

Human prion diseases: epidemiology and integrated risk assessment

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Human prion diseases are devastating and incurable, but are very rare. Fears that the bovine spongiform encephalopathy epizootic would lead to a large epidemic of its presumed human counterpart, variant Creutzfeldt-Jakob disease (vCJD), have not been realised. Yet a feeling of uncertainty prevails in the general public and in the biomedical world. The lack of data on the prevalence of asymptomatic carriers of vCJD compounds this uncertainty. In addition to this problem, Switzerland is currently faced with another issue of major public concern: a recent rise in the incidence of CJD. Here we examine the plausibility of several scenarios that may account for the increase in CJD incidence, including ascertainment bias due to improved reporting of CJD, iatrogenic transmission, and transmission of a prion zoonosis. In addition, we present the design and current status of a Swiss population-wide study of subclinical vCJD prevalence.

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Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of neurodegenerative disorders affecting many species, including sheep, cattle, deer, and human beings.^{1,2} All these diseases have an aetiological trait in common: the only identified component of the infectious agent is PrP^{Sc}, a modified form of a normal cellular protein termed PrP^C.^{3–5} The most common human TSE is Creutzfeldt-Jakob disease (CJD), of which there are four types: sporadic, familial, iatrogenic, and variant (s, f, i, and vCJD respectively). sCJD is very rare, and seems to be evenly distributed worldwide; in all countries that carry out surveillance, incidences of about 0.4–1.8 cases per 1 million people per year are reported.⁶ The aetiology of sCJD is unknown: no exogenous or endogenous causes have been identified. Familial forms are transmitted as autosomal dominant traits, and cosegregate with mutations in the gene that encodes the prion protein, *PRNP*.⁷ Instead, iatrogenic cases are attributed to neurosurgical intervention, transplantation of tissues, or administration of hormones derived from dead people who had undiagnosed TSEs.

Biochemical and histopathological evidence suggests that vCJD represents transmission of bovine spongiform encephalopathy (BSE) prions to human beings.^{8–10} The incidence of vCJD in the UK has been rising each year from 1996 to 2001,¹¹ which has evoked fears of a future, large epidemic. Since the year 2001, however, the incidence of vCJD in the UK seems to be stabilising and may actually be falling. It might be too early to draw any far-reaching conclusions, but as each month passes without a substantial rise in the number

of cases, hope increases that perhaps the total number of people who develop vCJD will not grow much more.¹²

Incidence of CJD in Switzerland

In Switzerland, CJD has been a statutory notifiable disease since December 1987. A national reference centre for prion diseases, NRPE (Nationales Referenzzentrum für Prionen-Erkrankungen), was established in 1995 at the Institute of Neuropathology of the University Hospital of Zurich. NRPE conducts CJD surveillance, including clinical assessment of patients, biopsies, autopsies, as well as biochemical and genetic analyses on cases in which a human prion disease is suspected.¹³ Between 1996 and 2000, the number of patients diagnosed with CJD ranged from eight to 11 yearly, corresponding to an incidence of 1.3–1.4 people per million per year (figure 1). However, in 2001 there were 19 “definite” or “probable” cases and one “possible” case of CJD.¹⁴ The numbers for 2002 (19 “definite” or “probable”) seem to suggest that CJD incidence in Switzerland may be stabilising at a high level, the yearly incidence for 2001 and 2002 being 2.6 people per million per year (figure 1). Preliminary data for January–August 2003 indicate that the incidence is not decreasing (<http://www.eurocjd.ed.ac.uk>).

The definite diagnosis of CJD can only be made by analysis of CNS tissue, before or after death. A potential problem is represented by “probable CJD” cases, which are diagnosed solely on the basis of clinical symptoms, and for which no histopathological or biochemical confirmation is available. Such “probable CJD” cases significantly contribute to mortality statistics in those countries that diagnose CJD based on surrogate markers such as high concentrations of protein 14-3-3 in CSF.¹⁵ In the Swiss collective, diagnosis was confirmed in frozen and paraffin embedded brain tissue in 95% of cases in 2001 and 2002.

