03-025IFA
03-025IFA-2
Terry S. Singeltary

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From: Terry S. Singeltary Sr. [flounder9@verizon.net]

Sent: Thursday, September 08, 2005 6:17 PM

To: fsis.regulationscomments@fsis.usda.gov

Subject: [Docket No. 03-025IFA] FSIS Prohibition of the Use of Specified Risk Materials for Human Food and Requirements for the Disposition of Non-Ambulatory Disabled Cattle

Greetings FSIS,

I would kindly like to submit the following to [Docket No. 03-025IFA] FSIS Prohibition of the Use of Specified Risk Materials for Human Food and Requirements for the Disposition of Non-Ambulatory Disabled Cattle

THE BSE/TSE SUB CLINICAL Non-Ambulatory Disabled Cattle

Broken bones and such may be the first signs of a sub clinical BSE/TSE Non-Ambulatory Disabled Cattle ;

SUB CLINICAL PRION INFECTION

MRC-43-00
Issued: Monday, 28 August 2000

NEW EVIDENCE OF SUB-CLINICAL PRION INFECTION: IMPORTANT RESEARCH FINDINGS RELEVANT TO CJD AND BSE

A team of researchers led by Professor John Collinge at the Medical Research Council Prion Unit1 report today in the Proceedings of the National Academy of Sciences, on new evidence for the existence of a ?sub-clinical? form of BSE in mice which was unknown until now.

The scientists took a closer look at what is known as the ?species barrier? - the main protective factor which limits the ability of prions2 to jump from one species to infect another. They found the mice had a ?sub-clinical? form of disease where they carried high levels of infectivity but did not develop the clinical disease during their normal lifespan. The idea that individuals can carry a disease and show no clinical symptoms is not new. It is commonly seen in conventional infectious diseases.

Researchers tried to infect laboratory mice with hamster prions3 called Sc237 and found that the mice showed no apparent signs of disease. However, on closer inspection they found that the mice had high levels of mouse prions in their brains. This was surprising because it has always been assumed that hamster prions could not cause the disease in mice, even when injected directly into the brain.

In addition the researchers showed that this new sub-clinical infection could be easily passed on when injected into healthy mice and hamsters.

The height of the species barrier varies widely between different combinations of animals and also varies with the type or strain of prions. While some barriers are quite small (for instance BSE easily infects mice), other combinations of strain and species show a seemingly impenetrable barrier. Traditionally, the particular barrier studied here was assumed to be robust.

Professor John Collinge said: "These results have a number of important implications. They suggest that we should re-think how we measure species barriers in the laboratory, and that we should not assume that just because one species appears resistant to a strain of prions they have been exposed to, that they do not silently carry the infection.

This research raises the possibility, which has been mentioned before, that apparently healthy cattle could harbour, but never show signs of, BSE.

"This is a timely and unexpected result, increasing what we know about prion disease. These new findings have important implications for those researching prion disease, those responsible for preventing infected material getting into the food chain and for those considering how best to safeguard health and reduce the risk that theoretically, prion disease could be contracted through medical and surgical procedures."

ISSUED FRIDAY 25 AUGUST UNDER EMBARGO. PLEASE NOTE THAT THE EMBARGO IS SET BY THE JOURNAL.

FOR FURTHER INFORMATION CONTACT THE MRC PRESS OFFICE ON 020 7637 6011 (OFFICE HOURS) OR 07818 428297 OR 0385 774357 (OUT-OF-OFFICE-HOURS) OR PROFESSOR JOHN COLLINGE ON 020 7594 3760. PLEASE NOTE THAT OWING TO TRAVEL COMMITMENTS PROFESSOR COLLINGE WILL ONLY BE AVAILABLE UNTIL 16.30 ON FRIDAY 25 AUGUST AND CONTACTABLE AGAIN ON MONDAY 28 AUGUST VIA THE MRC PRESS OFFICE. DR ANDREW HILL (A CO-AUTHOR ON THE PAPER) FROM THE DEPARTMENT OF PATHOLOGY AT THE UNIVERSITY OF MELBOURNE WILL BE AVAILABLE ON 00 61 3 8344 3995 (DURING OFFICE HOURS) OR 00 61 3 9443 0009 (OUT-OF-OFFICE HOURS). PLEASE NOTE THAT AUSTRALIA IS TEN HOURS AHEAD OF UK TIME.

NOTES FOR EDITORS

Professor Collinge is a consultant neurologist and Director of the newly formed MRC Prion Unit based at The Imperial College School of Medicine at St Mary?s Hospital. He is also a member of the UK Government?s Spongiform Encephalopathy Advisory Committee (SEAC). The MRC prion unit is was set up in 1999, and its work includes molecular genetic studies of human prion disease and transgenic modelling of human prion diseases.

Prions are unique infectious agents that cause fatal brain diseases such as Creutzfeldt-Jakob disease (CJD) in humans and scrapie and BSE (mad cow disease) in animals. In some circumstances prions from one species of animals can infect another and it is clear that BSE has done this to cause the disease variant CJD in the UK and France. It remains unclear how large an epidemic of variant CJD will occur over the years ahead.

The strain of prion used here to infect the mice is the Sc237 strain (also known as 263K) which infects hamsters, and until now was assumed not to infect mice.

This research was funded by the Medical Research Council and Wellcome Trust.

The Medical Research Council (MRC) is a national organisation funded by the UK tax-payer. Its business is medical research aimed at improving human health; everyone stands to benefit from the outputs. The research it supports and the scientists it trains meet the needs of the health services, the pharmaceutical and other health-related industries and the academic world. MRC has funded work which has led to some of the most significant discoveries and achievements in medicine in the UK. About half of the MRC?s expenditure of £345 million is invested in over 50 of its Institutes and Units, where it employs its own research staff. The remaining half goes in the form of grant support and training awards to individuals and teams in universities and medical schools.

The Wellcome Trust is the world's largest medical research charity with a spend of some £600 million in the current financial year 1999/2000. The Wellcome Trust supports more than 5,000 researchers, at 400 locations, in 42 different countries to promote and foster research with the aim of improving human and animal health. As well as funding major initiatives in the public understanding of science, the Wellcome Trust is the country's leading supporter of research into the history of medicine.

http://www.mrc.ac.uk/index/public_interest/public-press_office/public-press_releases_2000/public-mrc-43-00.htm

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Neurobiology

Species-barrier-independent prion replication in apparently resistant species

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Abstract

Transmission of prions between mammalian species is thought to be limited by a "species barrier," which depends on differences in the primary structure of prion proteins in the infecting inoculum and the host. Here we demonstrate that a strain of hamster prions thought to be nonpathogenic for conventional mice leads to prion replication to high levels in such mice but without causing clinical disease. Prions pathogenic in both mice and hamsters are produced. These results demonstrate the existence of subclinical forms of prion infection with important public health implications, both with respect to iatrogenic transmission from apparently healthy humans and dietary exposure to cattle and other species exposed to bovine spongiform encephalopathy prions. Current definitions of the species barrier, which have been based on clinical end-points, need to be fundamentally reassessed.

snip...

