OPINION ON

THE POTENTIAL REQUIREMENT FOR DESIGNATION OF SPECFIED RISK MATERIALS IN PIGS.

ADOPTED BY THE
SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 6 – 7 MARCH 2003
OPINION

It has previously been reported that BSE can be transmitted to pigs by multiple parenteral inoculation. However, experiments also of oral exposure of pigs to BSE-contaminated material have not resulted in clinical disease 7 years after exposure. But the question cannot yet be answered whether pigs exposed to contaminated material could be silent carriers. Research on the possible presence of infectivity in pig tissues is ongoing, but final results are not yet available.

The Scientific Steering Committee (SSC) was therefore invited:

(1) To advise whether in the light of the presently available limited scientific data (or its absence), it is scientifically justified to include certain pig tissues in the SRM-bans?

(2) If so, what is the list of tissues that should be classified as specified risk material and from pigs of what age?

(3) Would it be justified, for precautionary reasons, to apply the same SRM rules to pigs, as are applied to cattle?

On the basis of the scientific report prepared by the TSE/BSE ad hoc Group the SSC answers as follows to the questions of the mandate:

In the light of present knowledge - which, however, is partly based on non-final research results - there is no scientific justification to include certain tissue of pigs in a SRM-ban.

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REPORT OF THE TSE/BSE AD HOC GROUP ON:
THE POTENTIAL REQUIREMENT FOR DESIGNATION OF
SPECIFIED RISK MATERIALS IN PIGS.

Rapporteur: Dr.G.Wells

I. QUESTIONS AND MANDATE

It has previously been widely reported that BSE can be transmitted to pigs by multiple parenteral inoculation. However, experiments of oral exposure of pigs to BSE-contaminated material have not resulted in clinical disease 7 years after exposure. But the question cannot yet be answered whether pigs exposed to contaminated material could be silent carriers. Research on the possible presence of infectivity in pig tissues is ongoing, but final results are not yet available.

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The SSC asked the TSE/BSE ad hoc Group to prepare a scientific report to serve as basis for an opinion on the three questions.

II. BACKGROUND

Much of the relevant background information with regard to the exposure of pigs to the agent of bovine spongiform encephalopathy (BSE) and the potential for the occurrence BSE in pigs has been summarised in Matthews and Cooke (2003) which addresses the potential for transmissible spongiform encephalopathies (TSE) in non-ruminant livestock. The recognition of bovine spongiform encephalopathy (BSE) in domestic cattle in the United Kingdom (UK) in 1986 inevitably led to concerns about the potential risk of similar diseases occurring in non-ruminant livestock or farmed food species. Research was quickly directed toward the investigation of the susceptibility of pigs and poultry to infection with the bovine agent. Investigations into processing and trading practices within the rendering and feedstuffs industries identified the fact that consumption of meat and bone meal must have led to significant exposure of the British pig and poultry populations to the agent of BSE.

Numerous references in scientific literature during the 1930s and 1940s indicate that the use of meat and bone meal, meat meal and blood meal in diets for pigs and poultry was common practice in many countries. By the 1980’s the average inclusion rate of meat and
bone meal in pig feeds was 5% with a usage in excess of 175,000 t/year of meat derived products in pig diets in the UK.

The ban on the use of ruminant protein in ruminant feed in the UK in July 1988 raised concern about inter-species recycling. Also in the UK, between 1990 and 1996, some feed companies stopped using animal proteins, other than fish meal and milk products, in feeds for pigs and poultry. Others continued to use these ingredients until the use of mammalian meat and bone meal in livestock feed was banned in 1996. Despite the 1996 ban in the UK, the feeding of mammalian meat and bone meal to pigs and poultry remained legal in other countries of the European Union (EU). Nevertheless, sentiment and market forces contributed to a marked decline in its use in the last few years of the 1990’s. Following the introduction in 1994 of the EU ban on mammalian protein in ruminant feeds, many feed mills that manufactured both ruminant and non-ruminant animal feeds, ceased the use of animal proteins in any feeds.