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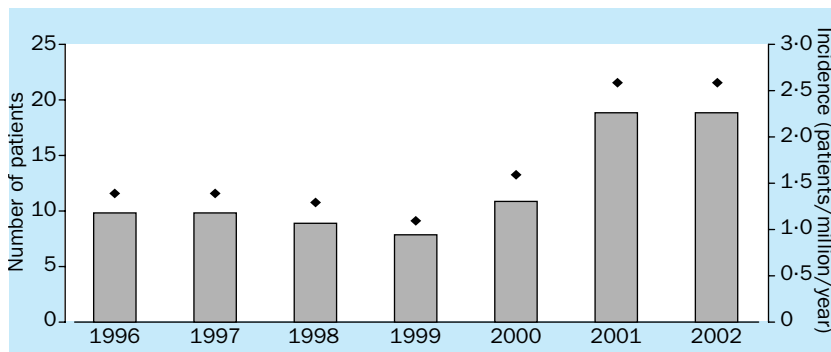


Figure 1. The epidemiology of CJD in Switzerland since 1996. Number of newly diagnosed cases of definite and probable CJD (bars) and incidence per million people per year (diamonds) in Switzerland from 1996 to 2002. There is a sharp rise in CJD incidence in Switzerland in 2001 and 2002.

1 year after our first report of this phenomenon,¹⁴ we still lack any obvious explanation for the observed increase in CJD incidence in Switzerland. Five scenarios are possible: a mere statistical fluctuation; improved ascertainment of CJD; genetically determined clusters of fCJD; clusters of iatrogenic transmission; and transmission of an as yet unknown prion zoonosis to human beings. As discussed in the final part of this article, investigation of this situation requires a vigorous interdisciplinary effort by neuropathologists, molecular biologists, epidemiologists, pathologists, and—maybe somewhat surprisingly—otorhinolaryngologists.

Statistical fluctuations and ascertainment bias

Could the surge in the number of people with CJD in Switzerland represent a simple statistical fluctuation? This possibility represents the best-case scenario, and there is no method that would allow formal exclusion. However, statistical analysis at the end of 2001 indicated that the observed rise in incidence of CJD was unlikely to occur stochastically ($p=0.007$, assuming a Poisson distribution). The stabilisation of the high incidence since 2002 helps rule out the random-fluctuation hypothesis as its sole cause.

Alternatively, the rise may be due to an ascertainment bias over previous years (eg, because of increased awareness of prion diseases among health professionals and within the general population), which may lead to more frequent detection of CJD in patients who would have otherwise been misdiagnosed. Several arguments support this possibility, including the fact that the rise in referrals is loosely associated with the introduction of the 14-3-3 test as a screening tool for CJD. Similar effects have been observed in other countries.¹⁶ Some European countries have experienced an increase in the median age of patients with CJD, which is believed to reflect the heightened intensity of CJD surveillance and better recognition of CJD in elderly demented patients who otherwise might have been diagnosed with more prevalent forms of dementia.¹⁷ Moreover, CJD can imitate dementing illnesses that are prevalent in residents of nursing homes. Therefore, any increase in diagnostic sophistication is likely to produce a rise in CJD diagnoses in this group of patients. Yet, none of

the patients with confirmed CJD in 2001 and 2002 were in nursing homes when the diagnosis was first suspected.

As a counter argument, we found that the current average age of Swiss patients with CJD does not differ from that of previous years, when CJD incidence was similar to that of other countries. This observation is one argument against the possibility that ascertainment bias can explain the recent rise in incidence.

Finally, the awareness-bias hypothesis would postulate that the Swiss surveillance system uncovers the “true” average incidence of sCJD, of

around 2.6 per million per year or more. If that were true, all countries but Switzerland would underreport an excess of 50%. Although this poor detection in other countries is possible, postulation that the Swiss system is so much better than others at recognising patients with CJD is somewhat presumptuous. In both Switzerland and Austria, for example, CJD surveillance is funded by generous governmental grants and is done by a network of experienced neuropathologists. However, there is no CJD surge in Austria (<http://www.eurocjd.ed.ac.uk>), despite the fact that the size and age structure of the population, the quality of the healthcare system, and even the resources per million inhabitants allocated to CJD surveillance are not obviously different between Switzerland and Austria (Dr Jesús de Pedro Cuesta, personal communication). Finally, owing to the Rokitansky legislation in Austria, which requires that every person who dies in a hospital has an autopsy, the general autopsy rate is even higher than that of Switzerland. The entirety of these observations suggests that the increase in Swiss CJD incidence may not be due to an allegedly superior sensitivity of the Swiss surveillance system.

