KURU AND "NEW VARIANT" CJD

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Abstract. Acquired transmissible spongiform encephalopathies in humans include Kuru (a disease which was associated with ritualistic cannibalism in Papua New Guinea), iatrogenic Creutzfeldt-Jakob disease and a newly recognized variant form of Creutzfeldt-Jakob disease (nvCJD).

Clinical and neuropathological features of nvCJD are reminiscent of Kuru: early and progressive cerebellar ataxia and numerous characteristic Kuru-type amyloid plaques surrounded by spongiform change. In contrast to typical cases of sporadic CJD, Kuru and nvCJD affect young patients.

The newly recognized form of CJD has been identified in ten young people in the UK in 1996, approximately 10 years after the beginning of the bovine spongiform encephalopathy (BSE) epidemic in the UK. Molecular analysis has shown that nvCJD has strain characteristics that are distinct from other types of CJD but similar to those of BSE.

In the UK an estimated half a million BSE-infected cows entered the human food chain before the bovine offal ban of 1989. To be effective the oral route probably requires high-infectivity titers which are encountered only in the brain, spinal cord and eyes of naturally infected cows. In patients with Kuru, titers of more than 10^8 infectious doses per gram were reported in the brain tissues.

As a result of the estimated very long incubation period of nvCJD (10 to 30 years or more) the predicted nvCJD epidemic will have the shape of a normal distribution curve with a peak expected in 2009. The epidemic may extend until 2030. There is already an example to illustrate such a curve in its descending line: the decline of Kuru deaths following the interruption of ritual cannibalism.

"...A number of people were found suffering from a probably new form of encephalitis attributed by inhabitants to sorcery and called Kuru..." (Vincent Zigas, December 1956).

"I first entered the Kuru region by March 1957 with Dr Vincent Zigas, the medical officer at Kainantu in the Eastern Highlands district..." (Daniel Carleton Gajdusek).

The transmissible spongiform encephalopathies or prion diseases are neurodegenerative conditions that affect both humans and animals. They are transmitted experimentally both within and between mammalian species by inoculation with infected tissues and sometimes by ingestion of contaminated tissues.

Acquired prion diseases in humans include Kuru, iatrogenic Creutzfeldt-Jakob disease and "new variant" Creutzfeldt-Jakob disease (Table 1).

Scrapie is a naturally occuring disease of sheep and goats occuring in many countries and recognized for over two centuries. The recent epidemic of a newly recognized prion disease, bovine spongiform encephalopathy (BSE), or "mad cow disease", among cattle in the United Kingom, has lead to fears that transmission to humans could occur through the ingestion of infected tissues. The BSE epidemic in the UK, the only country with a high incidence of the disease, appears to have been due mainly to the recycling of affected bovine material back to cattle (induced bovine cannibalism) before the July 1988 ruminant feed ban became effective.

KURU AND nvCJD

Clinical and neuropathological features of the recently identified "new variant" Creutzfeldt-Jakob

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Table 1

Human prion diseases.

Disease	Origin	Distribution
Familial Creutzfeldt-Jakob disease	inheritance of a mutation in the gene coding for the prion protein (PrP)	Some 100 extended families have been identified
Gerstmann-Sträussler-Scheinker disease	inheritance of a mutation in the PrP gene	Some 50 extended families have been identified
Fatal familial Insomnia	inheritance of a mutation in the PrP gene	Nine extended families have been identified
Sporadic Creutzfeldt-Jakob disease	spontaneous?	1 person per million worldwide
Kuru	infection through cannibalism	more than 2,600 cases identified since 1957. Fore group of Papua-New-Guinea (pop 35,000)
Iatrogenic Creutzfeldt-Jakob disease	infection through medical inoculation (injectable pituitary derived hormones)	90 cases (France 50)
New variant Creutzfeldt-Jakob disease	infection through consumption of bovine spongiform encephalopathy-infected tissues	14 confirmed cases in the UK, 1 in France (end 1996)

disease (nvCJD) are reminiscent of Kuru: psychiatric presentation with depression and other behavioristic changes, *early and progressive cerebellar ataxia*, dementia in the late stage, numerous characteristic Kuru-type amyloid plaques surrounded by spongiform change and extensive deposition of prion protein in all grey matter regions, particularly the cerebellum. In contrast to sporadiç CJD, nvCJD and Kuru occur in younger age groups.