Discussion

Implication of Demonstration of Subclinical Prion Infection. In prion diseases, infectious titers in the brain rise progressively throughout prolonged, clinically silent periods that precede the onset of disease. Thus asymptomatic animals may harbor significant infectious titers in brain and other tissues. However, there may be subclinical, as distinct from such preclinical, forms of prion infection, where animals become asymptomatic carriers of infectivity and do not develop clinical disease in their lifetimes (7, 28). Such carrier states are well recognized in other infectious diseases. However, in prion diseases, where incubation periods are extremely prolonged, distinction between subclinical and preclinical states is more difficult. It certainly can be argued that animals dying after a typical lifespan without clinical signs of prion disease but harboring high levels of infectivity represent the late preclinical stage of "transmissions" where the "incubation period" exceeds the normal lifespan (29). The distinction between the terms subclinical and preclinical is essentially a semantic one in this context. Here we use the term subclinical infection operationally to refer to animals in which prion replication is occurring but which have not developed clinical signs of prion disease during a normal lifespan.

We have demonstrated that conventional mice inoculated with Sc237 prions harbor high levels of PrP^{Sc} and high prion titers in their brains without developing clinical signs of prion disease within their normal lifespan. These results imply the existence of subclinical prion infections that can be induced by challenge with prions from another species. However, whether or not this infectivity is classified as preclinical or subclinical, it has important public health implications. Iatrogenic transmission could occur from apparently healthy humans who may harbor high prion titers and many animal species (including sheep, pigs, and poultry) were exposed to BSE prions via contaminated feed and could have developed subclinical prion infection. It is known that BSE

prions retain their distinctive strain characteristics after passage in a number of other species including humans (4, 13), arguing that such BSE passaged in species other than cattle also may be pathogenic to humans. The possibility that subclinical BSE might be present in other species and thereby present a threat to human health has been raised (30) but not yet rigorously investigated. Furthermore, these data argue in favor of screening apparently healthy cattle after slaughter to investigate whether significant levels of subclinical or preclinical BSE are present.

Secondly, because animals can harbor high levels of infectivity without developing clinical signs of prion disease, these results argue that PrP^{Sc} and indeed prions (whether or not they are identical) may not themselves be highly neurotoxic. Such results are in accordance with earlier findings of a lack of correlation between clinical disease and neuropathological features of prion disease (31), prion diseases in which PrP^{Sc} is barely or not detectable (32-35), and studies in mice with reduced levels of PrP^{C} expression that have extremely high levels of PrP^{Sc} and prions in the brain and yet remain well for several months after their wild-type counterparts succumb (36). Conversely, Tg20 mice, with high levels of PrP^{C}, have short incubation periods and yet produce low levels of PrP^{Sc} after inoculation with mouse prions (27). In addition, brain grafts producing high levels of PrP^{Sc} do not damage adjacent tissue in PrP knockout (*Prnp*$^{o/o}$) mice (37). The cause of neurodegeneration in prion diseases remains unclear. It remains possible that prion neurodegeneration is related, at least in part, to loss of function of PrP^{C}. That *Prnp*$^{o/o}$ mice (other than those associated with overexpression of the *Prnp*-like gene *Prnd*; ref. 38) do not develop neurodegeneration could be caused by compensatory adaptations during neurodevelopment. Complete or near complete ablation of PrP expression in an adult mouse using conditional gene expression methods has not yet been achieved. An alternative hypothesis is that a toxic, possibly infectious, intermediate is produced in the process of conversion of PrP^{C} to PrP^{Sc}, with PrP^{Sc}, present as highly aggregated material, being a relatively inert end-product. The steady-state level of such a toxic monomeric or oligomeric PrP intermediate then could determine rate of neurodegeneration. One possibility is that Sc237-inoculated CD-1 mice propagate prions very slowly and that such a toxic intermediate is generated at extremely low levels that are tolerated by the mouse. The fact that the PrP^{Sc}-negative Sc237-inoculated CD-1 mice were the ones culled earlier than those that were PrP^{Sc} positive, allows the assumption that they may have become PrP^{Sc} positive had they lived longer. A more detailed study of the time course of accumulation of infectivity will be necessary to investigate this further.

Transmission of Infectivity from Subclinical Animals. The transmission properties of prions from the subclinical Sc237-inoculated CD-1 mice were remarkable. With respect to transmissions to additional CD-1 or Tg20 mice, the 100% attack rate and highly consistent incubation periods suggest transmission in the absence of a barrier. However, the incubation periods, notably in the Tg20 mice, which succumb to RML mouse prions in around 60 days (27), are very prolonged. The 100% attack rate argues against this being a consequence of low prion titer in the inoculum. Incubation period at end point dilution in Tg20 mice of RML mouse prions is around 109 days (37). Remarkably, passage in hamsters of this isolate also showed a 100% attack rate and consistent incubation periods suggestive of transmission in the absence of a barrier. Again, incubation periods were extremely prolonged and differed markedly from the transmission properties of Sc237/263K prions in hamsters (8, 10, 39). Indeed, the incubation period seen would correspond to an Sc237 titer in Syrian hamsters of $<10^{3}$ LD_{50}/g brain, which is completely inconsistent with the titers measured; Sc237 incubation periods at end point dilution in Syrian hamsters are around 130 days (40). That a 100% attack rate was seen at a 127-day incubation period argues against persistent Sc237 inoculum, rather than newly formed prions, being responsible for the pathogenicity to hamsters. Together, these data suggested production of novel infectivity, pathogenic for both mice and hamsters on passage of Sc237 to CD-1 mice.

A recent report has suggested that hamster scrapie (263K) may persist in the brains of inoculated C57BL/10 mice for prolonged periods without replication (41). Our data are not consistent with infectivity in the PrP^{Sc}-positive Sc237-inoculated CD-1 mice being the result of persistence of residual Sc237 hamster scrapie inoculum. High levels of mouse PrP^{Sc} (and no hamster PrP^{Sc}) are detectable on Western blot, and prions pathogenic for mice are generated. Intracerebral inoculation is known to result in wide distribution of the inoculum outside the brain via the circulation and, presumably as a result of other clearance mechanisms, brain titers fall to undetectable levels within a few days (42). Prion titers present in the brains of these mice ($\approx 10^{8}$ LD_{50}/g mouse brain assayed in hamsters) considerably exceed those inoculated ($\approx 8.5 \times 10^{6}$). Together, these data argue strongly for prion replication in these mice. It is possible that the prions detected in the brains of the C57BL/10 mice in the earlier study were not caused by persistence of inoculated 263K, but by propagation of prions with the properties we describe. The species origin of PrP^{Sc} (hamster or mouse) in the 263K-inoculated C57BL/10 mice was not reported. The observation periods postinoculation were generally much shorter than those we report here. That those mice with the longest survival postinoculation produced the shortest incubation periods on passage of infectivity into hamsters is consistent with propagation, rather than simply persistence, of prions in this earlier study (41).

