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SPONGIFORM ENCEPHALOPATHY ADVISORY COMMITTEE
MINUTES OF THE 19TH MEETING HELD ON 21 JUNE 1995
AT THE CENTRAL VETERINARY LABORATORY

Present:  
Dr D A J Tyrrell (Chairman)  
Dr R H Kimberlin  
Professor J R Pattison  
Mr D B Pepper  
Dr W A Watson

Mr R Bradley (CVL)  
Dr A Wight (DH)  
Observers

Mr C Lister (DH)  
Mr T E D Eddy (MAFF)  
Secretariat

In attendance:  
Ms M Wilson (BBSRC)  
Dr P Dukes (MRC) (for Agenda items 1 to 3)  
Dr D Matthews (MAFF)  
Miss E J Wordley (MAFF)

Introductory

1. Apologies for absence had been received from Dr Will, Professor Allen, Professor Brown and Dr Hueston.

Minutes of Meeting on 10 February 1995

2. Dr Dukes suggested that paragraphs 5 and 6 of the minutes did not correctly reflect the position taken by MRC in the discussion of the inclusion of the third CJD case in a dairy farmer in the transmission studies. MRC was funding the current study of the two previous farmers and felt that the addition of a third needed to come with a scientific case to justify the considerable cost. It was proposed that the MRC Allen Committee, which was examining research priorities, would provide a suitable forum for examining the scientific case. The marginal cost of the addition of a third farmer would be high (the cost for the first two was £0.25m) and needed clear justification.

3. The Committee agreed that its judgement had been made on the basis that inclusion of an extra case would not add significant extra effort: the main difficulty was the high containment facilities. However, all felt that scientifically it was most
important to follow up all three farmers. It was essential to focus on any unusual cases where there was any connection with BSE. This was not simply another farmer but the third farmer. Since the numbers were very small and it was possible that some cases could be coincidental and some might result from transmission from animals (especially cattle), the Committee felt strongly that each should be followed up. Whilst recognising that other methodologies such as the use of transgenic animals were on the horizon, their role had still to be defined and early results fully interpreted and validated. The Committee did not feel it was prudent to delay the CJD investigations until then. The key points from the Committee’s discussion should be summarised in the minutes and made available for the Allen Committee to review.

4. Mr Bradley suggested that the list of priorities for strain typing annexed at Appendix 2 to the minutes needed to be kept under review in the light of other developments. The Committee discussed the priorities further under Any Other Business (see paragraph 28).

5. An amendment was agreed to paragraph 13.

Agenda Item 3 - The use of gelatin and blood and blood products in ruminant feedingstuffs.

6. Commission Decision 94/381 prohibits the feeding of protein derived from mammalian tissues to ruminant species. Mr Eddy explained that Commission Decision 95/60 of 6 March 1995 amended this decision to exclude certain products from the ban, including gelatin and dried plasma and other blood products. This amendment had not yet been implemented in the UK, though one company was pressing for implementation in relation to blood products. UKASTA had confirmed that, despite the legal ban, gelatin was a component of animal feed both in feed supplements and in large quantities of downgraded human food. They would therefore also like the exemption to be implemented. If a ban was applied, the latter would have to go to landfill with environmental and economic consequences. The position was delicate since, in practice, the existing ban was being breached. Paper SEAC 19/1 sought the views of the Committee on whether these exemptions should be allowed in the UK. If it was recommended that there was a risk, it would be necessary to inform the Commission and seek agreement to an amendment in respect of the UK, otherwise, there was a risk of legal challenge from the companies affected.
7. Mr Eddy added that it was already intended to tighten up on the SBO rules to prohibit the removal of the brain from the skull, as it was difficult to guarantee complete removal of all CNS tissue. In effect, the whole skull would become SBO. Consultations had been completed and it was hoped the necessary legislation would be made within a few weeks. The Committee agreed that this was a sensible change.

8. Dr Tyrrell drew attention to the wider implications of the issue. BABs were continuing to trickle out, including now the case born in 1992, so the Committee should consider carefully whether it was adequate to rely solely on the tightening up currently being made to SBO Order in the expectation that in a few years time the situation would have improved. There was a risk of simply passing on the problem for the future if some infectivity was associated with gelatin, though equally it was important not to over-react.

