BSE IN SHEEP?

Humiliated Lab Fights To Save Face

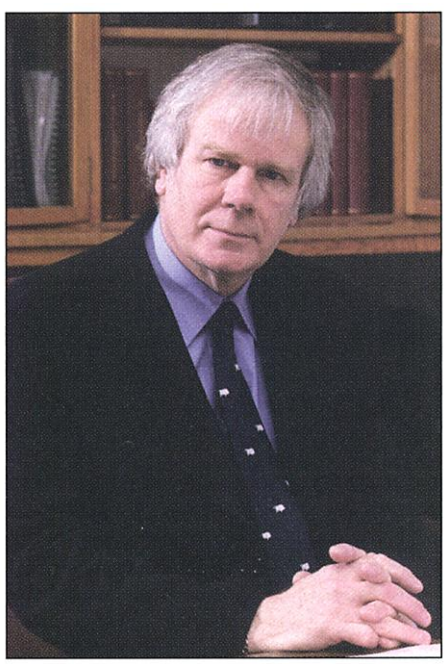
The U.K.'s Institute for Animal Health was ridiculed over an alleged sample mix-up. But the facts of the case are far more intriguing

EDINBURGH—Down a quiet corridor here at one of the United Kingdom's premier research labs sits a tall, padlocked freezer containing a handful of samples of brain tissue. The samples are all that's left of an experiment that went badly wrong—and yet no one really knows why. But solving the riddle could have important implications for public health.

Last October, scientists at the Institute for Animal Health's (IAH's) Edinburgh branch were about to publish results suggesting that British sheep might have become infected with bovine spongiform encephalopathy (BSE), or "mad cow disease," in the early 1990s. The human form of BSE, variant Creutzfeldt-Jakob disease (vCJD), is an invariably fatal neurodegenerative disease that has already caused more than 100 deaths in the United Kingdom. Finding BSE in sheep could raise the specter of an alarming new reservoir of infection. "The potential implications for public health if BSE were found in sheep would be large," notes John Krebs, chair of the Food Standards Agency (FSA). But the authors pulled the paper at the last minute after an independent lab concluded that the brain extracts they had tested—which the team believed were from sheep—actually came from cattle (*Science*, 26 October 2001, p. 771).

British newspapers derided "government scientists" who supposedly couldn't tell the difference between sheep and cattle—a hard blow for a team known for many key discoveries, including the critical finding in 1997 that vCJD is caused by BSE. IAH's plight worsened the following month when two government-ordered audits identified alleged deficiencies in how the samples had been labeled. And the timing could not have been worse: The U.K.'s Biotechnology and Biological Sciences Research Council (BBSRC), which provides a large chunk of IAH's core funding, had just begun examining the institute's requests for grant renewals. For weeks after the story broke, IAH staff lived in fear of cutbacks and job losses.

Instead, BBSRC has come out in strong support of the institute, while acknowledging that the lab may well have made some errors. IAH "will continue to receive core support for its excellent research," BBSRC spokesperson Andrew McLaughlin told *Science*. And outside researchers insist that the episode has not significantly tarnished IAH's overall reputation. The group "can look back at 4 decades of exceptionally productive research," says Adriano Aguzzi, a neuropathologist at the University of Zürich, Switzerland. Although the Edinburgh team concedes it may have made mistakes, including possibly mixing up the samples, it believes that there has been an unfair rush to judgment, particularly by the British media. The researchers are challenging the findings of the independent lab—the Laboratory of the Government Chemist (LGC)—and have launched their own investigation into the decade-long chain of events that led to the fiasco. "We are confident in our work and in our standards," says IAH researcher Moira Bruce. LGC, meanwhile, stands by its findings.



Team player. IAH chief Chris Bostock insists the jury is still out on brain mix-up.

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One experiment too many?

Much of the reputation of the Edinburgh lab, one of three IAH maintains in the United Kingdom, rests on its development of "strain-typing" techniques to discern variations in the infectious agents that cause BSE and other spongiform encephalopathies, such as scrapie and CJD. Most researchers now believe that these diseases are caused solely by aberrant forms of proteins called prions.

