The revelation that scrapie probably spawned "mad cow disease" and its human variant has renewed a campaign to eradicate scrapie before it or the mad cow pathogen—perhaps masquerading as scrapie—threatens people

On the Hunt for a Wolf in Sheep's Clothing

TRECASTLE, WALES, AND COMPTON, ENGLAND—As Welsh farmer David Jones opens a creaky iron gate, his black sheepdog, Zack, bolts up a hill toward a dozen ewes grazing near the top. The sheep take flight, disappearing over the crest with Zack in hot pursuit. They reappear a few seconds later, running down the hill with Zack nipping at their hindlegs. "Don't bite them, Zack!" yells Jones, eager to make a good impression on his visitors. As the sheep cower next to the gate, Zack glaring at any woolly thing that dares to move, Jones leaps into the middle of the flock and collars a ewe with his blue shepherd's staff. "See these brown patches on top of her head?" he asks, pointing to where the animal has lost some wool. "That's the first sign of scrapie."

If Jones's diagnosis is confirmed, it would be the third case of this fatal neurodegenerative disease among his 700 ewes during the past year. Scrapie, which is the ovine counterpart to bovine spongiform encephalopathy (BSE), or "mad cow disease," is a scourge that British sheep farmers—as well as their nation's health and agriculture officials—are treating with new respect. Like BSE in cattle and Creutzfeldt-Jakob disease (CJD) in humans, scrapie is linked to aberrant proteins called prions, which many researchers believe act alone to infect and destroy nervous tissues. BSE has apparently jumped the species barrier and caused more than 50 deaths in the United Kingdom and France from a new form of CJD in humans.

Although there is no evidence that scrapie, which has afflicted British flocks for more than 250 years, can also infect people, the devastating experience with BSE provides little room for comfort: It demonstrated that animal prions can harm humans, which means that scrapie should not be disregarded as a potential health threat. Even more worrisome, prion researchers have been able to infect sheep with BSE; the resulting disease, which causes the animals to tremble and stagger and eventually die from a progressive loss of brain cells, closely resembles scrapie. This has raised serious concerns among public health officials that if a BSE epidemic did break out among sheep, it might not be recognized immediately.

For these reasons, in 1996 the Spongiform Encephalopathy Advisory Committee (SEAC), an expert panel that advises the British government on transmissible prion diseases, recommended beefing up the low-profile scrapie research then under way, with the ultimate aim of eradicating the disease. A major scrapie initiative is now in progress at the Ministry of Agriculture, Fisheries, and Food's (MAFF's) Veterinary Laboratories Agency in Weybridge. And for the past 2 years, scientists at the Compton headquarters of the U.K.'s Institute for Animal Health (IAH) have been harnessing the tools of epidemiology, genetics, and mathematical modeling to lay the scientific groundwork for the task. Much of their initial work has meant studying scrapie at its source. IAH scientists attend sheep shows and slog through muck on sheep farms like the one Jones runs in Trecastle, looking for clues to how this poorly understood disease spreads and how it might someday be brought to heel.

Slaughterhouse bound? The patches on this ewe's head may be a sign of scrapie. If this suspicion is confirmed, she will be isolated from the flock and destroyed.

CREDIT: M. BAYLIS

Although the research is in its infancy, the Compton team's preliminary findings are providing new clues to risk factors for scrapie infection. They have also bolstered previous studies showing that some sheep are endowed with genes that help them resist a scrapie infection, while others have genes that make them highly susceptible—a finding that might allow farmers to breed in resistant genotypes and breed out susceptible ones. The scientists are also on the lookout for BSE in sheep masquerading as scrapie.

As they gather knowledge about this ancient killer, researchers are building bridges to farmers, whose cooperation will be essential to any eradication effort. Although the fractious dispute over BSE and British beef often sowed distrust between the two communities, the fight against scrapie is uniting scientists and farmers in a common cause. After all, says IAH mathematical biologist Angela McLean, who leads the Compton project, the primary goal is one that everyone agrees on: "We need to get prions out of the food chain."

A resilient enemy

Despite scrapie's long history in the British Isles, surprisingly little is known about how it is transmitted from sheep to sheep and flock to flock, or what puts individual animals at risk, aside from evidence that ewes may infect lambs during pregnancy or just after birth. "You can't just dive in and try to eradicate scrapie instantly," says Richard Cawthorne, MAFF's deputy chief veterinary officer. "We need to understand a lot more about the disease's basic mechanisms." Indeed, the most obvious strategy, killing off infected sheep in hopes of purging the pathogen, has failed. For example, soon after the first scrapie cases appeared in the United States in 1947—apparently a result of imports of British sheep—the U.S. Department of Agriculture (USDA) declared a state

Writing Scrapie's Coda, Codon by Codon?