The routes of contamination

In Papua New Guinea the mechanism of spread of Kuru was undoubtedly the contamination of the Fore population during ritual cannibalism (Gajdusek, 1976).

Women did the autopsies barehanded and did not wash thereafter. Infants and toddlers (none ingested Kuru-infected tissues) appear to have been massively contaminated by the hands of their mothers in wiping their eyes and cleaning their nose. The liquefying brain tissue, scooped by hand in bambou cylinders, was infectious at a dilution of 10^8. Scratching scabies, insect bites and impetigo with infected fingernails could have produced hundred of intradermal or deeper inoculation. Men rarely handled or ate the brain of dead Kuru victims and therefore were rarely contaminated.

In the UK, conjunctival, nasal and skin contamination could also have occurred in children and young people scratching their impetigo, acne or herpes or cleaning their nose or eyes with fingers contaminated by highly infectious BSE-infected bovine brain pool homogenates used as binding agent for the preparation of hamburgers (Verdrager,

1996). However most of the contaminations probably occurred via the oral route. In the UK hamburgers are eaten predominantly by young people and this may explain the present age group distribution of nvCJD (15-40 years).

Another possible route of contamination by BSE is the potential iatrogenic exposure to BSE through injectable bovine-pituitary derived hormones (somatotrophin, posterior lobe hormone, tyrotrophic hormone) injectable bovine brain and spinal cord extracts, implanted materials of bovine origin (non-synthetic surgical suture: "bovine catgut"), cosmetics using bovine eyes, bovine posterior pituitary nasal powder (snuff), etc. In France, most of these products were banned in 1992.

The infective tissues

To be effective the oral route probably requires high infectivity titers which are encountered only in brain and spinal cords. Gajdusek reported titers of more than 10^8 infectious doses per gram in the brain tissues of patients with Kuru whereas in peripheral tissues it has been found rarely at the time of death and in much lower titers. In contrast to scrapie in sheep, only brain and spinal cord of naturally infected cows with BSE have shown any detectable BSE high infectivity.

The incubation period

In recent field work on Kuru it has been possible to obtain clear documentation of incubation periods of 30 years and more in human Kuru (Gajdusek, 1990). The shortest minimum incubation periods (4-5 years) for Kuru were encountered in young children who never ingested Kuru-infected tissues but were massively contaminated by the spreading of liquefied brain tissues over their bodies and rubbing of their eyes and noses by their mothers' hands. The youngest patient with Kuru, who self-diagnosed the insidious onset of clumsiness in his gait as Kuru at 4 years of age, died aged 5, several years before his mother developed Kuru herself. Similar incubation periods (4-30 years) have been reported in iatrogenic CJD (peripheral route).

The BSE epidemic started in the UK in April 1985; but infected animals were already infective in 1984 (later stage of incubation). The earliest clinical onset in one of the ten young people with nvCJD occurred in February 1994. The interval of 9 or 10 years between 1984/1985 and 1994 may represent the minimum incubation period of nvCJD (oral route). This incubation period is longer than in Kuru or in iatrogenic CJD but this is in fact consistent with the results of experimental studies in animals. On primary inoculation with brain tissue from Kuru-affected human, the incubation period in monkeys is usually 2 years or longer, but on serial passage from monkey to monkey (same species) it has been reduced to under 1 year (Gajdusek, 1976). Experimental oral transmission of BSE has been successful in cattle, sheep, goats, mink, mice and squirrel monkeys. In all these species incubation periods were longer than after parenteral challenge, despite the use of much larger doses (WHO, 1996). Epidemiological data therefore suggest that the incubation period of nvCJD (or bovine CJD) may vary from a minimum of 10 years to a maximum of 30 years or more (oral route).

PREDICTED EPIDEMIC OF nvCJD

The newly recognized variant form of Creutzfeldt-Jakob disease (nvCJD) has been identified in ten young people in the UK in March 1996 (Will *et al*, 1996), approximately 10 years after the beginning of the bovine spongiform encephalopathy (BSE) epidemic in the UK. The further detection of four confirmed cases (and an unspecified number of suspected cases), within a period of a few months, can be considered as epidemiological evidence of a causal link between BSE and the new variant CJD and is also consistent with the beginning of an epidemic.