To identify the infectious agent, researchers feed or inject mice with brain extracts from humans or other animals suffering from prion diseases. The incubation time varies for each strain and, coupled with hallmark patterns of brain damage, enables researchers to tell many strains of scrapie apart; they can even distinguish vCJD from "sporadic" CJD, an extremely rare condition that's not linked to BSE. In a pivotal experiment in 1997, the group showed that BSE and vCJD behaved identically in strain-typing studies, providing the smoking gun that humans were getting sick from eating mad cows.

Normally, the brain extract used to infect mice comes from a single animal, so the chances of a mix-up are slight. But to probe whether BSE was lurking in sheep a decade ago, the researchers had to resort to a pool of sheep brain material collected under less than ideal conditions—and for an entirely different purpose.

In the early 1990s, after it became clear that the BSE epidemic had spread when cattle were fed so-called "meat and bone meal" (MBM) from slaughtered cattle and sheep, officials began to hypothesize that the high-temperature MBM rendering process may have transformed scrapie into a form that's infectious to cattle. To investigate how to inactivate these agents, government veterinarians collected 2867 brains from sheep apparently infected with scrapie and pooled the extracts. These early experiments, which were led by IAH microbiologist David Taylor—and which included a similar study of 861 BSE-infected cattle brains—were wrapped up in the mid-1990s after researchers found that some modified rendering techniques did seem to knock out the infectious agent. The leftover samples were stored in an IAH freezer.

The team may now be wishing that the samples had stayed there. In 1997, however, IAH launched a new round of experiments with the pooled sheep brain samples to further probe whether BSE originated from scrapie. In the meantime, the government had become increasingly worried that BSE might be masquerading in the sheep flock as scrapie. This concern was bolstered by findings that sheep infected experimentally with BSE had scrapielike symptoms. In 2000, the U.K. Department for Environment, Food, and Rural Affairs (DEFRA) asked IAH to extend the studies to try to resolve whether sheep had been infected with BSE in the early 1990s, the height of the mad cow epidemic.

Molecular biologist Chris Bostock, IAH's director, says that the alarm bells immediately began ringing. "We cautioned against this use of the samples, because of the uncertainty in their provenance," he says. The team, he says, had long been concerned that the sheep sam-

ples might have become tainted with cattle brain extract when collected at veterinary slaughterhouses where both species were killed. If those cattle brains were themselves infected with BSE, the results of experiments could be difficult to interpret. Yet the leftover sheep brain pool offered the only opportunity to learn whether BSE had entered sheep a decade earlier. "There was no choice," says IAH neuropathologist Janet Fraser. "You either used that or you didn't do the experiment." IAH agreed to carry out the study, although it asked DEFRA to pay for independent testing for contamination.

They're the ones with the woolly sweaters! The British media accused "government scientists" of getting their ruminants wrong.

CREDIT: TONY ARRUZA/CORBIS

Conflicting data

At this point, the team, despite its concerns about trace contamination, still believed that the samples it was working on were primarily sheep brain. But two audits commissioned by DEFRA after the affair broke last fall found deficiencies in the way the Edinburgh team had labeled and stored the samples since the early 1990s. One of the audits, carried out by the private firm Risk Solutions, suggested how a possible mix-up might have occurred when the second round of experiments began in 1997. The leftover sheep and cattle brain pools had been stored in the same freezer, and the technician working with Taylor told the auditors that the sample labels were not entirely clear.

The Edinburgh team declined to make the technician available for an interview. Taylor—who retired 2 years ago—says that he can now only recall that there was "some form of identification that retrospectively one might consider to be potentially ambiguous." But the team thought the matter was resolved after IAH's genetics team analyzed prion genes in the samples. When these came back with a DNA signature specific to sheep, the team assumed that it was working on the right samples.