Re-Evaluation of Species Barriers. Importantly, these data seriously question our current understanding of species barriers. The assessment of species barriers has relied on the development of a clinical disease in inoculated animals. On this basis there is a highly efficient barrier limiting transmission of Sc237 prions to mice. However, although not developing a clinical disease, and indeed living as long as mock-inoculated mice, Sc237-inoculated mice may accumulate high levels of prions in their brains. Previous studies on the species barrier between hamsters and mice (using the Sc237 or 263K strain) did not report whether PrP^{Sc} and/or infectivity were present in clinically unaffected animals (8, 12) or have attempted passage from mice only up to 280 days postinoculation (10). The barrier to primary passage appears in this case to be to the development of rapid neurodegeneration and the resulting clinical syndrome rather than a barrier to prion propagation itself.

The transmission characteristics of prions generated in the brains of Sc237-inoculated CD-1 mice argue that one or more distinct prion strains have been generated. The finding that Sc237-inoculated CD-1 mice in which PrP^{Sc} could not be detected on Western blot were the ones that had been culled after shorter periods than mice with detectable PrP^{Sc} argues that prion propagation is occurring in all of these mice, but is detectable only after prolonged incubation periods. That high levels of hamster infectivity were present in the PrP^{Sc}-negative Sc237-inoculated CD-1 mouse (examined at 463 days postinoculation) in the absence of detectable mouse infectivity, whereas very high and relatively comparable titers of both mouse and hamster infectivity were present in the PrP^{Sc}-positive Sc237-inoculated CD-1 mouse (examined at 730 days postinoculation) suggests that more than one strain may be propagating in these mice, with preferential replication of a strain with higher pathogenicity for hamsters early in the incubation period. One possibility is that early replication of a prion strain pathogenic only for hamsters is induced in Sc237-inoculated CD-1 mice, then later followed by the generation of a second strain that is pathogenic for mice. More extensive passage studies, including cloning of strains at end-point dilution in both mice and hamsters, will be required to investigate this further and to characterize the strain(s) of prions generated in the brains of Sc237-inoculated CD-1 mice.

http://www.pnas.org/cgi/content/full/97/18/10248

Neurobiology of Disease

Subclinical Bovine Spongiform Encephalopathy Infection in Transgenic Mice Expressing Porcine Prion Protein

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Abstract

The bovine-porcine species barrier to bovine spongiform encephalopathy (BSE) infection was explored by generating transgenic mouse lines expressing the porcine prion protein (PrP) gene. All of the porcine transgenic (poTg) mice showed clinical signs of BSE after intracerebral inoculation with a high-titer BSE inoculum. The protease-resistant PrP (PrP^{res}) was detected in 14% (3 of 22) of the BSE-infected poTg mice by immunohistochemical or immunoblot analysis. Despite being able to infect 42% (5 of 12) of control mice, a low-dose BSE inoculum failed to penetrate the species barrier in our poTg mouse model. The findings of these infectivity studies suggest that there is a strong species barrier between cows and pigs. However, after second-passage infection of poTg mice using brain homogenates of BSE-inoculated mice scoring negative for the incoming prion protein as inoculum, it was

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possible to detect the presence of the infectious agent. Thus, porcine-adapted BSE inocula were efficient at infecting poTg mice, giving rise to an incubation period substantially reduced from 300 to 177 d after inoculation and to the presence of PrP^{res} in 100% (21 of 21) of the mice. We were therefore able to conclude that initial exposure to the bovine prion may lead to subclinical infection such that brain homogenates from poTg mice classified as uninfected on the basis of the absence of PrP^{res} are infectious when used to reinoculate poTg mice. Collectively, our findings suggest that these poTg mice could be used as a sensitive bioassay model for prion detection in pigs.

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Discussion

The transgenic mouse lines developed expressed the porcine PrP transgene at different levels. This is characteristic of random transgene integration in the mouse genome by the microinjection technique. Given that high expression levels promote reduced incubation times for heterologous and homologous prion propagation in mice (Scott et al., 1997b; Castilla et al., 2003), we selected two poTg lines, poTg001 and poTg027, expressing fourfold and 16-fold, respectively, the levels of PrP protein found in pig brain.

We observed that mice expressing higher levels of poPrP spontaneously developed clinical signs. A similar neurological syndrome was described previously by Westaway et al. (1994) in older Tg PrP mice expressing high levels of hamster, ovine, or murine PrP transgenes. This phenomenon may be related to the observed toxicity of overexpressed PrP in certain cell lines, which suggests that lack of physiological PrPC expression may render pathogenic in mice. However, the lifespan of poTg027 mice was much longer than the time needed by porcine prions to propagate in these animals, and the confirmation of infection could be tested using proteinase K (PK)-resistant studies. In none of the cases did the noninoculated animals presenting late clinical signs show PK-resistant protein.

We observed substantial evidence of subclinical BSE infection in our poTg mice. PoTg mice inoculated with BSE_1 showed no clinical signs of BSE or detectable PrPres protein. However, subsequent passage of brain homogenates from these mice indicated the high level of infectivity of one of these animals. The presence of subclinical infection was particularly evident when we used the poTgBSE_1-N_2 inoculum (first-passage boTgBSE_1 in poTg PrPres-negative mice), which led to a mean incubation time of 269 d and to PrPres that was detectable by Western blotting in two of six mice. The presence of subclinical infection has been reported in other species (Race and Chesebro, 1998; Hill et al., 2000). Although there is no evidence of clinical BSE disease in the domestic pig population, pigs are susceptible to BSE, and our observations raise the possibility of subclinical infection occurring in pigs. The poTg model could be used as an assay for subclinical infection in suspected cases of prion disease in pigs.

Three inocula (Fig. 3) were used to infect the poTg mice. These inocula are known to efficiently infect transgenic mice expressing the bovine PrP gene (boTg110 line) (Castilla et al., 2003). We used the same vector to express the porcine and bovine PrP genes under the mouse PrP promoter. In the boTg110 model, increasing the PrPres titer had no effect on the incubation time. When the low-dose BSE_1 inoculum was tested in a normal mouse line, the animals showed neurological signs of disease, and 5 of 12 (42%) scored positive for PrPres. These data indicate that the BSE_1 inoculum can cross the bovine-murine species barrier, although the expression level of the mouse PrPC is approximately half that shown by our transgenic lines.

However, the low-dose BSE_1 inoculum provided evidence for a strong bovine-porcine species barrier, because it produced no signs of infection in the poTg001 or poTg027 mice. Survival times were unchanged compared with those observed in control PBS-inoculated poTg001 or poTg027 mice, and no PrPres was detected in any of the 39 inoculated mice (Table 1). In contrast, the higher titer BSE_2 and boTgBSE_1 inocula were able to breach the bovine-porcine species barrier, and PrPres was detected in 3 of 22 infected poTg mice (14%). Additional evidence for the bovine-porcine species barrier was obtained in second-passage transmission from BSE-infected poTg mice. The survival time dropped from 488 to 198 d postinoculation (dpi) for poTg001 and from 300 to 177 dpi for the poTg027 mice. The presence of a strong barrier may explain the resistance to infection shown by pigs during the BSE epidemic in the United Kingdom.