Genetics

One or several foci of genetically determined TSEs could contribute to the rise in incidence of CJD in Switzerland. Small presumed CJD epidemics in the past (eg, among Libyan Jewish people), have eventually been traced to inherited pathogenetic mutations of the *PRNP* gene.¹⁸ To date all fCJD cases have been shown to cosegregate with *PRNP* mutations; however, this mutation was detected in only one of the Swiss patients of 2001 and 2002, which is in agreement with reports of pathogenetic *PRNP* mutations in 10–15% of all CJD cases.

However, prion genetics is a rapidly developing subject and genetic susceptibility markers and modifiers are not necessarily limited to polymorphisms in the *PRNP* locus. What these additional modifiers could be is unclear; controversy has recently arisen about the possible protective effect of MHC allelotypes in vCJD. A study claimed that a certain HLA type may serve as protective factor for the development of vCJD,¹⁹ but a follow-up study reached diametrically opposite conclusions.²⁰ Yet whether the MHC

allele in question represents a risk modifier or not, there can be little doubt that endogenous genetic modifiers do indeed modulate the risk of prion infection after exposure to the agent. A large percentage of the European population may have been exposed to BSE infectious agents, and animal experiments indicate that the infectious dose for oral cross-species transmission of BSE is low (about 500 mg of brain tissue represent one infectious dose in sheep).²¹ Moreover, because only about 140 human beings have contracted vCJD, susceptibility is almost certainly controlled by endogenous or exogenous factors in addition to the mere size of the inoculum.²² Because certain genetic profiles, other than the known pathogenetic mutations in *PRNP*, predispose for the development of TSEs, further genetic analysis of the Swiss patients with CJD may be justified.

Refinements in the technologies available for detection of PrP^{Sc} have prompted us to study the distribution of the disease-associated prion protein in extracerebral organs of the Swiss patients with CJD. These studies have found that extraneuronal PrP^{Sc} is detectable much more commonly than previously thought: about a third of patients investigated displayed PrP^{Sc} in their skeletal muscle, and another third (partly overlapping) had PrP^{Sc} in lymphoid organs.²³ At present, whether these findings are universally valid for patients with CJD or whether they are specific to the Swiss patients with CJD, is unknown. If the findings are unique to Switzerland, the abnormal peripheral pathogenesis of CJD in Swiss patients might point to a specific aetiology.

Iatrogenic and zoonotic transmission

There is a fourth hypothesis, that a significant proportion of the cases is caused by some form of iatrogenic transmission. This hypothesis cannot be dismissed: indeed there have been studies suggesting that a proportion of allegedly sporadic CJD cases may actually be iatrogenic.²⁴ According to the official notification forms, none of the patients were exposed to any of the known iatrogenic risk factors. However, the sensitivity of this method is unsatisfactory. Therefore we have initiated an in-depth retrospective survey, which encompasses a review of each patient's clinical records as well as structured interviews of relatives with questionnaires developed by the National CJD Surveillance Unit in Edinburgh (www.cjd.ed.ac.uk). Over 200 individual variables are being assessed. The results of this survey might

shed light on possible risk factors that were shared by subsets of Swiss patients who have died of CJD.

The publication of the data of the shift in Swiss CJD epidemiology triggered, particularly in the popular press, far-reaching speculations that Swiss CJD may be related to a prion epizootic, such as BSE, scrapie, or chronic wasting disease. In fact, Switzerland has reported the largest number of BSE cases among continental European countries between 1991 and 1999 (415 cases of BSE since 1990).²⁵ The first diagnosed case of BSE, as well as the peak of the epidemic, hit Switzerland four years after the UK (figure 2).^{26,27}

The Swiss population may have been exposed to BSE-tainted bovine materials before the ban of specific bovine risk material from the human food chain, which was implemented in late 1990, after the first case of BSE was recognised. vCJD was first reported in the UK in 1996. Assuming that the incubation period of vCJD is similar in the UK and in Switzerland, one might have expected cases of vCJD to appear in Switzerland around 2000. However, BSE is thought to cause vCJD rather than sCJD,⁸⁻¹⁰ and all clinical, genetic, histopathological, and biochemical data of the recent CJD cases clearly indicate that no Swiss case fulfils the

