Of Mad Cows and Englishmen

Telltale protein betrays disease

By STEVE STERNBERG

A decade ago, many cows on farms throughout the United Kingdom began to fall ill. Worried owners watched as the disoriented animals staggered about, lowing pitifully, and eventually died. Veterinarians recognized the symptoms as similar to those of scrapie, a communicable brain disease that attacks sheep.

The cattle had become infected, it was thought, when they ate feed that had been fortified with sheep offal—brains, spinal cords, and other organs. Though the practice of adding offal to feed was banned in 1989, the epidemic of mad cow disease continued into the 1990s and peaked in 1992 at 37,000 new cases.

The story took an alarming new twist this spring, when doctors found that 10 people had died of what looks like a human version of mad cow disease (SN: 4/13/96, p. 228). The cases prompted the 15-nation European Union to ban exports of British beef and demand the slaughter of British cattle. The United Kingdom agreed to destroy 4.5 million head—almost half the nation's herd—that were old enough to have eaten infected offal. Some 170,000 cattle have been killed so far.

The epidemic might have been kept in check if there had been a simple test for mad cow disease. But the only way to detect infected cows was to remove brain tissue from each animal and study it, a virtual impossibility with the millions of cows involved.

Now, teams of researchers in the United States and Germany report that they have independently developed more practical tests for mad cow disease. If the tests prove accurate, the researchers say, they will allow farmers to cull individual infected cows from their herds.

The tests also enable doctors to diagnose Creutzfeldt-Jakob disease (CJD)—a rapidly degenerative disease of the human brain that was first recognized in 1920. Furthermore, the U.S. test appears to detect the strange human variant of mad cow disease that galvanized officials in the United States and Europe into taking steps to avert a new epidemic.

Mad cow disease and CJD have proven difficult to diagnose because the protein thought to cause both ailments is tricky to detect in living tissues. Known as Prp, for prion-related protein, it is the Dr. Jekyll and Mr. Hyde of brain biochemistry. In healthy people, Prp acts as an ordinary cell-surface receptor, but its function is not clearly understood. In some humans and animals, however, Prp undergoes a deadly transformation.

This misshapen Prp—a prion—transfigures normal Prp molecules into more prions. As prions proliferate, they clump together in tangled masses inside nerve cells, overloading them and perforating their delicate membranes (SN: 9/24/94, p. 202). Some scientists theorize that prions work alone, others that they act in concert with another protein or virus.

Either way, prions riddle the brain with holes until it is as porous as a sponge. These "holes in the cells themselves" prompted Clarence J. Gibbs of the National Institutes of Health in Bethesda, Md., to name this family of diseases transmissible spongiform encephalopathies (TSEs). Besides mad cow disease, scrapie, and CJD, this family includes Kuru, a disease in New Guinea thought to result from cannibalism.

The new tests don't look for prions but for particular proteins in an infected person or animal's spinal fluid. These proteins, members of the family called 14-3-3, normally reside only in brain cells. When found in spinal fluid, these substances, known as marker proteins, signal that something has damaged the brain cell membranes, causing them to leak.

Developed by Michael G. Harrington of the California Institute of Technology in Pasadena and Gibbs' team, the U.S. test detects 14-3-3 proteins. The German test relies on two of these proteins, known as 130 and 131. Inga Zerr of the Georg August University in Göttingen and her coworkers describe their test in the Sept. 28 LANCET.

"We surmise that the reason we find a high concentration [of 14-3-3] in spinal fluid is the pathological destruction of brain tissue," Gibbs says. He and his colleagues report their findings in the Sept. 26 NEW ENGLAND JOURNAL OF MEDICINE.

For more than a decade, researchers had been looking for a way of detecting TSEs without taking samples of brain tissue. That costly and risky procedure can go awry, leading to bleeding, infection, or death. "There's also the risk of exposing hospital personnel to an infectious agent," says Martin Zeidler of the National CJD Surveillance Unit in Edinburgh.

To diagnose both CJD and the human version of mad cow disease, Zeidler has relied primarily on observations made without sampling brain tissue. People with either disease spiral rapidly downward into dementia, their limbs jerk involuntarily, and a peculiar brainwave pattern, sharp spikes at 1-second intervals, shows up on an electroencephalogram. The spikes betray damage to nerve circuits throughout the brain.

Quick death also characterizes these diseases—patients typically survive just months after dementia sets in.

If the course of the disease is prolonged or if a person's brain waves vary from what doctors have come to expect, diagnosis becomes far trickier. In such cases, doctors may mistake CJD for Alzheimer's disease.

In their search for a practical diagnostic test, Gibbs and Harrington focused on proteins 130 and 131, which they found in the spinal fluid of the 21 CJD patients they had tested. Healthy people and most people with other brain diseases did not have these proteins in spinal fluid. Of 500 people with Alzheimer's disease, Parkinson's disease, or other neurological ailments, only a handful had 130 or 131 in their spinal fluid.

Although these proteins appeared to be excellent markers for the disease, they were difficult to detect. The only test available was so difficult to perform and so excruciatingly slow to show results—4 or 5 days—that it was virtually useless except for research and for

diagnosing particularly tricky cases.

To produce a simpler test, Gibbs and Harrington needed to identify these proteins in small amounts of spinal fluid. Working with Gibbs, Gary Hsich, now a pediatric resident at the University of Pennsylvania in Philadelphia, developed a way to separate the proteins from spinal fluid. Then he added an antibody that latches onto the telltale proteins. The resulting protein-antibody complex appears as a dark horizontal stripe on a piece of nitrocellulose paper.

Gibbs says the method is simple and reliable. "It can be used by any diagnostic lab in the world." Almost as important, the test is relatively quick. "Now we can do 40 samples per gel in 24 hours," he asserts.