Contrary to the strong species barrier observed when poTg mice were inoculated with BSE, there was little evidence of a species barrier in the opposite direction (i.e., when we infected boTg110 mice with poTgBSE_1). All of the boTg mice infected with this inoculum scored positive for PrPres, suggesting that the barrier has different difficulty levels depending on the direction of the infection. Western blotting analysis confirmed that the PrPres observed in the bo110Tg mice displayed the same pattern (band size, glycoform ratio) as the boTgBSE_1 or BSE_1 inocula but a pattern that is different from that of the newly generated porcine prion (po027Tg) (Fig. 2C). A characteristic feature of the BSE prion is that it retains its biological properties when transmitted to other species such as humans (Collinge and Rossor, 1996; Collinge et al., 1996; Will and Zeidler, 1996; Scott et al., 1999), sheep (Foster et al., 1993, 2001), or mice (Fraser et al., 1992; Lasmezas et al., 1997). Thus, the lack of a strong species barrier observed for transmission in the direction of pig to cow might be explained if the initial BSE inoculum infecting the pig confers BSE-like properties on the porcine prion, although the primary amino acid sequence of this prion is the porcine one. Alternatively, these results could be explained as follows: (1) the bovine PrP is a very permissive protein, more easily transformed by other heterologous prions or (2) the new porcine prion is highly infectious compared with others. This second possibility will be studied using other transgenic mice expressing ovine and human PrP.

The species barrier is related to amino acid sequence differences in the globular domain of the PrP protein, which undergoes a conformational change from α-helix to β-pleated sheet structures. The porcine PrP shows the most unique amino acid sequence (5) in this domain when compared with the mouse, cow, sheep, hamster, and human PrP sequences. Figure 6 compares the globular domains of porcine, bovine, and mouse PrP. It may be observed that four of the five unique amino acids occur in

helix 3, and that there are two additional differences in this helix between the porcine and bovine sequence, I to V and R to K. The K residue is known to alter the length and quality of definition of helix 3 (Calzolai et al., 2000), and it is possible that this combination of amino acid variants alters the structure of helix 3 sufficiently to inhibit interactions between porcine PrPC and PrPres. Nuclear magnetic resonance analysis indicates that the global architecture of this region is similar for all species analyzed to date (Riek et al., 1998; Lopez Garcia et al., 2000; Zahn et al., 2000), but individual amino acid changes have been shown to affect local conformation or surface charge (Lopez Garcia et al., 2000). These subtle differences may be sufficient to strengthen or weaken a species barrier.

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Subclinical prion infection in humans and animals

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Transmission of prion diseases between mammalian species is limited by a so-called 'species' or 'transmission' barrier. Recognition of prion transmission usually relies on the appearance of clinical symptoms in inoculated animals and the interval between inoculation and appearance of clinical disease is designated incubation period. At some point during this clinically silent period, neuropathological and biochemical changes as well as accumulation of prions in the brain can be detected and this stage can be called **preclinical** prion disease. Recently, several lines of evidence have suggested that **subclinical** forms of prion disease exist, in which high levels of infectivity and PrPSc are found in animals that do not develop clinically apparent disease during a normal life-span. Such asymptomatic prion 'carrier' states challenge our current understanding of pathogenesis as well as of the molecular basis of barriers to transmission. Subclinical as well as preclinical/clinical prion disease may be relevant when analysing the risk to public health of potential sources of prion exposure.

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Subclinical Prion Disease Induced by Oral Inoculation

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Natural transmission of prion disease is believed to occur by peripheral infection such as oral inoculation. Following this route of inoculation, both the peripheral nervous system and the lymphoreticular system may be involved in the subsequent neuroinvasion of the central nervous system by prions, which may not necessarily result in clinical signs of terminal disease. Subclinical prion disease, characterized by the presence of infectivity and PrPSc in the absence of overt clinical signs, may occur. It is not known which host factors contribute to whether infection with prions culminates in a terminal or subclinical disease state. We have investigated whether the level of host PrPc protein expression is a factor in the development of subclinical prion disease. When RML prion inoculum was inoculated by either the i.c. or intraperitoneal route, wild-type and *tga20* mice both succumbed to terminal prion disease. In contrast, orally inoculated *tga20* mice succumbed to terminal prion disease, whereas wild-type mice showed no clinical signs. However, wild-type mice sacrificed 375 or 525 days after oral inoculation harbored significant levels of brain PrPSc and infectivity. These data show that same-species transmission of prions by the oral route in animals that express normal levels of PrPc can result in subclinical prion disease. This indicates that the level of host PrPc protein expression is a contributing factor to the regulation of development of terminal prion disease. Events that increase PrPc expression may predispose a prion-infected animal to the more deleterious effects of prion pathology.

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Chronic Subclinical Prion Disease Induced by Low-Dose Inoculum

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We have compared the transmission characteristics of the two mouse-adapted scrapie isolates, ME7 and Rocky Mountain Laboratory (RML), in tga20 mice. These mice express elevated levels of PrP protein compared to wild-type mice and display a relatively short disease incubation period following intracerebral prion inoculation. Terminal prion disease in tga20 mice induced by ME7 or RML was characterized by a distinct pattern of clinical signs and different incubation times. High-dose RML inoculated intracerebrally into tga20 mice induced the most rapid onset of clinical signs, with mice succumbing to terminal disease after only 58 ± 3 days. In contrast, high-dose ME7 gave a mean time to terminal disease of 74 ± 0 days. Histological examination of brain sections from prion-inoculated tga20 mice at terminal disease showed that ME7 gave rise to a more general and extensive pattern of vacuolation than RML. Low-dose inoculum failed to induce terminal disease but did cause preclinical symptoms, including the appearance of reversible clinical signs. Some mice oscillated between showing no clinical signs and early clinical signs for many months but never progressed to terminal disease. Brain tissue from these mice with chronic subclinical prion disease, sacrificed at >200 days postinoculation, contained high levels of infectivity and showed the presence of PrPSc. Parallel analysis of brain tissue from mice with terminal disease showed similar levels of infectivity and detectable PrPSc. These results show that high levels of infectivity and the presence of the abnormal isomer of PrP can be detected in mice with subclinical disease following low-dose prion inoculation.

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Long-Term Subclinical Carrier State Precedes Scrapie Replication and Adaptation in a Resistant Species: Analogies to Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease in Humans

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Received 24 May 2001/Accepted 31 July 2001

Cattle infected with bovine spongiform encephalopathy (BSE) appear to be a reservoir for transmission of variant Creutzfeldt-Jakob disease (vCJD) to humans. Although just over 100 people have developed clinical vCJD, millions have probably been exposed to the infectivity by consumption of BSE-infected beef. It is currently not known whether some of these individuals will develop disease themselves or act as asymptomatic carriers of infectivity which might infect others in the future. We have studied agent persistence and adaptation after cross-species infection using a model of mice inoculated with hamster scrapie strain 263K. Although mice inoculated with hamster scrapie do not develop clinical disease after inoculation with 10 million hamster infectious doses, hamster scrapie infectivity persists in brain and spleen for the life span of the mice. In the present study, we were surprised to find a 1-year period postinfection with hamster scrapie where there was no evidence for replication of infectivity in mouse brain. In contrast, this period of inactive persistence was followed by a period of active replication of infectivity as well as adaptation of new strains of agent capable of causing disease in mice. In most mice, neither the early persistent phase nor the later replicative phase could be detected by immunoblot assay for protease-resistant prion protein (PrP). If similar asymptomatic carriers of infection arise after exposure of humans or animals to BSE, this could markedly increase the danger of additional spread of BSE or vCJD infection by contaminated blood, surgical instruments, or meat. If such subclinical carriers were negative for protease-resistant PrP, similar to our mice, then the recently proposed screening of brain, tonsils, or other tissues of animals and humans by present methods such as immunoblotting or immunohistochemistry might be too insensitive to identify these individuals.