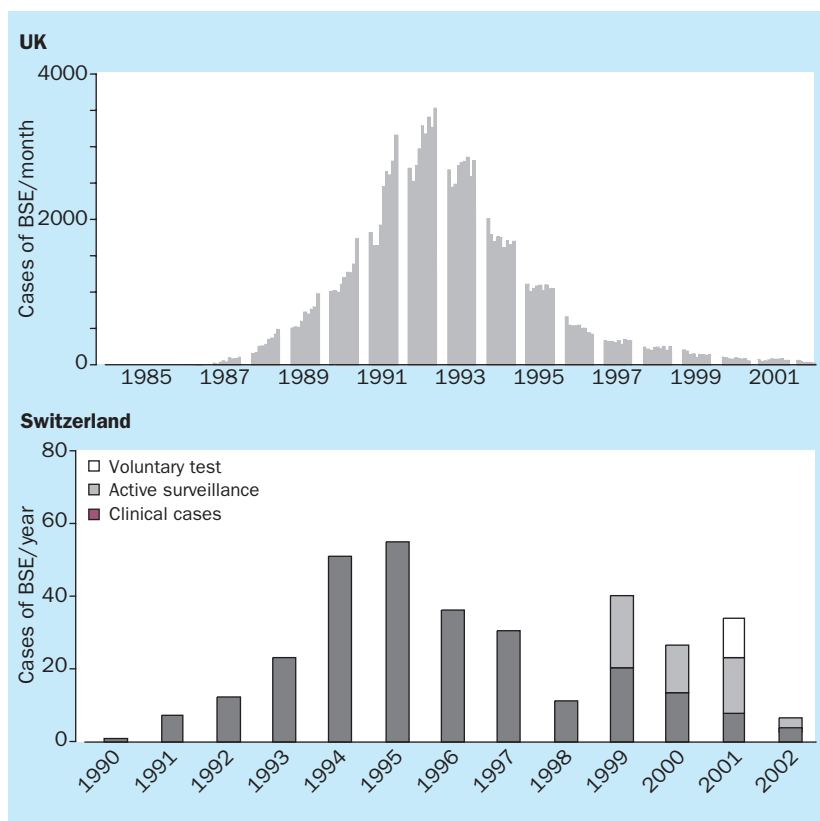


Figure 2. Comparison between the incidences of BSE in the United Kingdom²⁶ and in Switzerland²⁷ since the disease was first diagnosed (1986 in the UK and 1990 in Switzerland). BSE has been eradicated in neither country, but the incidence has been steadily decreasing since its peaks in 1992 (UK) and 1995 (Switzerland). Even accounting for the smaller size of the bovine and human populations in Switzerland, the extent of the Swiss epizootic has been much smaller than that of BSE in the UK. The apparently trimodal course of the epizootic in Switzerland reflects increased detection of cases owing to the introduction of an active surveillance programme in 1999 and of voluntary testing programmes by most large retailers in 2001.

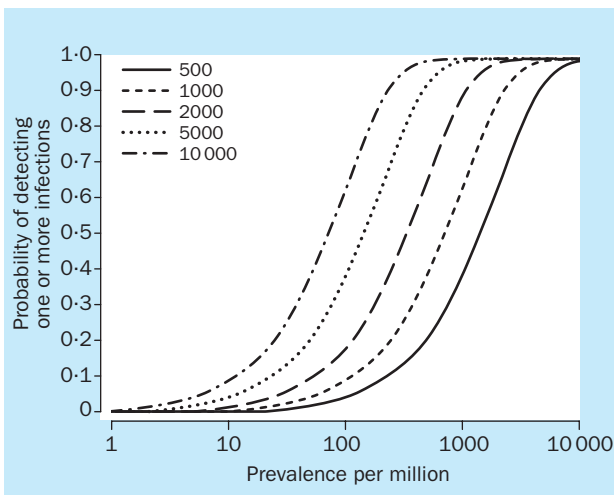


Figure 3. Influence of sample size on the probability of the detection of positive samples as a function of actual vCJD prevalence. The probability to detect a given prevalence of vCJD can only be considered adequate if the sampled pool is above 10 000 (ie, if the vCJD prevalence is about $100/10^6$ inhabitants, the probability of detecting one or more samples containing disease-associated prion protein is about 0.7).

diagnostic criteria of vCJD. Although the diagnostic criteria for vCJD established by the WHO are firm,²⁸ there is little reason to believe that vCJD is the only possible clinical manifestation of human infection with an animal prion disease. Moreover, recent studies in genetically modified mice have raised questions as to whether vCJD cases may be erroneously classified as sCJD on account of their PrP^{Sc} types as assessed by western blotting.²⁹ Finally, BSE may have infected human beings in the UK through routes different from those that may have possibly been at work in Swiss BSE, maybe because slaughtering procedures, eating habits, or the enforcement of regulatory measures differ between the two countries. If so, the disease phenotype in human beings in Switzerland might be different from that identified in the UK. Analogously, hamsters and mice fed or inoculated peripherally with prions accumulate PrP^{Sc} in different sites of the CNS than mice inoculated intracerebrally.^{30,31}