Since January 2001 the use of all processed mammalian protein in feeds for farmed animals has been banned throughout the EU with periodic adjustments, but its use in pig and poultry feeds in other parts of the world continues.

The following Opinions, Statements and Reports of the SSC have previously addressed issues concerning the possible risks associated with the potential for TSE agents to occur in the tissues of non-ruminant livestock species (including pigs), for the entry of such animal proteins potentially contaminated with TSE agents into food or feed chains and the recycling of TSE infectivity in animal feed:

Opinion on peptides from pig mucosa: risks with respect to TSEs (adopted on 21-22 February 2002)

Opinion of the Scientific Steering Committee (1) on the scientific basis for import bans proposed by 3 Member States with regard to BSE risks in France and the Republic of Ireland; (2) on the scientific basis for several measures proposed by France with regard to BSE risks; (3) and on the scientific basis for banning animal protein from the feed for all farmed animals, including pig, poultry, fish and pet animals. (Adopted by the Scientific Steering Committee at its meeting of 27-28 November 2000)


Scientific Report on the risks of non conventional Transmissible agents conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials. Submitted to the Scientific Steering Committee at its meeting of 24-25 June 1999 (Containing updates, 13.07.99)

Intra-Species Recycling - Opinion on: the risk born by recycling animal by-products as feed with regard to propagating TSE in non-ruminant farmed animals. Adopted on 17 September 1999

Scientific Opinion on the safety of organic fertilisers derived from mammalian animals,
adopted by the Scientific Steering Committee at its meeting of 24-25 September 1998

Report and Scientific Opinion on mammalian derived meat and bone meal forming a cross-contaminant of animal feedstuffs adopted by the Scientific Steering Committee at its meeting of 24-25 September 1998

Updated scientific report on the safety of meat-and-bone meal derived from mammalian animals fed to non-ruminant food producing farm animals. Scientific Steering Committee Meeting of 24-25 September 1998

III. RISK ASSESSMENT

III.1 Experimental studies of the transmissibility of BSE to pigs

III.1.2 Susceptibility of pigs to BSE by parenteral inoculation

Studies to test the transmissibility of the BSE agent to pigs began in the UK in 1989. Parenteral inoculation of the agent, by three routes simultaneously (intracranially [i.c], intravenously [i.v.] and intraperitoneally [i.p.]), produced disease with an incubation period range of 69 -150 weeks (Wells et al. 2003, in press). Pre-clinical pathological changes (spongiform encephalopathy) were detected in two pigs killed electively at 105 and 106 weeks post-inoculation (p.i.). Infectivity was detected by bioassay in inbred mice in the central nervous system (brain and spinal cord) of all pigs which developed spongiform encephalopathy. Infectivity was also found in the stomach, jejunum, distal ileum and pancreas but not in other tissues assayed (spleen, thymus, mesenteric lymph node, liver and kidney) of the terminally affected pigs. These findings show that pigs are susceptible to BSE and although infectivity was present in all the CNS tissues from exposed pigs that were tested, not all of the assay mice injected with brain from clinically-affected pigs developed the disease, suggesting the existence of a species barrier to the transmission of BSE from pigs to mice which reduced the sensitivity of the bioassay. It was expected that lower concentrations of agent would be present in tissues outside the CNS and this was shown by the lower incidences of disease and the longer incubation periods in the bioassay mice. What was unexpected was the relatively few peripheral tissues in which any infectivity was detected. This finding again suggests that a large species barrier compromised the sensitivity of the bioassays. The alimentary tissue infectivity in the affected pigs could represent persistence of the inoculum, replication of agent, or centrifugal spread of agent from the CNS in the late phase of the disease.

III.1.2 Resistance of pigs to BSE after exposure by feeding.