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http://jvi.asm.org/cgi/content/abstract/75/21/10106

From: TSS ()
Subject: PrPSc distribution of a natural case of bovine spongiform encephalopathy
Date: August 8, 2005 at 12:28 pm PST

PrPSc distribution of a natural case of bovine
spongiform encephalopathy

Yoshifumi Iwamaru, Yuka Okubo, Tamako Ikeda, Hiroko Hayashi, Morikazu Imamura, Takashi Yokoyama and Morikazu Shinagawa

Priori Disease Research Center, National Institute of Animal Health, 3-1-5 Kannondai, Tsukuba 305-0856 Japan gan@affrc.go.jp

Abstract

Bovine spongiform encephalopathy (BSE) is a disease of cattle that causes progressive neurodegeneration of the central nervous system. Infectivity of BSE agent is accompanied with an abnormal isoform of prion protein (PrPSc).

The specified risk materials (SRM) are tissues potentially carrying BSE infectivity. The following tissues are designated as SRM in Japan: the skull including the brain and eyes but excluding the glossa and the masseter muscle, the vertebral column excluding the vertebrae of the tail, spinal cord, distal illeum. For a risk management step, the use of SRM in both animal feed or human food has been prohibited. However, detailed PrPSc distribution remains obscure in BSE cattle and it has caused controversies about definitions of SRM. Therefore we have examined PrPSc distribution in a BSE cattle by Western blotting to reassess definitions of SRM.

The 11th BSE case in Japan was detected in fallen stock surveillance. The carcass was stocked in the refrigerator. For the detection of PrPSc, 200 mg of tissue samples were homogenized. Following collagenase treatment, samples were digested with proteinase K. After digestion, PrPSc was precipitated by sodium phosphotungstate (PTA). The pellets were subjected to Western blotting using the standard procedure. Anti-prion protein monoclonal antibody (mAb) T2 conjugated horseradish peroxidase was used for the detection of PrPSc.

PrPSc was detected in brain, spinal cord, dorsal root ganglia, trigeminal ganglia, sublingual ganglion, retina. In addition, PrPSc was also detected in the peripheral nerves (sciatic nerve, tibial nerve, vagus nerve).

Our results suggest that the currently accepted definitions of SRM in

BSE cattle may need to be reexamined.

179

T. Kitamoto (Ed.)
PRIONS
Food and Drug Safety
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ALSO from the International Symposium of Prion Diseases held in Sendai, October 31, to November 2, 2004;

Bovine spongiform encephalopathy (BSE) in Japan

snip...

"Furthermore, current studies into transmission of cases of BSE that are atypical or that develop in young cattle are expected to amplify the BSE prion"

NO. Date conf. Farm Birth place and Date Age at diagnosis

8. 2003.10.6. Fukushima Tochigi 2001.10.13. 23

9. 2003.11.4. Hiroshima Hyogo 2002.1.13. 21

Test results

8b, 9c cows Elisa Positive, WB Positive, IHC negative, histopathology negative

b = atypical BSE case

c = case of BSE in a young animal

b,c, No PrPSc on IHC, and no spongiform change on histology

International Symposium of Prion Diseases held in Sendai, October 31, to November 2, 2004.

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From: TSS ()
Subject: Atypical Proteinase K-Resistant Prion Protein (PrPres) observed in an Apparently Healthy 23-Month-Old Holstein Steer
Date: August 26, 2005 at 10:24 am PST

Atypical Proteinase K-Resistant Prion Protein (PrPres) observed in an Apparently Healthy 23-Month-Old Holstein Steer

Jpn. J. Infect. Dis., 56, 221-222, 2003

Laboratory and Epidemiology Communications

Atypical Proteinase K-Resistant Prion Protein (PrPres) Observed in an Apparently Healthy 23-Month-Old Holstein Steer

Yoshio Yamakawa*, KenÕichi Hagiwara, Kyoko Nohtomi, Yuko Nakamura, Masahiro Nishizima ,Yoshimi Higuchi1, Yuko Sato1, Tetsutaro Sata1 and the Expert Committee for BSE Diagnosis, Ministry of Health, Labour and Welfare of Japan2

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Communicated by Tetsutaro Sata

(Accepted December 2, 2003)

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Since October 18, 2001, 'bovine spongiform encephalopathy (BSE) examination for all cattle slaughtered at abattoirs in the country' has been mandated in Japan by the Ministry of Health, Labour and Welfare (MHLW). 'Plateria' ELISA-kit (Bio-Rad Laboratories, Hercules, Calif., USA) is routinely used at abattoirs for detecting proteinase K (PK)-resistant prion protein (PrPSc) in the obex region. Samples positive according to the ELISA screening are further subjected to Western blot (WB) and histologic and immunohistochemical examination (IHC) at the National Institute of Infectious Diseases (NIID) or Obihiro University. If PrPSc is detected either by WB or by IHC, the cattle are diagnosed as BSE. The diagnosis is approved by the Expert Committee for BSE Diagnosis, MHLW. From October 18, 2001 to September 30, 2003, approximately 2.5 million cattle were screened at abattoirs. A hundred and ten specimens positive according to ELISA were subjected to WB/IHC. Seven showed positive by both WB and IHC, all exhibiting the typical electrophoretic profile of a high content of the di-glycosylated molecular form of PrPSc (1-3) and the distinctive granular deposition of PrPSc in neuronal cells and neuropil of the dorsal nucleus of vagus.