Gibbs, Harrington, Hsich, and their colleagues then used the test to demonstrate that proteins 130 and 131 are members of the 14-3-3 family found inside brain cells. The test also indicated that 14-3-3 proteins are not prion-related.

The researchers tried the test on spinal fluid from 71 people with TSEs. It accurately detected 14-3-3 proteins in 96 percent of the specimens. In 66 samples from people with viral encephalitis, stroke, brain hemorrhages, and other non-TSE neurological disorders, the test showed 14-3-3 in only a few cases. This indicates that the test is specific for TSE, the group reports.

To satisfy himself that the test is accurate, Zeidler sent Gibbs and his colleagues 16 additional specimens from patients with known or suspected CJD and 12 from people with the human version of mad cow disease—including samples from two cases that have not yet been made public. He also sent off specimens from patients with other neurological diseases. Zeidler declines to reveal the results of this comparison because he and his colleagues are preparing to submit them in a letter to LANCET.

Gibbs and his colleagues also tried the test on spinal fluid from cattle, sheep, and chimpanzees. Some of the animals had been infected with prions, some had not. The test detected 14-3-3 in 87 percent of the infected animals. In animals that were not infected, only 3 percent of the tests gave a positive result.

Pharmaceutical companies have no reservations about the value of such a test. Gibbs says they have begun queuing up to license the new technology, which was patented by NIH and Cal Tech. The researchers are preparing to simplify the test even further so that it can be administered as routinely as an HIV test.

Even before the new test becomes available, regulators in the United States have announced that they will take steps to ensure the safety of livestock feed—and ultimately of the people who dine on beef.

The Food and Drug Administration is likely to ban the use in cattle feed of all protein derived from certain animals or just that from their eyes, brains, spinal cords, and lower intestines. The banned group could include sheep, beef, other ruminants, and perhaps swine, says Bert Mitchell, director of surveillance and compliance in the FDA's Center for Veterinary Medicine.

Since the emergence of mad cow disease in the United Kingdom, the U.S. beef industry has relied on a Department of Agriculture surveillance program that each year culls cattle showing symptoms of neurological disease, slaughters them, and examines their brain tissue.

Although about 5,000 head are tested each year, none has been found to have mad cow disease—proving that the ailment has not yet emerged in the United States, says Gary Weber, executive director of regulatory affairs for the National Cattlemen's Beef Association in Washington, D.C.

Cattle farmers would welcome a simple, accurate test, says Weber. "That's where this new test from NIH could help us."

Not surprisingly, Gibbs is delighted with the outcome of years of painstaking effort. "It's a very great satisfaction," he says, "knowing that you have been able to do something that's going to help in human medicine—and veterinary medicine as well." □

Mathematics

Crop circles: Theorems in wheat fields

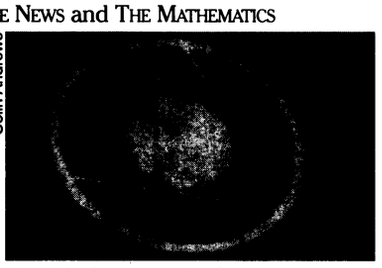
Since the late 1970s, farmers in southern England looking out on their wheat fields in the morning have sometimes been startled to find large circles and other geometric patterns neatly flattened into the crops. How these crop circles were created in the dead of night at the height of the summer growing season remains a puzzle, though hoaxers have claimed responsibility for some of them.

Several years ago, astronomer Gerald S. Hawkins, now retired from Boston University, noticed that some of the most visually striking of these crop-circle patterns embodied geometric theorems that express specific numerical relationships among the areas of various circles, triangles, and other shapes making up the patterns (SN: 2/1/92, p. 76). In one case, for example, an equilateral triangle fitted snugly between an outer and an inner circle (see photo). It turns out that the area of the outer circle is precisely four times that of the inner circle.

Three other patterns also displayed exact numerical relationships, all of them involving diatonic ratios, the simple whole-number ratios that determine a scale of musical notes. "These designs demonstrate the remarkable mathematical ability of their creators," Hawkins comments.

Hawkins found that he could use the principles of Euclidean geometry to prove four theorems derived from the relationships among the areas depicted in these patterns. He also discovered a fifth, more general theorem, from which he could derive the other four (see diagram). "This theorem involves concentric circles which touch the sides of a triangle, and as the [triangle] changes shape, it generates the special crop-circle geometries," he says.

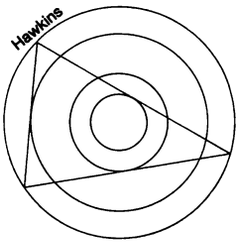
Curiously, Hawkins could find no reference to such a theorem in the works of Euclid or in any other book that he consulted. When he challenged readers of SCIENCE NEWS and THE MATHEMATICS TEACHER to come up with his unpublished theorem, given only the four variations, no one reported success.


Colin Andrews

Aerial photograph of a geometric crop-circle pattern in an English wheat field.

This past summer, however, "the crop-circle makers... showed knowledge of this fifth theorem," Hawkins reports. Among the dozens of circles surreptitiously laid down in the wheat fields of England, at least one pattern fit Hawkins' theorem.

The persons responsible for this old-fashioned type of mathematical ingenuity remain at large and unknown. Their handiwork flaunts an uncommon facility with Euclidean geometry and signals an astonishing ability to enter fields undetected, to bend living plants without cracking stalks, and to trace out complex, precise patterns, presumably using little more than pegs and ropes, all under cover of darkness.



Hawkins' fifth crop-circle theorem involves a triangle and various concentric circles touching the triangle's sides and corners. Different triangles give different sets of circles. An equilateral triangle produces one of the observed crop-circle patterns; three isosceles triangles generate the other crop-circle geometries.