In contrast to the transmission of BSE by parenteral inoculation, disease failed to occur in pigs retained for seven years after exposure by feeding BSE affected brain on three separate days, at 1-2 week intervals (Wells et al. 2003, in press). The amounts fed each day were equivalent to the maximum daily intake of meat and bone meal in rations for pigs aged eight weeks. No infectivity was found in tissues (brain, spinal cord, semitendinosus muscle, spleen, thymus, retropharyngeal, mesenteric and popliteal lymph
nodes, stomach, distal ileum, pancreas, liver and kidney) assayed from the pigs exposed orally. It is suggested that these pigs did not become infected.

That exposure of pigs to the BSE agent by feeding did not transmit the disease to pigs is in marked contrast to the now considerable body of evidence that BSE has transmitted, by natural or accidental means, via foodstuffs to several other animal species and to man and indeed has been transmitted by feeding BSE-affected brain tissue to several additional animal species. Thus there is no doubt as to the susceptibility of several simple stomach species of mammals to BSE infection after oral exposure. The apparent exception of pigs is of considerable interest.

**III.1.3 The cattle/pig species barrier to oral transmission**

One possible explanation of the apparent resistance of the pig to oral exposure with the BSE agent is clearly that the pig is susceptible to infection with the BSE agent, but the oral exposure was insufficient to establish infection. This is consistent with the findings from scrapie transmission studies within other species which showed that the oral route of exposure is less efficient than parenteral routes of exposure. Other studies make it likely that the effective exposure of pigs was further reduced by a species barrier to the oral transmission of BSE from cattle to pigs. The existence of such a barrier can be inferred from comparisons of the present findings with the results of an oral titration, in cattle, of a pool of 60 BSE-affected brain stems. All the calves exposed to the 100g dose of brain material developed clinical signs and histopathological lesions of BSE. The amount of the brain pool required to cause BSE in 50% of the exposed cattle is estimated to be less than 1g. (Wells et al. 2003). However, it should be noted that different pools of BSE brain were used in these two experiments. The infectivity titre of the pool fed to cattle was $10^{3.5}$ mouse ic/ip units ID₅₀/g whereas the titre of the pool fed to the pigs was $10^{2.4}$ mouse ic/ip units ID₅₀/g., that is, about 10 times lower. Thus, if the cattle-pig species barrier was zero, a dose of just over 10 g of brain should have caused disease in half of the exposed pigs. The fact that none of the pigs appeared to become infected after being fed an average of 400 g of brain on each of three successive occasions (a total of 1,200 g) suggests the existence of a cattle-pig species barrier that reduced the effective oral exposure to BSE by as much as 100-fold, or even more.

**III.1.4 The absence of a naturally occurring TSE cases in pigs**

A species barrier of 100-fold or more may be relevant to the fact that there have been no reports of a naturally occurring TSE in pigs in the United Kingdom even though in the period that cattle were being exposed to contaminated MBM, pigs were also being exposed. Indeed, pigs continued to be exposed after the introduction of the feed ban for ruminants in July 1988 (HMSO, 1988) until September 1990 when legislation (HMSO, 1990) banned the use of specified bovine offals (SBO), including brain and spinal cord, in all animal feed. Moreover, the inclusion rates of MBM in commercial pig feeds were usually greater than in ruminant rations.

It is difficult to estimate the degree of BSE contamination of MBM. However, the epidemiological evidence suggests that the generally sporadic occurrence of cases throughout the BSE epidemic was the consequence of a relatively low-dose exposure.
Therefore, the risk of infection of any one animal at a given time was also low, even at the peak of the epidemic. The reason why a major epidemic occurred in the UK was because a large number of cattle received multiple potential exposures via contaminated MBM in concentrated feeds.

It was not possible to mimic the multiple low-dose exposures which pigs would have experienced naturally. Therefore, the design of the oral exposure study focussed on three maximal exposures by replacing the MBM content of feed with brain material from cattle clinically-affected with BSE. Assuming comparable titres among bovine brains from cases of BSE this exposure was greater, either singly or cumulatively, than the exposure pigs could have received in the field.

