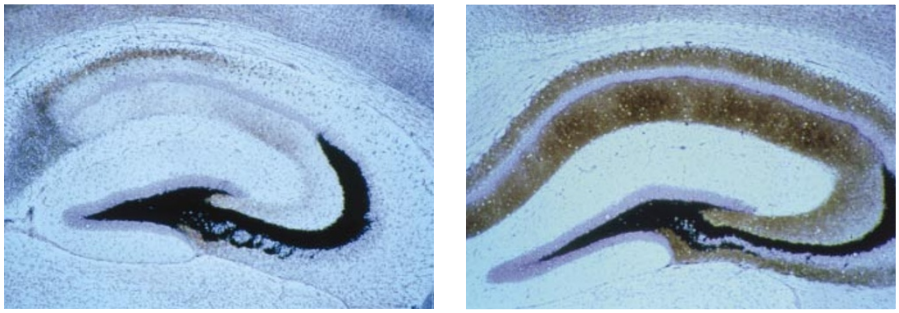
A wolf in sheep's clothing

Is BSE lurking in sheep, but masked by scrapie? Reliable and fast tests that can tell the diseases apart are urgently needed, says Declan Butler.

P. DEAN/AGRIPICTURE



J. FRASER/EURELIOS/SPL

Scrapie in a slice of sheep brain (left) showing loss of grey matter compared with a healthy brain.

After years of research, we're still no closer to knowing whether the epidemic of bovine spongiform encephalopathy (BSE) that ripped through British cattle herds from the mid-1980s also spread to sheep. In October, researchers were poised to reveal that they had found the tell-tale signature of the BSE agent in a sample of sheep brains dating from the early 1990s, when a last-minute government statement threw their work into confusion. The tests, it was announced, had been conducted in error on cattle brains, because of a mix-up of samples[1].

But while the lamb industry is off the hook for the time being, the risk that BSE is lurking in sheep flocks is real, as sheep were fed the same contaminated feed blamed for the disease's spread through cattle. If so, it would be impossible to distinguish the symptoms from scrapie, a naturally occurring disease of sheep.

That is a worrying proposition. Experiments in which sheep have been infected with BSE indicate that a greater proportion of tissues become infective than in a cow with BSE[2] — meaning that lamb might even have to be pulled off the market altogether. And unlike BSE in cows, which should be eradicated in a matter of years now that contaminated feed is not in use, scrapie passes readily from sheep to sheep in ways that are not fully understood. If BSE in sheep behaved similarly, it might not go away. The British government's contingency plans, released for consultation in September, reflect these worries — even suggesting that the entire national flock of up to 40 million animals might have to be slaughtered.

If nothing else, the 'wrong brains' fiasco revealed the importance of developing a fast diagnostic test that can distinguish BSE from scrapie. The researchers had tested a pooled sample of brains by injecting the liquidized tissue into different strains of mice and looking at the disease incubation times and the pattern of the brain lesions that formed. This method, developed by Moira Bruce of the Institute for Animal Health (IAH) in Edinburgh, provides a reliable way of distinguishing between different strains of scrapie, and of identifying the BSE agent. But with readout times of up to two years, it can hardly be described as fast.

Prion probes

No rapid diagnostic test has yet been validated for detecting transmissible spongiform encephalopathies in sheep, let alone one that can distinguish between BSE and scrapie. In both diseases, a cell-membrane protein called PrP, which consists mainly of conformations called α-helices, is converted to the abnormal 'prion' form, PrP^{Sc}, which consists mainly of β-sheets (see figure, opposite). Three rapid tests, which use antibodies to detect the rogue form, are reliable detectors of BSE in cows showing symptoms, and have been approved for use in cattle by the European Commission[3].