Over the past decade, geneticists have begun to unravel why some sheep are more vulnerable than others to scrapie. They have found that different variations, or polymorphisms, in the gene coding for PrP—a cellular protein that many scientists believe becomes infectious when it converts to an abnormal form called a prion—seem to confer varying degrees of susceptibility. This correlation raises the possibility that genetically susceptible sheep could be bred out of the population, leaving only scrapie-resistant animals (see main text).

Studies of sheep experimentally infected with scrapie have shown that three codons, or positions, in the PrP gene—codons 136, 154, and 171—are critical in determining whether the animal comes down with the disease. Each codon gets translated into one of the 256 amino acids of the sheep PrP protein. Individuals most vulnerable to scrapie have the amino acids valine, arginine, and glutamine at the respective positions dictated by the three codons. Using the single-letter code for amino acids, this polymorphism is referred to as VRQ. At the other extreme, sheep with the polymorphism alanine-arginine-arginine (ARR) are the most resistant. Indeed, out of hundreds of scrapie-infected sheep tested worldwide, only one, in Japan, has turned out to be ARR. Three other polymorphisms (shown at right) apparently lead to intermediate levels of vulnerability to the disease.

SHEEP PrP POLYMORPHISMS

	PrP codon		
	136	154	171
Least vulnerable	V	R	Q
	A	R	Q
	A	H	Q
	A	R	H
Most vulnerable	A	R	R

A = alanine; H = histidine; Q = glutamine; R = arginine; V = valine

Letter carriers. The particular amino acid at these three positions in the PrP protein strongly influences whether a sheep can become infected with scrapie.

Muddling this neat picture, however, are some bizarre differences in the effect of polymorphisms in different sheep breeds. For example, Suffolk sheep with the genotype ARQ are susceptible to scrapie, whereas ARQ Cheviot sheep are resistant. "We really don't understand this," says Nora Hunter, a geneticist at the Institute for Animal Health's Neuropathogenesis Unit in Edinburgh. Hunter and her colleagues are currently testing several hypotheses, including the possibility that the two breeds are being infected by different prion strains, or that Suffolk sheep may produce higher levels of PrP and thus have more protein available for conversion to the prion form.

More clear, however, is why PrP polymorphisms correlate with scrapie susceptibility in the first place. Findings reported in the 17 July 1997 issue of *Nature* and in the 13 May 1997 *Proceedings of the National Academy of Sciences* show that the VRQ version of normal PrP protein is easily converted into the prion form when mixed with other prions in the test tube. (Most researchers studying prion diseases believe this mechanism is responsible for the creation of new prions in infected animals.) The ARR polymorphism, on the other hand, strongly resists this conversion, while polymorphisms corresponding to intermediate scrapie susceptibility fall in between. This biochemical confirmation of the importance of PrP polymorphisms has bolstered the view that breeding VRQ and other susceptible genotypes out of the sheep population might be the best course toward eradication. Says Hunter: "At the moment, there really isn't any good alternative." **–M.B.**

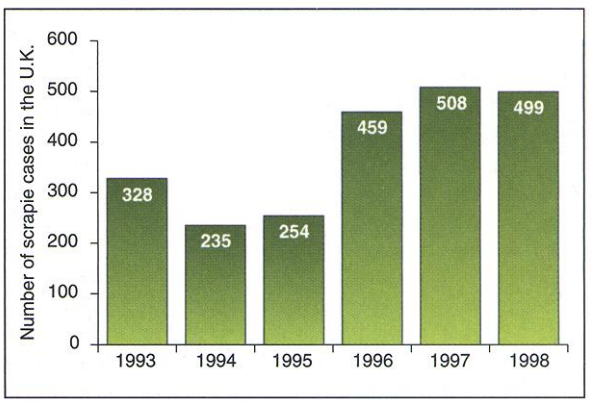
of emergency and ordered the slaughter of whole flocks each time a single sheep fell ill. Yet this scorched-earth policy, which continued until the early 1980s, hardly made a dent in the U.S. scrapie toll.