The difference between the field and experimental exposures of pigs can be illustrated by calculating the average proportion of MBM that was derived from BSE-affected CNS tissue. The contamination of MBM was dominated by the CNS from animals that had been infected as calves in dairy herds and were more than two years of age at slaughter. On average, the proportion of MBM derived from bovine material was 0.48 (MMC, 1985). Approximately 180 kg of waste material from each bovine was rendered to produce MBM (MMC, 1985) and less than 0.75 kg of this material was from the CNS. Therefore, the proportion of MBM derived from bovine CNS was 0.2%. If 1% was the highest average proportion of all cattle going to slaughter that had been infected in dairy herds and were more than two years old, then the proportion of MBM derived from BSE-infected CNS would have been no more than 0.002%, on average. In the experimental study, the oral exposure to BSE was based on the consumption of 80g MBM per day by commercially-raised pigs. In the field, CNS tissue would have contributed only about 1.6 mg of this amount. Therefore, replacing 80 g of MBM by whole brain increased the experimental exposure to CNS tissue to 50,000 times more than the calculated exposure in the field.

It must be emphasised that these simple calculations take no account of the considerable variations in the contribution of infected bovine CNS tissue to different batches of MBM, in the BSE titre and in the inclusion rate of MBM in commercial feeds; all of which would have been epidemiologically significant in exposed cattle. Nevertheless, the experimental exposure of pigs on just one of the three occasions was probably well in excess of the average life-time exposure of pigs in the field to BSE, either in terms of cumulative low risks or, more controversially, the accumulation of low infectious doses in multiply exposed animals.

The evidence that exposure of commercial pigs to infected MBM did not result in cases of TSE (in contrast to the BSE epidemic in cattle) depends, *inter alia*, on the survival to maturity of a substantial population of animals. In 1983, there were 680,210 breeding sows in England, Wales and Scotland (MAFF, 1983, DAFS, 1983), and in 1995 the number was 637,870 (MAFF, 1995; SOAFD, 1995). Approximately 20 per cent of these breeding pigs (≡130,000), most of which were located in large commercial units, were kept to between four and five years of age (MLC, 1999). If pigs were as susceptible to BSE by the dietary route as are cattle, with a similar median incubation and assuming the highest level of prevalence of infection was 1 per cent, then over 1,000 cases of BSE in pigs should have occurred by now. Although there was no active surveillance for TSE in domestic pig populations in this country (or elsewhere), it is unlikely that many cases,
had they occurred, would have escaped detection because the clinical signs of experimental BSE in the pig are distinctive, a description was published in February 1990 (Dawson et al., 1990) and the neurological signs may resemble those of some statutorily notifiable diseases in the pig.

Cases of TSE in pigs may have occurred undetected if incubation periods were much longer than for BSE in cattle. Unusually long incubation periods are often, though not invariably, found on transmission of TSE agents across a species barrier (Kimberlin et al., 1989). But, equally, the species barrier can reduce the efficiency of infection (Kimberlin, 1996). Indeed, the observations on pigs orally exposed to large amounts of infected brain and observed for seven years thereafter, support the view that the lower exposures encountered in the field were insufficient to cause infection and, therefore, a naturally occurring TSE of pigs.

III.1.5 The possibility of sub-clinical infection of pigs

It is important to consider an alternative explanation of the outcome of the oral exposure experiment because studies of several models of scrapie have shown that disease may fail to develop despite life-time persistence of infection in peripheral tissues (Dickinson et al., 1975; Race & Chesebro, 1998), even at high titres (Bruce, 1985; Collis & Kimberlin, 1985). Therefore, it is possible that infection of pigs occurred but did not produce clinical or pathological evidence of disease and the mouse bioassay, across the pig-mouse species barrier, was too insensitive to detect infectivity in any of the tissues.