An ELISA-positive specimen from a 23 month-old Holstein steer slaughtered on September 29, 2003, in Ibaraki Prefecture (Ibaraki case) was sent to the NIID for confirmation. The animal was reportedly healthy before slaughter. The OD titer in ELISA was slightly higher than the 'cut-off' level given by the manufacturer. The histology showed no spongiform changes and IHC revealed no signal of PrPSc accumulation typical for BSE. However, WB analysis of the homogenate that was prepared from the obex region and used for ELISA revealed a small amount of PrPSc with an electrophoretic profile different from that of typical BSE-associated PrPSc (1-3). The characteristics were (i) low content of the di-glycosylated molecular form of PrPSc, (ii) a faster migration of the non-glycosylated form of PrPSc on SDS-PAGE, and (iii) less resistance against PK digestion as compared with an authentic PrPSc specimen derived from an 83-month-old Holstein (Wakayama case) (Fig. 1). Table 1 summarizes the relative amounts of three distinctive glycoforms (di-, mono, non-glycosylated) of PrPSc calculated by densitometric analysis of the blot shown in Fig. 1. As 2.5 mg wet weight obex-equivalent homogenate of the Ibaraki case (Fig. 1, lane 4) gave slightly stronger band intensities of PrPSc than an 8 mg wet weight obex-equivqlent homogenate of a typical BSE-affected Wakayama case (Fig. 1, lane 2), the amount of PrPSc accumulated in the Ibaraki case was calculated to be 1/500 - 1/1000 of the Wakayama case. In the Ibaraki case, the PrPSc bands were not detectable in the homogenates of the proximal surrounding region of the obex. These findings were consistent with the low OD value in ELISA, i.e., 0.2 - 0.3 for the Ibaraki case versus over 3.0 for the Wakayama case. The DNA sequence of the PrP coding region of the Ibaraki case was the same as that appearing in the database (GenBank accession number: AJ298878). More recently, we encountered another case that resembled the Ibaraki case. It was a 21-month-old Holstein steer from Hiroshima Prefecture. WB showed typical BSE-specific PrPSc deposition though IHC did not detect positive signals of PrPSc (data not shown).

Though the clinical onset of BSE is usually at around 5 years of age or later, a 20-month-old case showing the clinical signs has been reported (4). Variant forms of BSE similar to our cases, i.e., with atypical histopathological and/or biochemical phenotype, have been recently reported in Italy (5) and in France (6). Such variant BSE was not associated with mutations in the prion protein (PrP) coding region as in our case (5,6).

The Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF) announced a ban of feeding ruminants with meat bone meal (MBM) on September 18, 2001, and a complete ban was made on October 15 of the same year. According to the recent MAFF report, the previous seven cases of BSE in Japan were cattle born in 1995 - 1996 and possibly fed with cross-contaminated feed. However, the two cattle in this report were born after the complete ban. Whether contaminated MBM was implicated in the present cases remains to be investigated.

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SEE SLIDES IN PDF FILE;

http://www.nih.go.jp/JJID/56/221.pdf

IN fact, we are now finding that as little as 1 mg (or 0.001 gm) caused 7% (1 of 14) of the cows to come down with BSE ;

Published online

January 27, 2005

Risk of oral infection with bovine spongiform

encephalopathy agent in primates

Corinne Ida Lasmézas, Emmanuel Comoy, Stephen Hawkins, Christian Herzog, Franck Mouthon, Timm Konold, Frédéric Auvré, Evelyne Correia,

Nathalie Lescoutra-Etchegaray, Nicole Salès, Gerald Wells, Paul Brown, Jean-Philippe Deslys

The uncertain extent of human exposure to bovine spongiform encephalopathy (BSE)—which can lead to variant

Creutzfeldt-Jakob disease (vCJD)—is compounded by incomplete knowledge about the ef.ciency of oral infection

and the magnitude of any bovine-to-human biological barrier to transmission. We therefore investigated oral

transmission of BSE to non-human primates. We gave two macaques a 5 g oral dose of brain homogenate from a

BSE-infected cow. One macaque developed vCJD-like neurological disease 60 months after exposure, whereas the

other remained free of disease at 76 months. On the basis of these .ndings and data from other studies, we made a

preliminary estimate of the food exposure risk for man, which provides additional assurance that existing public

health measures can prevent transmission of BSE to man.

snip...

BSE bovine brain inoculum

100 g 10 g 5 g 1 g 100 mg 10 mg 1 mg 0·1 mg 0·01 mg

Primate (oral route)* 1/2 (50%)

Cattle (oral route)* 10/10 (100%) 7/9 (78%) 7/10 (70%) 3/15 (20%) 1/15 (7%) 1/15 (7%)

RIII mice (ic ip route)* 17/18 (94%) 15/17 (88%) 1/14 (7%)

PrPres biochemical detection

The comparison is made on the basis of calibration of the bovine inoculum used in our study with primates against a bovine brain inoculum with a similar PrPres concentration that was

inoculated into mice and cattle.8 *Data are number of animals positive/number of animals surviving at the time of clinical onset of disease in the .rst positive animal (%). The accuracy of

bioassays is generally judged to be about plus or minus 1 log. ic ip=intracerebral and intraperitoneal.

Table 1: Comparison of transmission rates in primates and cattle infected orally with similar BSE brain inocula

snip...end

www.thelancet.com Published online January 27, 2005

The BSE Inquiry / Statement No. 14. Issued 20 March 1998 ... number of feed compounders and it became clear that cross contamination of feeds could occur. ...

http://www.bseinquiry.gov.uk/files/ws/s014.pdf

[PDF] The BSE Inquiry / Statement No 76F (Supplementary) Mr Alan ...

but the main problem was probably cross-contamination. ...

http://www.bseinquiry.gov.uk/files/ws/s076f.pdf

03-025IF 03-025IF-631 Linda A. Detwiler [PDF]

http://www.fsis.usda.gov/OPPDE/Comments/03-025IF/03-025IF-631.pdf

Specified Risk Materials (SRMs)

I am in full support of the interim final rule which prohibits SRMs from being included in food for human consumption. In addition to the list of tissues published in this rule, I am requesting that additional tissues be added to the list. These would include dura ("sheath") covering the spinal cord and the ENTIRE INTESTINE (from pylorus to rectum). The scientific justification is provided below. THESE SRMs should also be prohibited from ANY FDA regulated food or product intended for human consumption, including but not limited to flavorings, extracts, etc. ...

Dr. Linda Detwiler comments in full;

http://www.fsis.usda.gov/OPPDE/Comments/03-025IF/03-025IF-634.pdf

NEW STRAIN OF TSE USA CATTLE OR JUST INCOMPETENCE IN TESTING???

DR. CLIFFORD: "Basically the IHC test, besides looking at location of the brain stem you're also doing a staining technique to identify abnormal prion proteins. In this case they had some staining, but the staining did not match up with what they would typically see in a BSE case. It didn't have the normal distribution it would see within the samples. So basically that's why the request for doing additional testing, and that's why we're sending it to Weybridge as well."

DR. CLIFFORD: "There was some staining present. But it did not match a normal pattern, and we're taking through that to do additional tests in additional parts of the brain stem to try to see if we can find a normal staining pattern as well as sending that sample to Weybridge to run against their IHC."

http://www.usda.gov/wps/portal/usdahome?contentidonly=true&contentid=2005/07/0280.xml

IN CONFIDENCE

PERCEPTION OF UNCONVENTENTIONAL SLOW VIRUS DISEASES OF ANIMALS IN THE USA

1985 The Stetsonville outbreak (farmer's name: Brecke). In addition to the downer cows and horses Brecke's mink recieved a cereal supplement. Hartsough's view was that this would contain bone meal and would be from a commercial source. If this were so and it was contaminated with a TME agent why were no other ranches affected?

Many mink ranches now feed a commerical pelleted diet. Brecke was equipped to process LARGE CARCASSES USING A CRUSHER/MIXER WHICH COULD ACCOMMODATE A WHOLE COW!

snip...

Dead mink go for rendering but are used only in poultry feed.