9. Dr Kimberlin said that the issue was really about SBOs. There had never been any concern about bone, and given that the manufacture of gelatin involved significant processing, in the rest of Europe any minor BSE contamination would be dealt with by that process, given the very small scale of the disease outside the UK. The same situation should be broadly true in the UK because any SBO should be removed. On this basis, there should be no concern about gelatin, but the logic hinged on the proper removal of SBOs. If this was not being done satisfactorily, there was a risk.

10. Mr Pepper felt it was necessary to look at the practicalities on the ground. The institution of the Meat Hygiene Service on 1 April was a watershed for the introduction of new, more uniform standards. However in view of initial problems with the MHS, it remained to be seen whether adequate standards would be achieved. Dr Watson felt that the Committee could only say what should be done in practice. However it was noted that the Committee had received information before that led it to believe that certain things were standard practice in the industry when it subsequently transpired that they were not. The question was therefore whether the scientific assessment should be changed or whether the science was right and it was a question of implementation and enforcement.

11. Dr Matthews said that in looking at the procedures in abattoirs for the review of the SBO Order MAFF had become uneasy about what it had found. There were problems with the quantities of SBO arriving with the renderers not being commensurate with the throughput of the abattoirs and with use of the patent blue
stain, orders for which were negligible. In head boning plants which split skulls and
removed brains as SBO, it appeared that some brain tissue was being left behind.
There did not appear to be any problems with the removal of spinal cord. As a result
of these findings MAFF was carrying out an audit of practice. Over a period of two
months, unannounced visits were being made and any deficiencies noted and put in
writing to the OVS and the MHS, with a further visit after two weeks. If deficiencies
were still found, prosecutions would be recommended. So far, on the basis of a very
small sample, it appeared that there were some problems with the separation of SBOs
and that less than 50% were complying with staining requirements.

12. The Committee was very concerned at these reports given the previous
understanding that the position had been satisfactory. It was now recognised that
previous reports based on pre-arranged visits to premises had given a falsely
reassuring picture. The Committee felt that, if there was evidence that something was
going wrong, action should be taken as a matter of the highest priority. The best
hypothesis for the continuing number of BABs now seemed to be that SBOs had
continued to leak through the system. If this was the case, BAB cases could continue
until 2000.

13. Mr Bradley pointed out that the new rendering rules implemented by 1 January
1995 provided one safeguard against continuing contamination, though the weakness
of the lack of correlation between the BSE rendering experiment and actual practice
was recognised and the possibility of cross-contamination in feed mills remained.
However, it was also known from the pathogenesis study that the distal ileum had
been identified as infective and it was important also to take account of the way the
offals from the gut were handled as well as the CNS, though these were more readily
identifiable in the abattoir.

14. Professor Pattison said that he would be concerned about divorcing the
Committee's recommendations from practice. He was worried about efficacy and
thought that the change requiring the brain to be left in the skull was an improvement,
but needed reassurance that there was security on spinal cord. Mr Eddy noted that, as
part of the package of SBO changes, it had been decided that there should be a ban on
the removal of spinal cord in knackers yards and hunt kennels because they were not
subject to the same degree of oversight. Dr Watson asked for a short paper setting
out who was responsible for what and where in relation to abattoirs and other
premises handling carcases and SBOs.
15. The Committee considered whether the exemption from the ban of blood and blood products would represent any risk and concluded that it did not believe it to be a matter for concern. The exemption could be agreed.

16. Dr Tyrrell concluded that in order to agree to the exemption for gelatin the Committee needed to be convinced that a negligible amount of infectivity was present in the raw material used to produce it. Their acceptance of the exemption for gelatin was therefore conditional on the adequacy of controls to prevent this. The Committee's main concern was not whether UK legislation was aligned with the EC Decision but whether our animals and humans were protected. This turned on the quality of the practice. Although the risk from gelatin itself was vanishingly small, the Committee could only be satisfied if it was convinced that the existing regulations were being effectively implemented and the new ones were in place. This meant a holding position needed to be taken on Decision 95/60 until the new SBO Order was in place and there was satisfactory feedback from the audit of abattoir practice. Information on the latter should be to hand by the end of July. Provided this was satisfactory, the Committee would be content for the exemption for gelatin to be given effect.