The rude awakening came last October, when a DEFRA-commissioned study concluded that IAH had been working on cattle brains all along. The devastating report came from LGC, an independent outfit that had been contracted to check one last time into IAH's concerns that the samples might harbor trace contamination. LGC used the highly sensitive polymerase chain reaction (PCR) to analyze DNA in the samples submitted to it by IAH. The news media trumpeted the stunning result: all cow, no sheep.

Although neither of the government-commissioned audits was able to pinpoint what had gone wrong, the Risk Solutions auditors concluded that a sample mix-up when the experiment first began in 1997 was the most likely explanation for LGC's finding. IAH biochemist Robert Somerville, who took over the experiment after Taylor retired, agrees that this scenario is "plausible," although he argues that it "does not explain" the in-house genetic test results pointing to sheep.

Bostock insists that the jury is out on exactly what is in the samples. "It didn't surprise me that there was bovine material," he says. "What was surprising and damaging was the claim that there was no sheep material." Bostock and other IAH researchers contend that this conclusion is premature, especially because the samples were highly degraded after being processed for earlier experiments and had been thawed and refrozen repeatedly. They raise the possibility that if highly degraded sheep material had been contaminated with a tiny amount of better quality cattle extract, PCR would have picked up only the cattle signature.

But LGC molecular biologist Helen Parkes, who supervised the PCR work, rejects this scenario. "We got a very good yield of DNA," she says. "The levels we were seeing are not consistent with trace bovine contamination at all." She confirms that LGC did not detect any sheep DNA. IAH is now conducting its own PCR tests.

Right all along?

Some outside researchers familiar with the findings of IAH's ill-fated study suggesting that sheep were infected with BSE posit another scenario: that IAH studied the right brains but submitted the wrong samples to LGC for analysis. The conclusion drawn in the unpublished paper was that strains similar to both scrapie and BSE were present in the brain pool. If IAH's genetic findings were valid, as Bostock asserts, and if contamination levels were low, the BSE-like strains must either have derived from scrapie strains resembling BSE or from BSE-infected sheep, the paper's authors concluded.

The Risk Solutions auditors considered it unlikely that the wrong samples had been delivered to LGC. But Edinburgh researchers do say that the BSE "signal" they saw in the mice was atypical—and therefore could not have come from testing a pure-cattle sample. In previous experiments in which mice were inoculated with BSE, the animals were easily infected and showed a characteristic BSE pattern. But in this study, the scientists had to reinject the infected mice brains into a second group of mice before the BSE pattern emerged.

This finding suggests that the researchers may have been working with sheep extracts all along, says Danny Matthews, chief of spongiform encephalopathy research at the U.K.'s Veterinary Laboratories Agency in Weybridge. "My interpretation is that they were actually strain-typing a pool of [sheep] brains and that at some subsequent point the study was compromised," he says. Taylor agrees: "These results could not have been obtained from infected cow brains. They would have seen different incubation periods and [pathology] profiles."

Whatever the explanation, Bostock says that the institute was given no time to try to figure out what had happened before being exposed to public humiliation. He and other IAH scientists still fume at FSA's decision to announce on its Web site last August that the unfinished study was under way, before the possibility of contamination had been eliminated.

But Krebs, FSA's chair, defends the decision to go public. Early announcements are "tricky," he says. But if the results had confirmed BSE in sheep, "no one would have doubted that we did the right thing by telling people at a time when the government was promoting the consumption of lamb."

Bostock and other researchers argue that the study could not have determined whether lamb is safe to eat today. Only ongoing studies on the current sheep flock can answer that question. "What matters to us in the U.K. is whether BSE is present in the sheep population now," says James Ironside of the National CJD Surveillance Unit in Edinburgh. But the now-discredited study could have helped fine-tune estimates of how many people might have become infected with BSE in the early 1990s from eating sheep—and thus might eventually contract vCJD. Unless the mystery of the wayward brains is cleared up—for example, by IAH's internal investigation—we may never know the answer.

–MICHAEL BALTER