Could the Swiss patients with CJD have developed the disease as a result of BSE transmission after one or more serial passages through species other than cattle (eg, sheep) or through a prion zoonosis other than BSE? At present, there is no evidence for such a possibility. Indeed, regulatory measures aimed to prevent the entry of bovine CNS tissue into human and animal food chains were implemented in Switzerland several years before other continental European countries.³²

In sheep, scrapie cannot be easily distinguished from BSE.³³ Scrapie is very rare in Switzerland (only seven cases were reported in the past 10 years), although cases of scrapie may be underreported. Sheep and lamb is rarely consumed in Switzerland and about 50% of that is Swiss; the remainder is primarily imported from Australia and New Zealand (Dagmar Heim, personal communication). Chronic wasting disease of deer and elk is prevalent in various regions of North America but has never been reported in Europe.

Therefore, chronic wasting disease seems to be an unlikely cause of CJD in Switzerland. As a qualification, it is important to realise that surveillance data on TSEs in European game animals are incomplete at best.

The UK cases of vCJD are likely to be primary transmissions from cattle. However, experimental transmission studies show that TSE strain characteristics can change upon serial passages after the original primary transmission.³⁴ Therefore, horizontal vCJD transmission among human beings could present with a different phenotype than vCJD. Nevertheless, this scenario is unlikely to account for the high CJD incidence in Switzerland, because no vCJD cases have been recorded in Switzerland.

As a result of the surge in CJD incidence, we have initiated a study that characterises current cases of CJD and tests factors that could explain such a development. The study uses classic methods as well as new, up-to-date strain typing tools.

Assessment of subclinical-vCJD prevalence in Switzerland

vCJD has claimed over 130 lives in the past 7 years. Although most cases have been reported in the UK, other European and non-European countries including France, Italy, and Canada have also been hit by vCJD. It is of direct practical relevance whether subclinical vCJD exists in countries with vCJD cases and maybe in BSE-exposed countries that seem to have been spared from vCJD so far. A quantitative answer to that question could be used to reduce the uncertainty about the future size of the epidemic and will put health authorities in a position to assess whether the current measures taken to prevent the horizontal spread of vCJD (ie, through medical interventions) are sufficient.

In vCJD, significant amounts of pathological prion protein can be detected in lymphoid tissue at preclinical stages of the disease; the detection of pathological prion protein in lymphoid tissues of non-demented individuals is therefore a potential marker for vCJD in asymptomatic people.^{35,36} Conventional methods of PrP^{Sc} detection by western blotting and immunohistochemistry do not reveal its presence in lymphoid tissues of patients with sCJD. Although more sensitive methods based on detecting PrP^{Sc} before dementia develops may have positive results also in lymphoid tissue.²³

The Swiss Federal Office of Public health has mandated the NRPE to do a study aimed at quantification of the prevalence of subclinical carriers of vCJD-prions in Switzerland. However, the detection of people that might eventually develop a progressive, lethal dementia with no effective treatment invariably poses several ethical questions. To accommodate these issues, a working group that includes members with expertise in law, ethics, epidemiology, and basic research was set up with the goal to design an appropriate study protocol. For the successful completion of this study, several critical factors need to be respected. In order to use highly sensitive and specific methods, tissues to be tested need to be collected under native (non-fixed) conditions. A statistically sound statement can only be made if the size of the sampled population is large enough for a

reasonable power of analysis: in view of the size of the Swiss population (about 7 million), this requires sampling of at least 10 000 people (figure 3). Finally, ethical issues, such as patients' rights and public information policy, need to be taken into account.

In order to satisfy both ethical and scientific criteria, we decided to do a cross-sectional, "linked anonymised" prevalence study. Here we discuss the details of the study design, with particular focus on its bioethical implications.