Infectivity in retina

17. In addition, Dr Bradley informed the Committee that, following incorrect claims made by Professor Lacey about the use of bovine eyes in human food, retina and optic nerve from clinically affected cattle had been tested by bioassay in mice. Some of the mice inoculated with retina had now come down with a scrapie-like disease, at least one with histological evidence of SE, demonstrating that retina showed signs of infectivity. Results on optic nerve were not yet available. The Committee noted that it had earlier recommended, on general principles, that eyes should not be used for dissection in schools, though unfortunately it had taken some time for this information to be sent to the English Education Authorities by the DFE. The new evidence on infectivity of retinas would be covered by the new SBO changes since the eyes would have to be left in the skull with the brain. The Committee took note of this information, which had not yet been published.

Agenda Item 4 - Mechanically recovered meat (MRM)

18. The Committee considered Paper SEAC 19/4 (previously tabled as SEAC 17/6 but not discussed at the August 1994 meeting). This flowed from a recommendation
of the Scientific Veterinary Committee in July 1994 that UK derived spinal column should not be used for the production of MRM except for herds free of BSE for more than six years. The Commission had not taken up this recommendation and was unlikely to do so but the Committee was invited to consider whether there were any grounds for it to change its previous advice on MRM. It had previously been understood that MRM was not produced from spinal column of cull cows though it was now understood that it was in some cases. However, skulls were definitely not used because of damage to the machinery caused by the teeth.

19. On MRM, Dr Tyrrell noted that the key question was once again how effectively the SBO controls were being carried out. Dr Watson said that there was more likelihood of spinal cord being properly removed than brains from the skull. The impact of prohibiting the use of spinal columns on the industry would be enormous. In practice, there was a greater risk from spinal cord spraying onto meat. The question was once again one of policing. Mr Bradley noted that the head of the Meat Hygiene Service had been informed of the requirement to ensure that each side was inspected for the full removal of the spinal cord by meat inspectors.

20. Dr Tyrrell concluded that, provided in the slaughtering process the removal of the spinal cord was done properly, the MRM process was safe and there was no reason for the Committee to change its advice.

Agenda Item 5 - Amino Acids

21. The Committee confirmed that it agreed with the explanation in paper SEAC 19/5 for not including amino acids in the exceptions to "protein" as defined in the Bovine Spongiform Encephalopathy Order 1991. It was agreed that the exclusion of amino acids from the ban did not present a problem.

Agenda Item 6 - BSE in an animal born in 1992

22. Mr Eddy noted that paper SEAC 19/4 had been prepared as background information for the Minister. Earlier discussion had been relevant to this item. It was recognised that the 1992 case was not a one-off and evidence suggested that the existing controls had not been fully applied in some slaughterhouses and in some feed mills. Dr Tyrrell said that for contamination of feed to continue there must have been failures at all three levels: the slaughterhouses, the renderers and the feed mills. A potential alternative source of contamination was scrapie.
23. Mr Pepper said that he was concerned that paragraph 4 of the paper said that this case was not unexpected. If this was the case, it should have been made clear earlier. The Committee considered a histogram showing suspects born after the ban but it was noted that the immediate impression this gave was too sanguine since it did not yet reflect the full potential incubation period of animals infected after the feed ban. A better picture was gained from a graph of the standardised morbidity ratios which was a means of determining the changes in risk of exposure in successive birth cohorts, allowing for the right truncation (due to the long incubation period). Dr Tyrrell felt that despite large confidence limits, this gave the impression that the risk was levelling out, but agreed that it was too early to conclude this definitely.

24. The Committee considered the position in feed mills. Mr Eddy explained that the ruminant protein ELISA had not proved as straightforward as hoped. The test had not been validated against all potential raw materials when first introduced. There had been cross reactions on three vegetable proteins: salseed, shea nut and mango. Luddington VIC believed they could differentiate these. The industry would continue to submit more samples to Luddington to permit further refinement. Sampling on farms with BAB cases had been carried out between June 1994 and April 1995 and the results from 936 samples (359 farms) showed three positive samples related to two feed mills. In one case this resulted in positive results in some raw materials from biscuit mix made from waste human food. One company changed its practices instantly. Some mills had admitted, as a result of the test, that they had difficulties in preventing small scale cross contamination of ruminant feed. One problem in deciding how best to use the test was that MAFF had no statutory right of entry to feed mills. Dr Tyrrell accepted that the test was not sufficiently robust for use in prosecutions but it was clearly helpful to have an independent test. Mr Eddy noted that the feed mills were likely to respond positively because they were concerned about insurance liability. It had therefore proved possible to work with them. The three major players in the industry had indicated their willingness to accept unannounced testing at the mill, although the limited testing capacity would minimise the scale of the sampling.