However, this scenario can be examined by further consideration of the foodborne exposure of pigs to BSE in the field. Had primary infection of pigs from cattle with BSE occurred, there would have been the potential for recycling and, hence, amplification of a porcine-adapted BSE agent because of the inclusion in pig rations of MBM of porcine origin. This is directly analogous to the recycling which occurred in cattle and drove the bovine epidemic into an exponential phase (Kimberlin & Wilesmith, 1994; Wilesmith, 1991; Wilesmith, 1998). With the ban on SBO in animal feed (HMSO, 1990), pig material contributed in greater proportion to MBM, infection in pigs would have been transmitted to pigs with no species barrier effect and, had disease resulted, it might have been expected to occur with shorter incubation periods than primary foodborne transmission to pigs (Kimberlin, 1993b). Recycling would also have accompanied primary transmissions from cattle and would have continued long after these ceased in September 1990, until April 1996 when further legislation in Great Britain prohibited the feeding of mammalian MBM to all farmed animals (HMSO, 1996). The failure of recycling and amplification to produce clinical disease in pigs both before and, currently, six years after the end of such exposure, tends to negate the hypothesis of inapparent BSE infection in pigs. Experimental investigation of this hypothesis would require sub-passage of selected tissues, notably those of the alimentary tract, from the orally exposed pigs, employing the same species, or possibly transgenic mice expressing porcine PrP.

III.1.6 Possible transfer of infective gut content to feed

A separate, but real, concern in countries where feed controls are restricted to feed manufactured for consumption by ruminants, is that pigs could, prior to slaughter have been fed BSE contaminated feed and that without the pig becoming infected, the
infectivity in the intestinal lumen could be transferred to feed via rendering (Matthews and Cooke, 2003). As a result, these animals could perpetuate cycles of transmission through feed and thereby undermine the effectiveness of feed bans. For example, where ruminant protein may still be fed to pigs and poultry, their offal may still represent a risk of recycling infectivity to ruminants if intestinal contents are still present at the time of rendering. In other words, if ruminant protein may be fed to pigs and porcine MBM may still be fed to ruminants, the intestine of the pig at slaughter, and consequently the porcine MBM, may contain ruminant protein. Clearly, under current regulations, this eventuality is prevented within member states of the EU.

III.1.7 Further studies in progress

In an EU project (FAIR CT97-3306) pigs have been inoculated with BSE agent, intracerebrally, or orally, the latter comparing single and multiple exposures. Interim kills of pigs during the putative incubation period have provided various tissues which have been examined by pathological methods, by immunohistochemical detection of PrPSc and by inoculation into transgenic mice expressing porcine PrP. Results are incomplete, but the experiment has thus far not demonstrated that the pigs were infected using these inocula.

IV. SUMMARY

It can be concluded from the studies of the transmissibility of BSE to pigs that although pigs are susceptible to BSE when injected by combined i.c., i.v. and i.p. routes, there is no evidence of transmission after exposure by feeding three doses of BSE-infected brain in amounts equivalent to the maximum daily intake of MBM formerly used in commercial pig rations. The simplest explanation of this finding is that the effective exposure of pigs by the oral route was insufficient to establish infection. Infectivity studies in conventional mice of the peripheral tissues of pigs exposed orally to the BSE agent further support this outcome. This explanation is also consistent with the field evidence that repeated primary exposures of commercial pigs to BSE, together with the considerable potential for pig-to-pig recycling of infection (until August 1996), has not resulted in natural cases of TSE in pigs. These observations are in contrast to the susceptibility of cattle to oral infection with gram quantities of BSE-affected brain and to the major feed-borne epidemic in the UK.

V. CONCLUSIONS

Evidence from the unsuccessful transmission of BSE to pigs after experimental oral exposure to a dose of BSE agent approximately 50,000 times more than the calculated exposure in the field and evidence that repeated primary exposures of commercial pigs to BSE including the considerable potential for pig-to-pig recycling of infection in Britain, did not resulted in natural cases of TSE in pigs, indicates that there is no basis on which to suspect that pigs have become infected with BSE.

Whereas there are no studies which have as yet provided results of more sensitive assays which might give additional evidence that the peripheral tissues of pigs exposed orally to
the BSE agent do not support a sub-clinical infection, the evidence already available would seem conclusive for all practical applications.

VI. REFERENCES


DAFS (Department of Agriculture and Fisheries) (1983). *Results of June 1983 Annual Agricultural and Horticultural Census, Scotland.* DAFS, Edinburgh