These tests — marketed by Prionics of Zurich, Enfer Scientific of Tipperary, Ireland, and Bio-Rad of Hercules, California — have also been put through preliminary trials on sheep with scrapie, and seem to perform well, according to European Commission officials. Applying them to sheep would be useful, as almost nothing is known about the epidemiology of scrapie in Europe. And if used in abbatoirs, they could help keep infected sheep out of the food chain.

But to get to the bottom of the issue, we need a test that can distinguish between the two diseases. The best-known candidate was developed by John Collinge of Imperial College, London. His test involves loading samples of PrP^{Sc} onto a gel, using electrophoresis to separate the protein into different bands and then performing a standard western blot procedure to identify the rogue prion. In 1996, he showed that PrP^{Sc} from human patients with vCJD, the variant of Creutzfeldt–Jakob disease apparently caused by the BSE agent, forms a distinctive pattern of bands that distinguishes it from other forms of CJD[4]. The differences are thought to arise from variations in the sugar groups attached to the protein.

The method is cheap and, in experienced

hands, hundreds of samples can be processed in 48 hours. But to obtain good results, large quantities of protein must be loaded onto the gels, which can make the test difficult to perform. "You need something that works in everyone's hands 100% of the time," notes one prion researcher.

Nevertheless, scientists at the UK Central Veterinary Laboratory (CVL) in Weybridge, Surrey — part of the Veterinary Laboratories Agency (VLA) — have been trying to determine whether a western blot procedure can also identify BSE in sheep. Collinge has publicly criticized the Department for Environment, Food & Rural Affairs (DEFRA), which is sponsoring the research at the VLA, for the time it has taken to adapt the test for use in sheep.

Digestive aids

The VLA is working on a number of tests that might be able to distinguish BSE from scrapie. It is modifying the Prionics test to incorporate a variant of Collinge's method. In addition, Michael Stack, a researcher at the CVL, has identified a 'signature' at one end of the fragment that remains when PrP^{Sc} is digested with a protease enzyme. The point at which the enzyme cleaves the protein seems to vary depending on whether the prion is BSE or scrapie, and antibodies can target this difference. The CVL is now collaborating with Prionics to turn this into a commercial test.

Another method, developed by Martin Jeffrey's group at the VLA in Lasswade, near Edinburgh, uses two different antibodies to stain samples of brain and lymphoid tissue, giving patterns for scrapie and for BSE that Jeffrey argues can be distinguished by a trained eye.

Last month, David King, the UK government's chief scientific adviser, announced plans to screen 20,000 sheep for prion disease, using the basic Prionics test and a test related to the one developed by Jeffrey that does not distinguish between BSE and scrapie. Positive results will be further investigated using the VLA's prototype tests to look for suspicious BSE-like signals.

In the meantime, other groups are also trying to devise tests that could distinguish BSE in sheep. Enfer Scientific is working with an antibody that it believes is specific for scrapie PrP^{Sc}. Samples that test positive on its current test but negative using this antibody would be BSE, says Riona Sayers, who coordinates the company's research. The technique discriminates cattle with BSE from sheep with scrapie, she says.

The researchers at the French Atomic Energy Commission (CEA) who developed the test sold by Bio-Rad are also working on a two-step test, but one which exploits the fact that scrapie PrP^{Sc} from sheep is more resistant than BSE PrP^{Sc} from cattle to digestion by an enzyme called proteinase K. They assume that the BSE prion in sheep will be similarly susceptible to the enzyme. Samples testing positive using the existing Bio-Rad test are retested after applying a concentration of proteinase K that obliterates all BSE PrP^{Sc}, but leaves scrapie PrP^{Sc} detectable. In cows with BSE, the signal disappears, whereas in sheep with scrapie it persists. Preliminary tests on 300 sheep showing symptoms of scrapie have shown a handful of BSE-like responses.