25. Dr Tyrrell concluded that although the Committee had no further comments at present on the 1992 case it was essential that any problems of control should be rectified speedily.
Agenda Item 7 - Any Other Business: the Hounds Survey

26. Paper SEAC 19/7 responded to a request from the Committee for a re-evaluation of the pathology material in the hounds survey to determine whether anything further could be derived from the available data.

27. In discussion of the options for further work set out in the paper most members felt that the study had been badly carried out and there would be little value in spending more money to try and improve the interpretation of the data. It was particularly significant that no clinical data were available, although the Committee were reminded that most of the hounds were clinically normal culls. Dr Kimberlin was concerned about the lack of results from the study. Any further work would require a control but this could be obtained by exposing hounds to BSE which would also help to answer questions about species sensitivity, thereby serving more than one purpose. The use of immunocytochemistry was fairly robust and would enable the work to be brought to a satisfactory conclusion. Dr Kimberlin's view that this would be necessary was confirmed by an article, circulated at the meeting, showing that the predictive protein sequence was the same in dogs as in cattle. Mr Eddy noted that such an experiment could be expensive and it would be necessary to know what questions were to be addressed.

28. Concluding, Dr Tyrrell said that there was a range of opinions in the Committee from those who thought further work a waste of time to those who wished to do limited further experiments using immunocytochemistry. The Committee did not suggest transmission studies and thought that the lack of clinical data was a major weakness. Hounds were initially studied on the recommendation of the Southwood Committee because they were perceived as a "high risk" population exposed to large quantities of potentially infective bovine tissues. Since then, however, a range of other species had been identified with TSEs, and the study of hounds was therefore less critical.

Idiopathic Brain Stem Neuronal Chromatolysis (IBNC)

29. Mr Bradley described the results of transmission studies in mice from brains of two cows with IBNC (paper SEAC 19/8). At the previous meeting of SEAC, and at the review of R&D, it had been announced that there was no clinical observation of a scrapie-like disease in mice: this information had proved to be incorrect for a number of reasons. Of the mice inoculated with brain tissue from the first cow, there had
been mild transient clinical signs, one had shown equivocal lesions of SE but PrP studies had proved negative. From the second cow there were two definite cases of SE though the lesion distribution and incubation period were not the same as seen in mice inoculated with brain from BSE cases or any characterised strain of scrapie. The lesions in these two mice were PrP positive. There was no obvious evidence of any mix up though one possible area of cross-contamination was during the necropsy in the Perth VIC. More evidence would be needed and further transmission studies to validate the results and proposals were put forward for further study.

30. The Committee noted that the results were unusual. They questioned whether there could be coincidental BSE infection or contamination with scrapie. Dr Tyrrell noted that the feeling of the Committee was that this did not represent a new agent but it was important to be prepared to say something publicly about these findings. A suggested line to take was that these were scientifically unpublishable results but in line with the policy of openness they would be made publicly available and further work done to test their validity. Since the BSE precautions were applied to IBNC cases, human health was protected. Further investigations should be carried out on isolations from brains of IBNC cases with removal of the brain and subsequent handling under strict conditions to avoid the risk of any contamination.

31. Mr Bradley informed the Committee that the CVO had informed the CMO about the IBNC results and the transmission from retina and he, like the Committee was satisfied that the controls already in place or proposed were adequate.

Research priorities

32. The Committee reviewed the list of priorities attached at Appendix 2 to the previous minutes. It was agreed that the work on BSE in native-born Portuguese cattle was of higher priority than that on post 1991 BABs with unusual lesions. Although of lower priority, transmission of the BSE isolate from a French cow imported into the UK was of interest, but as with the IBNC cases was compromised because brain removal was not under conditions intended to prevent cross-contamination. A future indigenous French case would be more appropriate. Comparisons with UK BSE might help to give a better understanding of the origin of the BSE outbreak. Whatever the outcome, the results would be interesting. These two items should be promoted to three stars. Dr Kimberlin said he would also like to see higher priority given to transmission of marmoset-passaged BSE as it would give worthwhile information on the stability of the BSE agent after passage in a primate.
33. After some discussion, it was agreed that it was valuable to classify research priorities both on scientific and policy grounds.