Such an epidemic will reflect the complex interplay of many factors. The chief actors being the infected cattle, the susceptible human population and the BSE transmissible agent.

The infected cattle

The BSE epidemic which started in April 1985 (earliest clinical cases) with 161,412 confirmed cases by June 1996, has obviously been preceeded by a progressive increase of a BSE infective reservoir of cattle incubating the disease (average incubation period: 4-6 years). Animals in their last year of incubation being apparently the most infective. The period 1984-1989 was therefore the most criti-

cal because potentially highly infective offal were freely available prior to the specified bovine offal (SBO) ban of 13 November 1989 in England and Wales. Anderson *et al* (1996) estimated that approximately 446,000 (440,000-580,000) infected animals entered the human food chain before the specified bovine offal ban at the end of 1989 with approximately 283,000 more before the end of 1995. In 1995, unannounced visits to slaughterhouses revealed that, at least in a few instances, pieces of spinal cord, ranging in size from 1 cm to one third of the spinal cord, had been left attached to carcasses after dressing. These potentially serious failings may have resulted in CNS material entering the human food chain long after the 1989 SBO ban (MAFF, 1995).

Among the estimated 446,000 BSE-infected cows which entered the human food chain before 1990, it can be assumed that 100,000 were infective (older cows in latter stage of incubation). At least 50,000 kg of highly infective brain may therefore have entered the human food chain and contaminated some 25 millions hamburgers before 1990, 2 g being the average dose of brain-pool homogenate per beefburger. Another 50,000 kg of highly infective spinal cord may have contaminated some 2.5 millions vol-au-vent (bouchée à la reine) and/or some other foodstuff.

The susceptible human population

The UK genetically susceptible population may represent approximately 38% to 49% of the total population, an estimated 38% of the Caucasian population being homozygous for PrP methionine while 11% are homozygous for PrP valine (Collinge *et al*, 1996a). All of the nvCJD cases reported so far are homozygous for methionine at codon 129 of the PrP gene (bovine PrP also has a methionine at this position). In pituitary hormone-related iatrogenic cases both valine and methionine homozygotes are over represented. The remaining 51%, who are heterozygous for the polymorphism at codon 129, are expected to be at lower risk for acquired prion diseases.

The transmissible agent

BSE has been transmitted to cattle, sheep, goats, pigs, mink, mice and squirrel monkeys by parenteral inoculation. Experimental oral transmission has been attempted in all these species; it has been successful except in the case of pigs (WHO, 1996). Doses as low as 0.5 g of BSE-infected cow brain are sufficient to cause infection in sheep. At least one primate, the squirrel monkey, is susceptible to both Kuru and BSE via the oral route.

The most characteristic neuropathological feature of this new variant CJD is the presence of large numbers of Kuru-type amyloid plaques in the cerebral cortex. Intracerebral inoculation of three macaques with brain extracts from cattle suffering from BSE has produced a brain disease with identical Kuru-type plaques whereas no plaques were seen in two macaques inoculated with sporadic human CJD (Lasmezas *et al*, 1996). In other words, BSE may produce a distinct neuropathological "signature" in humans and in some non-human primates such as the cynomolgus macaque whose PrP has a high degree of homology (96.4%) with that or man.

Last but not least, nvCJD is associated with a unique and highly consistent appearance of protease-resistant PrP on Western blots involving a characteristic pattern of glycosylation (Collinge *et al*, 1996b). This glycoform "signature" which clearly differenciates nvCJD from sporadic and iatrogenic CJD, is seen in BSE itself, in experimental murine BSE (whereas CJD transmission to these types of mice produces a CJD "signature") and in naturally transmitted BSE in the domestic cat and experimental BSE in macaques. Thus, new variant CJD has strain characteristics distinct from other types of CJD, which closely resemble those of BSE transmitted to mice, domestic cats and macaques.

These neuropathological and physicochemical " signatures" are additional evidence of a causal link between BSE and the new variant CJD. It appears therefore evident that the BSE agent is responsible for the emergence of the "new variant" CJD. However the exact degree of transmissibility of the BSE agent from ruminants to humans remains unknown. To be effective the oral route probably requires high-infectivity titers which, in contrast to scrapie in sheep, are encountered only in the brain, retina and spinal cord of naturally infected cows (in experiments involving orally challenged cattle, the distal ileum consistently carry infectivity during the incubation period from 6-18 months after the oral challenge).