A commercial mink ranch was visited. This was Johny Werth's, Capitol Fur Farm comprising 1400 breeding females. The feed is bought in from a commercial supplier in the form of frozen packs of "poultry", "fish", "dried egg" or "tripe". A commercial mink cereal supplement is used and contains "animal meat meal" which was said to contain material mainly from poultry or fish origin but OCCASIONALLY FROM BEEF SOURCES. the partially thawed packs were tipped into an augur mixer which has a fully loaded capacity of 6000lb and this would be approximately 15000 mink per day.

In the fall at pelting time the skinned carcasses of the mink are placed in large barrels which are left in the open to freeze. When full, a renderer collects "for use in poultry feeds".

Sections from the brains of the two Brecke TME inoculated cattle were examined and Marsh provided all the blocks from the 2nd steer for study at CVL and comparison with BSE. In general the vacuolar changes were more severe than in most cases of BSE but very similar in distribution. Unfortunately material aken fro histopathology from those anials omitted representaion of most of the brain stem. .....

Wilbur Clarke (reference the Mission, Texas scrapie transmission transmission to cattle study) is now the State Veterinarian for Montana based at Helena.

I was given confidential access to sections from the Clarke scrapie-cattle transmission experiment. Details of the experimental design were as supplied previously by Dr. Wrathall (copy of relevant information appended). Only 3 animals (2 inoculated with 2nd pass Suffolk scrapie and 1 inoculated with Angora goat passaged scrapie) showed clinical signs. Clinical signs were characterised by weakness, "a stilted hindlimb gait", disorientation, ataxia and, terminally, lateral recumbency. The two cattle from which I examined material were inocluated at 8 months of age and developed signs 36 months pi (goat scrapie inoculum) and 49 months pi (one of the Suffolk scrapie inoculated) respectively. This latter animal was killed at 58 months of age and so the clinical duration was only 1 month. The neuropathology was somewhat different from BSE or the Stetsonville TME in cattle. Vacuolar changes were minimal, to the extent that detection REQUIRED CAREFUL SEARCHING. Conversely astrocyte hypertrophy was a widespread and prominent feature. The material requires DETAILED NEUROPATHOLOGICAL ASSESSMENT BUT WHETHER OR NOT THIS WILL BE DONE REMAINS A QUESTION.

Transmission Studies

Mule deer transmissions of CWD were by intracerebral inoculation and compared with natural cases

{the following was written but with a single line marked through it "first passage (by this route)}...TSS

resulted in a more rapidly progressive clinical disease with repeated episodes of synocopy ending in coma. One control animal became affected, it is believed through contamination of inoculum (?saline). Further CWD transmissions were carried out by Dick Marsh into ferret, mink and squirrel monkey. Transmission occurred in ALL of these species with the shortest
incubation period in the ferret.

snip...

Appendix 3

VISIT TO USA - DR A E WRATHALL - INFO OH BSE AND SCRAPIE

1. Dr Clark lately of the Scrapie Research Unit, Mission Texas has successfully transmitted ovine and caprine scrapie to cattle. The experimental results have not been published but there are plans to do this. This work was initiated in 1978. A summary of it is:-

Expt A
6 Her x Jer calves born in 1978 were inoculated as follows with a 2nd Suffolk scrapie passage:-

i/c 1ml; i/m, 5ml; s/c 5ml; oral 30ml.
1/6 went down after 48 months with a scrapie/BSE-like disease.

Expt B
6 Her or Jer or HxJ calves were inoculated with angora Goat virus 2/6 went down similarly after 36 months.

Expt C
Mice inoculated from brains of calves/cattle in expts A • B were resistant, only 1/20 going down with scrapie and this was the reason given for not publishing.

Diagnosis in A, B, C was by histopath. No reports on SAT were given.

2. Dr Warren Foote indicated success so far in eliminating scrapie in offspring from experimentally- (and naturally) infected sheep by ET. He had found difficulty in obtaining embryos from naturally infected sheep (cf SPA).

3. Prof. A Robertson gave a brief account of BSE. The US approach was to accord it a very low profile indeed. Dr A Thiermann showed the picture in the "Independent" with cattle being incinerated and thought this was a fanatical incident to be avoided in the US at all costs. BSE was not reported in USA.

4. Scrapie incidents (ie affected flocks) have shown a dramatic increase since 1978. In 1953 when the National Control Scheme was started there were 10-14 incidents, in 1978 - 1 and in 1988 so far 60.

5. Scrapie agent was reported to have been isolated from a solitary fetus.

6. A western blotting diagnostic technique (? on PrP) shows some promise.

7. Results of a questionnaire sent to 33 states on the subject of the national sheep scrapie programme survey indicated

17/33 wished to drop it

6/33 wished to develop it

8/33 had few sheep and were neutral

Information obtained from Dr Wrathall's notes of a meeting of the U.S.
Animal Health Association at Little Rock, Arkansas Nov. 1988.

33

end...TSS

>> Differences in tissue distribution could require new regulations
>> regarding specific risk material (SRM) removal.

snip...end

full text 33 PAGES ;

http://www.bseinquiry.gov.uk/files/mb/m11b/tab01.pdf

http://www.bseinquiry.gov.uk/files/yb/1988/10/00001001.pdf

It was, however, performed in the USA in 1979, when it was shown that cattle inoculated with the scrapie agent endemic in the flock of Suffolk sheep at the United States Department of Agriculture in Mission, Texas, developed a TSE quite unlike BSE. 32 The findings of the initial transmission, though not of the clinical or neurohistological examination, were communicated in October 1988 to Dr Watson, Director of the CVL, following a visit by Dr Wrathall, one of the project leaders in the Pathology Department of the CVL, to the United States Department of Agriculture. 33 The results were not published at this point, since the attempted transmission to mice from the experimental cow brain had been inconclusive. The results of the clinical and histological differences between scrapie-affected sheep and cattle were published in 1995. Similar studies in which cattle were inoculated intracerebrally with scrapie inocula derived from a number of scrapie-affected sheep of different breeds and from different States, were carried out at the US National Animal Disease Centre. 34 The results, published in 1994, showed that this source of scrapie agent, though pathogenic for cattle, did not produce the same clinical signs of brain lesions characteristic of BSE.

http://www.bseinquiry.gov.uk/

1: J Infect Dis. 1994 Apr;169(4):814-20.

Intracerebral transmission of scrapie to cattle.

Cutlip RC, Miller JM, Race RE, Jenny AL, Katz JB, Lehmkuhl HD, DeBey BM, Robinson MM.

USDA, Agriculture Research Service, National Animal Disease Center, Ames, IA 50010.

To determine if sheep scrapie agent(s) in the United States would induce a disease in cattle resembling bovine spongiform encephalopathy, 18 newborn calves were inoculated intracerebrally with a pooled suspension of brain from 9 sheep with scrapie. Half of the calves were euthanatized 1 year after inoculation. All calves kept longer than 1 year became severely lethargic and demonstrated clinical signs of motor neuron dysfunction that were manifest as progressive stiffness, posterior paresis, general weakness, and permanent recumbency. The incubation period was 14-18 months, and the clinical course was 1-5 months. The brain from each calf was examined for lesions and for protease-resistant prion protein. Lesions were subtle, but a disease-specific isoform of the prion protein was present in the brain of all calves. Neither signs nor lesions were characteristic of those for bovine spongiform encephalopathy.