Research Update

34. Dr Bradley provided an update on NPU transmission studies provided by Dr Taylor and Dr Manson. The information they had provided (attached at Appendix 1) did not give a clear picture but the message appeared to be that some sheep and goats showing positive clinical signs of disease were not being confirmed pathologically and others with positive pathology showed no clinical signs. However the picture was incomplete and confused and would need to be clarified for the next meeting. A summary of updates on other research projects is attached at Appendix 2.

Date of next meeting

35. The next meeting will be held at 10.30 am on Friday, 8 September 1995 in Room 125a Skipton House.

6 July 1995
Comparative efficiencies of the bioassay of BSE infectivity in cattle and mice

Titration of infectivity studies in mice have now been completed. The titre of infectivity of the brain stem homogenate was $10^{3.3}$ mouse ID$_{50}$/g of tissue when measured by the combined i/e and i/p injection of RIII mice.

The titration of infectivity in cattle is continuing (29 months p.i). To date 9 animals have been necropsied due to progression of clinical signs characteristic of BSE. These are spread over those groups inoculated with brain stem homogenate at dilutions $10^{-3}$ - $10^{-6}$. Histopathological examination of 4 of these animals has confirmed a diagnosis of BSE and is pending on the remaining 5 animals.

One animal inoculated with the spleen pool was necropsied due to intercurrent disease 26 months p.i. Histopathological examination is pending.

Although some of the surviving animals show some signs consistent with early stages of clinical BSE, none are as yet showing characteristic signs of the disease.

Pathogenesis of experimental BSE in cattle

The sequential kill protocol has now been completed. Remaining cattle were killed as three groups at two month intervals.

At 36 months p.i. three challenged animals were necropsied, two of which showed possible early clinical signs. Histopathological examination confirmed a spongiform encephalopathy consistent with BSE in only one of these animals.

At 38 months p.i. a further 3 challenged animals were necropsied, one of which showed unequivocal clinical signs and spongiform encephalopathy consistent with BSE. The other two showed possible early clinical signs and in one typical lesions of BSE were also confirmed.

The two remaining challenged animals were necropsied 40 months p.i. One showed unequivocal clinical signs of BSE and, on histopathological examination, a distribution of lesions closely similar to that seen in severe field cases of the disease. The other animal showed possible early clinical signs but no evidence of spongiform encephalopathy.

Mouse bioassay for infectivity of cattle tissues continues and as reported previously, infectivity in the distal ileum has been demonstrated in challenged cattle from the second (6 months p.i.), third (10 months p.i.), fourth (14 months p.i.) and fifth (18 months p.i.) sequential kill groups, but not from the first (2 months p.i.).

The mouse bioassays of all other tissues from the control calf and challenged calves from kills 1-3 are complete. No evidence of infectivity was found. Mouse bioassays of tissues collected at subsequent kills are incomplete.
Effect of oral inoculum dose on attack rate and incubation period of BSE

The onset of clinical signs of BSE in these animals has been insidious. Unequivocal clinical signs have been confirmed in 10 animals, 7 of which have been necropsied to date (41 months p.i.). Histopathological examination of 4 of these animals has shown severe spongiform encephalopathy typical of BSE. Histopathological examination of the remaining 3 animals necropsied is pending.

Of the remaining 29 animals 13 show irregular premonitory signs suggesting the approach of the clinical stage. To avoid clinical bias in the critical assessment of incubation period the groups remain coded.

SAC Hawkins
15 June 1995
TYRRELL COMMITTEE MEETING: 21 JUNE 1995

Brief Report from DM Taylor

1. Although the experiment is not yet completed, there is fairly sound evidence that treatment of 22A (as half mouse brains) with ethanol for 48 hours renders it insensitive to inactivation by subsequent porous-load autoclaving at 136°C for 18 minutes; control material appears to have been inactivated by this autoclaving regime.

2. In the scrapie-spiked rendering experiment, positives have only occurred so far in the bioassay of the untreated scrapie brain pool.

Brief Report from J Manson

Mice have been produced that have a PrP gene mutation analogous to the codon 102 mutation of the human PrP gene which is associated with familial spongiform encephalopathy. These mice have leucine rather than proline at codon 101; the mutation was introduced by homologous recombination, a technique which has not been used previously in the study of point mutations of the PrP gene. No spontaneous neurological disease has been observed in homozygous mice which are now six months old.