his team worked with diagnosticians from UC Davis to determine the cause. "We were able to pin this down as a *perfringens*-related death," he says.

"We've also had extensive contacts with the San Diego Zoo's Center for Reproduction of Endangered Species (CRES). The center breeds endangered animals in captivity and then releases them into the wild. Songer's team has assisted them in preventing and diagnosing enteric diseases.

The test was up and running by 1994, and Songer says the UA lab has used it to diagnose thousands of C. perfringens-related illnesses at the request of community and scientific professionals.

Although state veterinary diagnostic laboratories in Washington, Texas, Kentucky, Alabama, Indiana and other states are using the test, Songer says many labs still send their isolates to the UA. In some cases biologics companies and livestock producers have been able to work together to take isolates from the ranch animals and have the company make a vaccine. "The idea is that if the organism is in their own herd, producers can use that specific one to target the disease with a vaccine," Songer says.

Through the UA Department of Veterinary Science and Microbiology web site, Songer says he has gotten e-mails and inquiries from all over the world, including South Africa, New Zealand, Norway, Canada and Japan. The web site describes the multiplex PCR assay and gives instructions on how to send in samples for *C. perfringens* genotyping. For more information, view the web site at microvet.arizona.edu. Using the navigation bar on the left side, click on "Research."

— *Susan McGinley*