All samples to be studied originate from leftovers of lymphoid tissue taken for diagnostic procedures and were obtained from tonsillectomies and autopsies. Therefore, there is no need to remove tissues for the specific purpose of the study, which greatly facilitates sample collection. Nevertheless, patients' rights were analysed in detail and taken to the furthest possible degree of consideration. It was deemed essential that donors understand how the samples will be used, and how this research might affect their interests. Donors may opt out of the study at any time. The patients must sign a consent form detailing these points before the study of samples can proceed. Potential donors are informed that there will be no link between donor data and study results as long as no samples containing pathological prion protein are detected. However, should prion-positive samples occur, the Federal Office of Public Health would inform the public about this fact. Starting from that moment, an officially announced neutral institution would be set up to offer information and counselling to people who donated samples.

An unlinked anonymous prevalence study for vCJD was launched in the UK: the prevalence data generated by that study contain only minimal demographic information (such as breakdown of age into only one of two groups), and no provisions were taken to allow for allocation of test results to individual donors. There is a lot to be said in favour of the UK study design in terms of economy and avoidance of very involved and entangled questions of bioethics. On the other hand, in the light of the vast advances that are being accomplished in prion research,^{37–39} newly developed therapeutic⁴⁰ or

prophylactic^{41–44} options may become available in the future. In such case, it might be unethical not to be able to inform donors who tested positive. Conversely, not to undertake efforts to ensure the safety of the general public in the (admittedly very unlikely) case of a very high prevalence of subclinical vCJD would also be unethical. Therefore, it was decided to maintain a link between the tonsillectomy samples and the patients from whom they were obtained,