Unfolding issues

Other researchers have different approaches. Jiri Safar, working in the lab of Stanley Prusiner at the University of California, San Francisco, is developing a test that depends on the way in which different versions of PrP^{Sc} unfold when they are treated with a chemical called guanidine hydrochloride[5]. Meanwhile, Alex Bossers and his colleagues at the Institute for Animal Science and Health in Lelystad, the Netherlands, are working from the observation that PrP^{Sc} converts PrP to its own prion form more quickly if it comes from the same species. BSE in sheep might therefore show different conversion rates from scrapie, and it should be possible to measure this.

Given that any test can be subject to false positives and false negatives, and the high stakes involved, experts say it will be important to use several tests in parallel to cross-check results — as the British programme will do. But Safar is worried that none of the tests is yet sufficiently well developed to be relied upon. Applying them too quickly, he says, "is a very dangerous strategy that risks generating a lot of controversial findings which cannot be verified in a reasonable time".

UPPA

Head start: John Collinge's test might identify BSE in sheep.

The main immediate bottleneck for those developing diagnostics is getting sufficient BSE-infected sheep tissues — most of which are held by the CVL — to validate the prototype tests. Jacques Grassi, a member of the CEA team, says that its new test works well on samples of mice infected with either BSE or scrapie. But so far the team has been able to try it on just five sheep infected experimentally with BSE. "We need more tissues," he says.

Aside from the reliability of the nascent diagnostic technology, the main theoretical problem is our ignorance of the variety of scrapie strains circulating in the field, and of the behaviour of these strains — and of BSE — in sheep of different genotypes. "These two areas are the major limiting factors," says Stephen Edwards, chief executive of the VLA.

So far, for instance, only one breed of sheep has been deliberately infected with BSE. The CVL has begun a new series of experiments to generate infected tissues from other breeds, but these tissues will not become available for another two years. Until these samples can be examined, there is a danger that using the tests in the field might give false positives — or false negatives — in particular breeds of sheep.

BSE seems to behave as a single strain, which is unchanged on transmission to other species. But few strain-typing tests have been done, and experiments with scrapie in rodents have shown that the characteristics of a strain can change if it is repeatedly transmitted from animal to animal[6]. "BSE might change when passed from sheep to sheep," says Bruce.

Given these complications, and the dire consequences of producing a positive test for BSE in sheep, the scientists developing the tests are under intense pressure. Weighing on their shoulders is the potential danger to public health — not to mention the livelihoods of thousands of farmers. ■

Declan Butler is *Nature*'s European correspondent.

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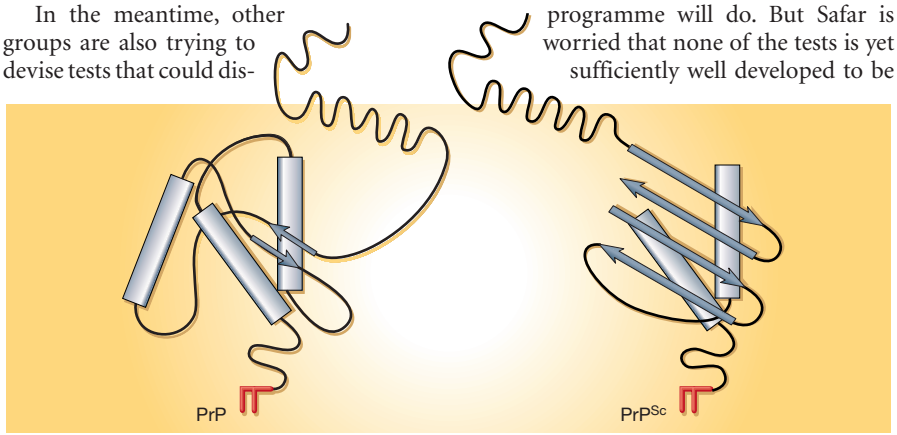
UK government contingency plan

www.defra.gov.uk/corporate/consult/sheepbse/index.htm



Deadly change: some α-helices (grey tubes) in PrP are refolded as β-sheets (blue arrows) in prions.