The mass slaughters discouraged farmers from reporting the disease and "drove it underground," says veterinarian Linda Detwiler, coordinator of the USDA Animal and Plant Health Inspection Service's working group on transmissible prion diseases. The agency now relies on voluntary programs to identify and isolate infected flocks, and—as is current practice in the United Kingdom—only infected animals are killed. The average number of cases has dropped by about half in the past decade, but several dozen new cases are still reported each year among the 11.5 million sheep in the United States. "We have tried every scrapie control and eradication program known to mankind, but they did not work," says Detwiler.

Although scrapie flourished, the disease intrigued few researchers—until it became clear in the mid-1990s that people had become infected with BSE, apparently from eating meat or other products from infected cattle. "If the BSE epidemic had not occurred, I suspect scrapie would still be a scientific backwater," says molecular biologist Chris Bostock, IAH's director.

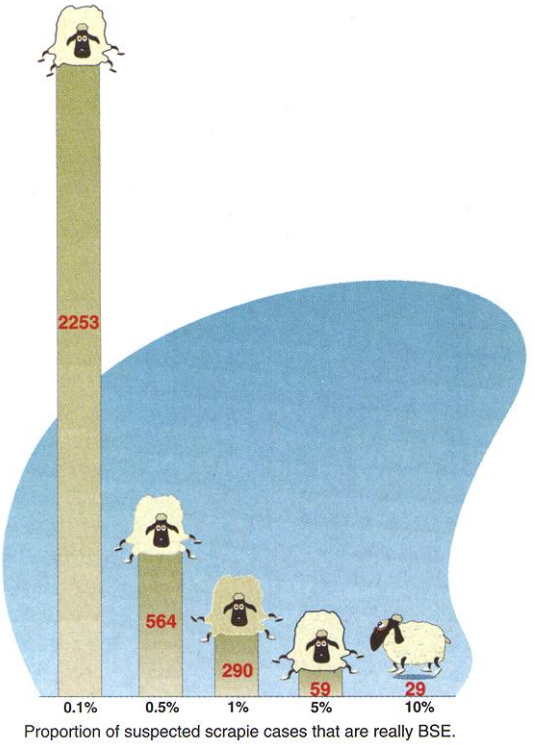
But there was more at play than merely a missed research opportunity. The persistence of scrapie may well be responsible for the rise of BSE in cattle. Many researchers believe that the BSE epidemic began when scrapie-infected meat, bones, and nervous tissue from sheep were added to cattle feed during the decade leading up to July 1988, when the practice was banned. Support for this idea emerged last year, when IAH researchers reported in the January 1999 issue of the *Journal of General Virology* that one "strain" of scrapie prion isolated during the 1970s—called CH1641—is strikingly similar to the BSE prion. Although the concept that protein-based prions come in strains like viruses or bacteria is controversial, researchers agree that certain characteristics—such as how many sugar molecules are bound to the prion's amino acids—generally remain stable even when the prions infect other species. The key evidence that humans have been infected with BSE, for example, came from the fact that prions isolated from the brains of variant CJD (vCJD) victims are nearly identical to BSE prions.

Yet even if the BSE prions that jumped from cattle to humans originated in sheep, this does not necessarily mean that scrapie prions can infect humans. The most persuasive evidence that scrapie prions can't—for now—harm people is that decades of exposure to scrapie-infected sheep has not caused a single known infection in humans. Indirect support also comes from experiments demonstrating species barriers against prion



On the hoof. Since notification became mandatory in 1993, yearly numbers of reported cases have edged up.

SOURCES: (TOP TO BOTTOM) N. HUNTER; MAFF



Lurking in the madding crowd? The scarcer BSE is in the sheep population, the harder it will be to flush it out. The bars indicate how many samples are required to be 95% certain of detecting at least one BSE case hidden among 5000 suspected scrapie cases.

infection: Mice fed BSE prions become infected, while hamsters do not. "There appears to be an absolute block between cows and hamsters," says Bostock. However, if brain extracts from BSE-infected mice are fed to hamsters, they do become infected. Some researchers suggest that a similar block may exist between sheep and humans, but not between sheep and cattle: Scrapie prions must be modified somehow in cattle before they are capable of infecting people. Because cattle are no longer being fed ground sheep, the presumed BSE link between scrapie and vCJD should now be severed. Therefore, says Byron Caughey, a virologist at the U.S. National Institutes of Health's Rocky Mountain Laboratories in Hamilton, Montana, "there are no grounds for hysteria."

A far more serious concern is that sheep might harbor BSE prions. Until the practice was banned in 1988, the remains of both cows and sheep often wound up in ruminant feed. And sheep certainly are capable of being infected. In 1993, researchers at the IAH's branch in Edinburgh, Scotland, first reported that BSE could be transmitted to sheep via infected cattle brain extracts—results that have been confirmed by many other groups.