MeSH Terms:
Animals
Brain/microbiology*
Brain/pathology
Cattle
Cattle Diseases/etiology*
Cattle Diseases/pathology
Encephalopathy, Bovine Spongiform/etiology*
Encephalopathy, Bovine Spongiform/pathology
Immunoblotting/veterinary
Immunohistochemistry
Male
Motor Neurons/physiology
Prions/analysis
Scrapie/pathology
Scrapie/transmission*
Sheep
Sleep Stages
Time Factors

Substances:
Prions

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=8133096&dopt=Citation

Intracerebral transmission of scrapie to cattle FULL TEXT PDF;

SNIP...

Discussion

WE conclude that American sources of sheep scrapie are transmissible to cattle by direct intracerebral inoculation but the disease induced is NOT identical to BSE as seen in the United Kingdom. While there were similarities in clinical signs between this experimental disease and BSE, there was no evidence of aggressiveness, hyperexcitability, hyperesthesia (tactile or auditory), or hyperemetria of limbs as has been reported for BSE (9). Neither were there extensive neurologic lesions, which are primary for BSE, such as severe vacuolation of neurons and neuropil or neuronal necrosis and gliosis. Although some vacuolation of neuropil, chromotolysis in neurons, and gliosis were seen in the brains of some affected calves, these were industinguishable from those of controls. Vacuolated neurons in the red nucleus of both challenged and normal calves were considered normal for the bovines as previously described (50).

PrP-res was found in ALL CHALLENGED CALVES REGARDLESS OF CLINCIAL SIGNS, and the amount of PrP-res positively related to the length of the incubation. ...

snip...

WE also conclude from these studies that scrapie in cattle MIGHT NOT BE RECOGNIZED BY ROUTINE HISTOPATHOLOGICAL EXAMINATION OF THE BRAIN AND SUGGEST THAT DETECTION OF PrP-res by immunohistochemistry or immunoblotting is necessary to make a definitive diagnosis. THUS, undiagnosed scrapie infection could contribute to the ''DOWNER-COW'' syndrome and could be responsible for some outbreaks of transmissible mink encephalopathy proposed by Burger and Hartsough (8) and Marsh and harsough (52). ...

snip...

Multiple sources of sheep affected with scrapie and two breeds of cattle from several sources were used inthe current study in an effort to avoid a single strain of either agent or host. Preliminary results from mouse inoculations indicate multiple strains of the agent were present in the pooled inoculum (unpublished data). ...

Transmission of the sheep scrapie to cattle was attempted in 1979 by using intracerebral, intramuscular, subcutaneous, and oral routes of inoculation of 5, 8- to 11-month old cattlw with a homologous mixture of brain from 1 affected sheep (61, 62). ONE of the 5 cattle develped neurologic signs 48 months after inoculation. Signs were disorientation, incoordination, a stiff-legged stilted gait, progressive difficulty in rising, and finally in terminal recumbency. The clinical course was 2.5 months. TWO of the 5 cattle similarly inoculated with brain tissue from a goat with scrapie exhibited similar signs 27 and 36 months after incoluation. Clinical courses were 43 an 44 days. Brain lesions of mild gliosis and vacuolation and mouse inoculation data were insufficient to confirm a diagnosis of scrapie. This work remained controversial until recent examination of the brains detected PrP-res in all 3 cattle with neurologic disease but in none of the unaffected cattle (62). Results of these studies are similar to ours and underscore the necessity of methods other than histopathology to diagnose scrapie infection in cattle. We believe that immunologic techniques for detecting PrP-res currently provide the most sensitive and reliable way to make a definitive diagnosis...

http://www.bseinquiry.gov.uk/files/sc/seac17/tab03.pdf

Visit to USA ... info on BSE and Scrapie

http://www.bseinquiry.gov.uk/files/yb/1988/10/00001001.pdf

http://www.ngpc.state.ne.us/cgi-bin/ultimatebb.cgi?ubb=get_topic;f=12;t=000385

12/10/76
AGRICULTURAL RESEARCH COUNCIL
REPORT OF THE ADVISORY COMMITTE ON SCRAPIE
Office Note
CHAIRMAN: PROFESSOR PETER WILDY

snip...

A The Present Position with respect to Scrapie
A] The Problem

Scrapie is a natural disease of sheep and goats. It is a slow and inexorably progressive degenerative disorder of the nervous system and it ia fatal. It is enzootic in the United Kingdom but not in all countries.

The field problem has been reviewed by a MAFF working group (ARC 35/77). It is difficult to assess the incidence in Britain for a variety of reasons but the disease causes serious financial loss; it is estimated that it cost Swaledale breeders alone $l.7 M during the five years 1971-1975. A further inestimable loss arises from the closure of certain export markets, in particular those of the United States, to British sheep.

It is clear that scrapie in sheep is important commercially and for that reason alone effective measures to control it should be devised as quickly as possible.

Recently the question has again been brought up as to whether scrapie is transmissible to man. This has followed reports that the disease has been transmitted to primates. One particularly lurid speculation (Gajdusek 1977) conjectures that the agents of scrapie, kuru, Creutzfeldt-Jakob disease and transmissible encephalopathy of mink are varieties of a single "virus". The U.S. Department of Agriculture concluded that it could "no longer justify or permit scrapie-blood line and scrapie-exposed sheep and goats to be processed for human or animal food at slaughter or rendering plants" (ARC 84/77)" The problem is emphasised by the finding that some strains of scrapie produce lesions identical to the once which characterise the human dementias"

Whether true or not. the hypothesis that these agents might be transmissible to man raises two considerations. First, the safety of laboratory personnel requires prompt attention. Second, action such as the "scorched meat" policy of USDA makes the solution of the acrapie problem urgent if the sheep industry is not to suffer grievously.

snip...

76/10.12/4.6

http://www.bseinquiry.gov.uk/files/yb/1976/10/12004001.pdf

THE infamous USA SPORADIC CJDs, something to ponder;

IF the USA TSE in cattle all does not look like UK BSE, why would all USA human TSE look like UK nvCJD???

over 20 strains of scrapie documented to date with new atypical strains now being documented in sheep and goat i.e. BSE.

atypical strains of BSE/TSE showing up in cattle in different countries?

ALL animals for human/animal consumption must be tested for TSE.

ALL human TSEs must be made reportable Nationally and Internationally, OF ALL AGES...

IN a time when FSIS/APHIS/USDA/FDA et al should be strengthening the TSE regulations, it seems corporate interest has won out again over sound science and consumer protection from an agent that is 100% fatal for the ones that go clinical. With the many different atypical TSEs showing up in different parts of the world, and with GWs BSE MRR policy (the legal policy of trading all strains of TSEs), the battle that has waged for the last 25 years to eradicate this agent from this planet will be set back decades, if not lost for good. ...

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