The epidemic

The nvCJD epidemic began in February 1994 (Date of the earliest clinical onset in one of the ten first confirmed cases). Presently (end 1996) there are 14 confirmed cases, 7 cases with onset in 1994 and 7 cases with onset in 1995. These figures are obviously provisional because there is a very long delay (16 months average; longest: 30 months) between the clinical onset and the post-mortem confirmation of diagnosis. The final number with onset in 1994/95 might be about 20. The already infected human reservoir (contaminated during the critical period 1984-1989, prior to the November 1989 offal ban) will continue to reveal its presence by the progressive emergence of an increasing number of symptomatic cases. As a result of the very long incubation period of the disease (10 to 30 years or more) the epidemic will have the shape of a normal frequency distribution curve (Gaussian type epidemic) with a peak expected in 2009. The epidemic may extend at least until 2025 or 2030 (Fig 1).

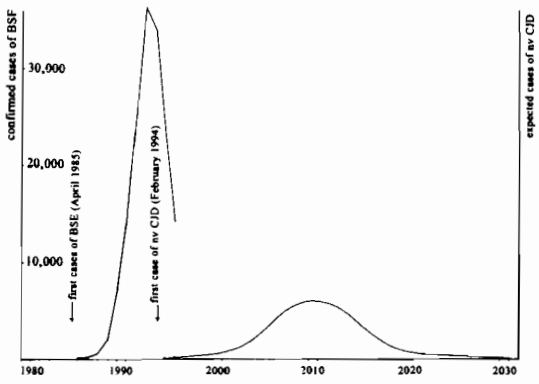

Fig 1–BSE epidemic and predicted epidemic curve of nv CJD in the UK.

The severity of this epidemic may be estimated within 2 or 3 years, depending on the more or less rapid increase of cases. However it looks as though the total number of cases, over the whole course of the epidemic, will be in the thousands rather than in the hundreds. If, for example, there are 25 or more cases in 1996, with a doubling in each of the following years (50 in 1997, 100 in 1998, etc) this would be compatible with a severe epidemic with 150,000 confirmed cases by 2009 (peak of the epidemic) and a total of 300,000 cases by 2025 or 2030.

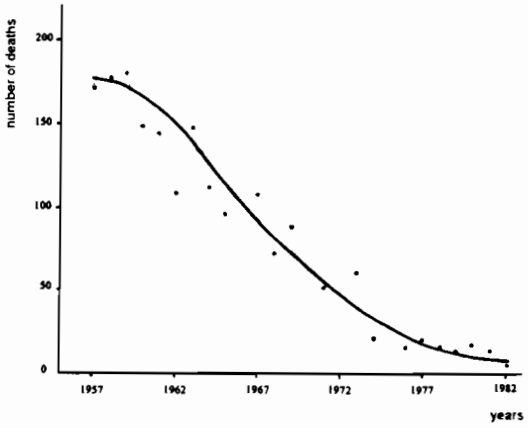

Fig 2–Death from Kuru in female patients of the Fore group (adapted from Gajdusek).

The shape of the predicted curve is not simply hypothetical and, in fact, there is already an excellent example to illustrate such a curve in its descending line: the decline of incidence of Kuru deaths in female patients of the Fore group following the interdiction of ritual cannibalism in the highlands of Papua New Guinea (Fig 2). The discovery of Kuru in 1956 coincided with the height of the epidemic. It occured in 160 villages with a total population just over 35,000. Since its discovery more than 2,600 patients have died of Kuru.

Origin of Kuru

According to Gajdusek a spontaneous sporadic case of CJD may have given rise to the chain of Kuru, thus explaining its origin. However, close similarities of Kuru with nvCJD may rather suggest a possible animal origin (as in nvCJD). Thus, it might be profitable, in view of its epidemiological importance, to carry out the molecular analysis of Kuru protease-resistant PrP, including its appearance on Western blots and its pattern of glycosylation, and to compare the results with those of sporadic CJD and new variant CJD.

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