There's no evidence yet that BSE is lurking in sheep, but if these prions do find their way into a new animal reservoir, they will be easy to miss. Although the number of reported cases of scrapie is relatively low—averaging about 500 per year among the U.K.'s 40 million sheep—an anonymous survey of more than 11,000 sheep farmers, carried out by the Compton and MAFF teams, indicates that the actual occurrence may be eight times the official numbers. Underreporting of scrapie cases, combined with the ability of BSE symptoms to masquerade as scrapie, makes it unlikely that BSE in sheep would be detected unless it amounted to more than 5% of scrapie cases, according to SEAC calculations. For these reasons, says McLean, "deep down" the Compton project "is about BSE in sheep." Eradicating scrapie, she says, would be the best insurance against an undetected BSE epidemic in these animals.

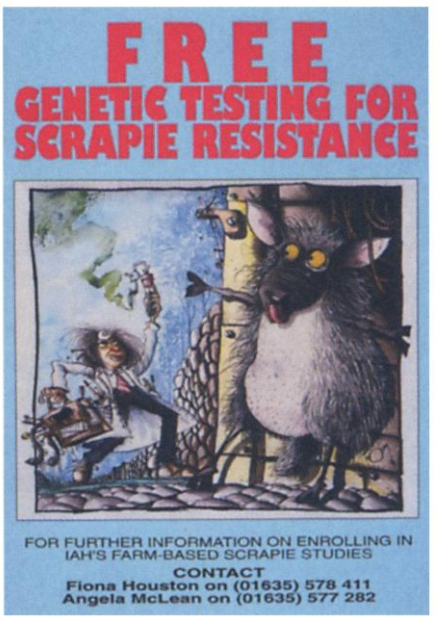
Ewegenics for rams?

The key to defeating scrapie—and defusing the BSE threat—may lie in figuring out which sheep should be allowed to reproduce. Over the past decade, geneticist Nora Hunter of the IAH's Neuropathogenesis Unit in Edinburgh and other researchers have established that sheep vary in their susceptibility to scrapie depending on variations—called polymorphisms—in the nucleotide sequence of the gene coding for PrP, the normal protein that apparently causes disease if it converts to the abnormal, or prion, form (see sidebar). Some polymorphisms make sheep nearly impervious to scrapie, while others make them highly susceptible. Testing for these polymorphisms, and only allowing sheep with beneficial ones to breed, might help eradicate scrapie.

Both supporting and complicating this picture are findings from a pilot study last year of four British flocks—two of which were scrapie-infected and two scrapie-free—led by IAH field epidemiologist Matthew Baylis. The surprising news is that the scrapie-free flocks had only slightly lower proportions of susceptible genotypes than did the scrapie-infected flocks. But the number of older animals with susceptible genotypes in the infected flocks was much lower than expected. This implied, the team concluded, that the real scrapie toll in these flocks had been much higher than thought—either due to underreporting or because farmers were not recognizing scrapie. If so, the two infected flocks must have started out with significantly higher proportions of younger susceptible sheep, which then succumbed to scrapie.

These findings support the idea that selective breeding should at least cut the toll from scrapie, although researchers caution that there are many uncertainties. "Breeding for resistance might remove clinical signs of scrapie," says Hunter, "but there is a slight worry that the resistant animals might still be carriers of infection." And McLean says that although breeding programs might eradicate today's strains of scrapie, it's unknown whether the resistant sheep could withstand altered or "mutant" prions that might arise later. Still, says veterinary epidemiologist Linda Hoinville, who leads the scrapie epidemiology group at the Veterinary Laboratories Agency, "this is the most promising strategy at the moment."

McLean and her team have begun asking farmers like David Jones—who has spray-painted his sheep to mark those with susceptible or resistant genotypes—to breed lambs only from scrapie-resistant ewes and rams. And Hoinville's team believes it has evidence that this effort may pay off: In preliminary, unpublished work on one flock in which scrapie was rampant (carrying a whopping 6% infection rate at the outset), the Weybridge group found that only allowing resistant rams to breed reduced scrapie incidence in later generations to negligible levels. If these results are confirmed, it could mean that researchers and farmers may soon be able to write the final chapter on this deadly disease's 250-year history and the human health concerns that have become an unsettling subtext to the story.



Not sheepish one bit. Through whimsical ads and visits to sheep farms, British scientists are winning cooperation from a community still shell-shocked by BSE.

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–MICHAEL BALTER