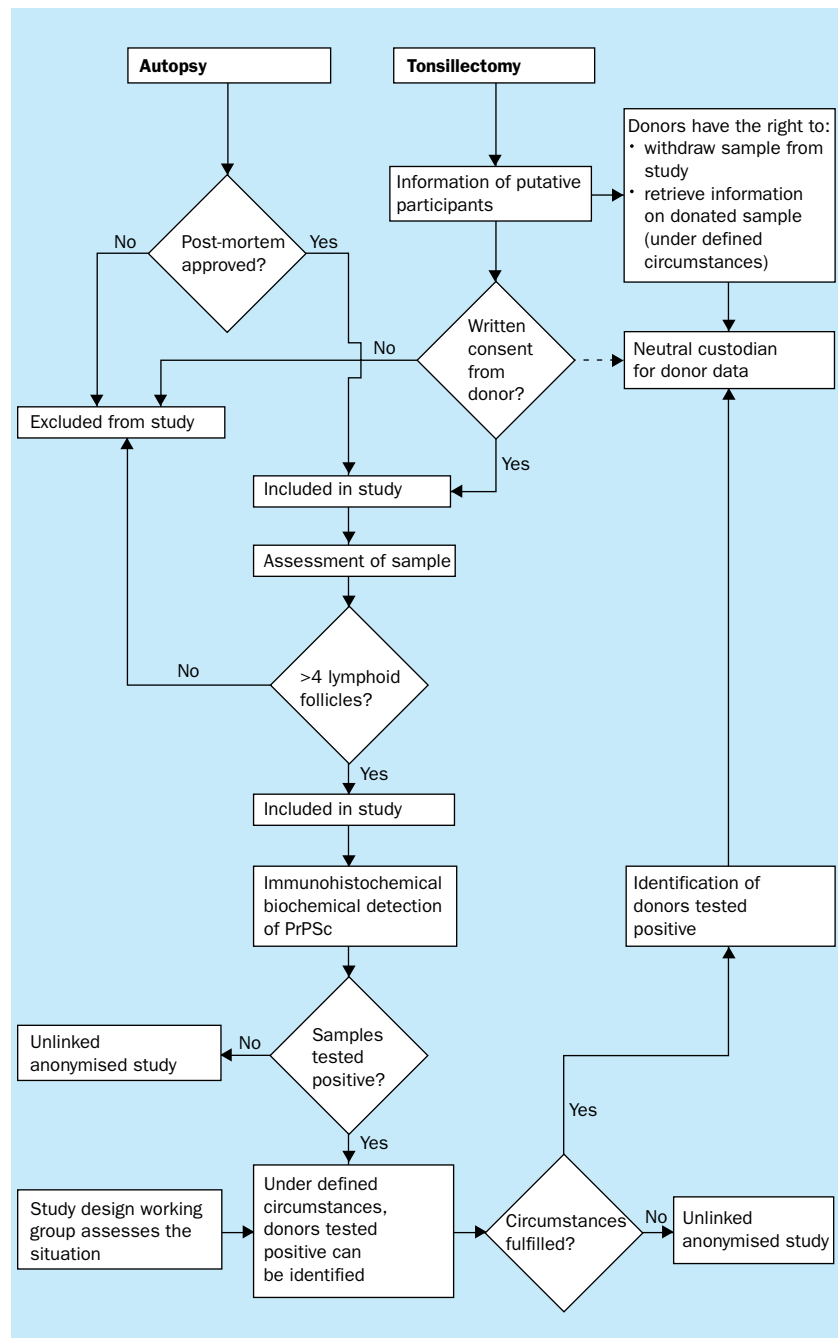


Figure 4. Trial profile of the study of the prevalence of vCJD in the Swiss population. Samples obtained through autopsies and samples obtained from patients having routine tonsillectomies are being assessed. Tonsillectomy samples are only included if consent is given. Withdrawal from the study is possible throughout the entire study period.

Search strategy and selection criteria

Data for this review were identified by searches of Medline with the search terms "prion" and "Creutzfeldt-Jakob" and from the reference lists from relevant articles. Articles published until June 2003 were included. Only papers published in English and German were reviewed.

which will enable future identification of individuals enrolled in the study if needed. The information to establish this link is deposited in the safeguard of a neutral warden: the Swiss Academy of Medical Sciences agreed to fulfil this custodial role.

The study design described above puts the investigators in a unique position. Depending on the study outcome and on the development of therapies that may influence prion diseases, several possible outcomes are conceivable (figure 4). There are two principal outcomes of this study.

The most optimistic scenario predicts that no tissue samples will test positive for PrP^{Sc}. In this case links between donor data and study results will never be established and all donor data will be destroyed at the end of the study. This will allow the study to be thought of retrospectively as "unlinked-anonymised" for all bioethical purposes.

The less favourable scenarios take into account the occurrence of one or more tissue samples that prove to be positive for the presence of PrP^{Sc}. For these situations the study-design work group has agreed upon a set of defined conditions which must be fulfilled before a link between data of tissue donors and study results may be established. Whether links will only be established upon demand from concerned individuals or whether identifications will also take place in order to implement practical measures will depend mostly on the availability of not yet existing therapies and new possibilities to prevent possible secondary transmissions of vCJD. Considering the fast growth of knowledge of prions in general, it has also been agreed that the finding of positive tissue samples will necessitate a reassessment of the situation by the working group.

The Swiss investigation into the possible presence of patients with subclinical vCJD is designed to fulfil all criteria required by the ethical complexity and the political effect of the vCJD problem. From a practical viewpoint, there was concern that full informed consent of patients undergoing acute tonsillar surgery would be difficult to obtain, given the complexity of the topic which is daunting for the patients and even for many physicians. However, nationwide sample collection has been in progress for over 6 months, and preliminary results indicate that the compliance of both physicians and patients is excellent. These preliminary results suggest that concerns a study with this degree of complexity would be unfeasible are unfounded.

Conclusions

In terms of human prion diseases, Switzerland is facing two key problems. First, the incidence of sCJD during the last 2 years is more than twice as high as that of other countries doing CJD surveillance. This problem is being addressed by research into epidemiology and by basic research.

The second key problem, which affects Switzerland and other countries with significant exposure to BSE, is the absolute lack of epidemiological data on subclinical vCJD infection in the population. Human prion diseases have incubation times that are unparalleled by any other infectious disease, and people who have vCJD prions but are clinically unidentifiable might cause further spread of prion infections in human beings. Hence, there is a crucial and urgent need for statistically sound epidemiological data on the prevalence of vCJD. How to assess vCJD prevalence in the face of its unique disease kinetics, while simultaneously safeguarding the rights of study participants, is a formidable challenge that has preoccupied epidemiologists, ethicists, and public health officials, and has paralysed efforts towards this goal in several countries. The Swiss medical community has initiated a study to determine vCJD prevalence that has the potential to satisfy epidemiologists, ethicists, and public health officials. Bioethical issues and patients' rights are becoming more important even in epidemiological studies that do not, at first, seem to have ethically problematic components. With this in mind, the proposed study design may, if proven feasible in practical and economical terms, have some potential for representing a new useful paradigm for future studies of disease epidemiology beyond human prion diseases.

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Authors' contributions

PMO, TL, JG, AG, WW, GH, HM, MP, and BS contributed to the section describing the prevalence study and MG and AA planned and wrote the review.

Conflict of interest

We have no conflict of interest.

Role of the funding source

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