



COMBINING EVIDENCE FROM TISSUE SURVEYS TO ESTIMATE THE PREVALENCE OF SUBCLINICAL vCJD

ISSUE

1. The Department of Health (DH) has asked for advice on how the results from the National Anonymous Tonsil Archive (NATA) can be combined with those from the retrospective survey of appendix tissue by Hilton *et al* (2004)¹, in order to arrive at a reasonable range of scenarios for the prevalence of subclinical vCJD within the population. This is essential if risk management measures are to be based on all available data.
2. To determine whether and how the numerical results from these studies could be combined, it is necessary to consider how they could have arisen. Firstly, advice is therefore sought as to what reasonable assumptions (or ranges of assumptions) can be made about:
 - the relative timing of PrP^{vCJD} accumulation in appendix and tonsil tissue during the vCJD incubation period;
 - the relative sensitivities of the analytical assays used to detect PrP^{vCJD} in appendix and tonsil samples as well as the possible effects on sensitivity of using the particular assays on a large-scale; and
 - the potential effect of prion protein gene codon 129 genotype on the pathogenesis of vCJD and how it may influence the relative accumulation of PrP^{vCJD} in appendix and tonsil tissues.
3. Given the committee's views on these topics, advice is sought on the following further questions:
 - What research could be commissioned to test the suggested assumptions?
 - How can a realistic range of scenarios for the prevalence of vCJD be estimated, given the evidence currently available?

¹ Hilton *et al.* (2004) Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol.* 203, 733-739.

- What further research could significantly improve the estimation of current vCJD prevalence, especially if the planned surveys of post mortem tissues and blood prove to be unfeasible?
- Finally, is it reasonable to assume that all subclinical carriers of vCJD are infective, whether or not they go on to develop clinical disease? What research could be commissioned to test this proposition?

BACKGROUND

4. The prevalence of subclinical vCJD in the UK is highly uncertain. Therefore, analyses of the potential risks of human-to-human transmission of vCJD, and of the effectiveness of measures to reduce them, must incorporate a wide range of estimates for the prevalence of subclinical vCJD. As a result, the outputs of these analyses are imprecise, making public health decisions about managing the risks of health care associated vCJD transmission between humans difficult.
5. Current analyses use estimates of the prevalence of subclinical vCJD based solely on the survey by Hilton *et al.* This study of stored appendix and tonsil tissues removed between 1995 and 1999 found three PrP^{vCJD} positive appendix samples (all from individuals born between 1961 and 1985 - the 1961 to 1985 birth cohort) from analysis of 11 109 appendix samples. No PrP^{vCJD} positive samples were found from analysis of 1 565 tonsil samples. A crude estimate of the prevalence of subclinical vCJD based on these data is 1 subclinical infection per 4 000 of the population in this age cohort. However, the confidence interval is wide (95% CI, 1 in 1 400 to 1 in 20 000), and the prevalence in other age cohorts remains conjectural. The paper by Hilton *et al* is given at Annex 1.
6. A smaller study by Frosh *et al* (2004)² of 2 000 tonsil samples collected between July 2000 and August 2002 found no samples positive for PrP^{vCJD}. About 1 000 samples were from individuals less than nine years of age with the other samples from individuals aged nine years or more at the time of collection.
7. NATA was set up to prospectively collect and analyse around 100 000 tonsils to obtain more precise estimates of the prevalence of subclinical vCJD. Collection of tonsils began in December 2003. SEAC considered the first large tranche of data from NATA at SEAC 99³. No PrP^{vCJD} positive samples were found from analysis of nearly 50 000 samples, including nearly 9 500 samples from the 1961 to 1985 birth cohort (from which Hilton *et al* found three PrP^{vCJD} positive appendix samples).

² Frosh *et al.* (2004) Analysis of 2000 consecutive UK tonsillectomy specimens for disease-related prion protein. *Lancet* 364, 1260-62.

³ SEAC 99 draft minutes paragraphs 49-58. <http://www.seac.gov.uk/minutes/99.pdf>

8. SEAC considered the NATA testing methodology to be robust and noted that it was sufficiently sensitive to detect PrP^{vCJD} at levels between 100 to 1000-fold lower than present in the tonsils of clinical vCJD cases. Whilst the analytical methodology used in NATA is capable of detecting PrP^{vCJD} when present at relatively low concentrations in tonsil tissue, the capability of tonsil testing to identify individuals infected with vCJD is not well understood. SEAC noted that, although no PrP^{vCJD} positive samples have been found to date, the NATA data gathered to September 2007 were statistically consistent with those of Hilton *et al.* However, the continuing consistency or otherwise of the studies will necessarily depend on whether and how results are disaggregated by age.
9. The data from Hilton *et al.* (appendix samples only) and available from NATA is shown in table 1 (Annex 2). If the data from the two tissue surveys are assumed to be compatible, the two surveys remain formally consistent with each other as the 95% confidence intervals overlap for the birth cohorts for which data are available from both studies. The final column of table 1 shows the range of numbers of infected individuals in each age cohort that would be compatible with the data from both surveys. However, should the lack of PrP^{vCJD} positive samples continue the two data sets will at some stage become discrepant.

COMBINING DATA FROM THE APPENDIX AND TONSIL SURVEYS

10. As the Hilton *et al.* and NATA surveys differ in the type of tissue tested, the analytical methods employed, the age range of individuals from which the tissues are collected and the time of collection, a process of combining the data needs to take into account the potential impact of these differences. Thus, combining the data from the appendix and tonsil surveys would include making assumptions about the:
 - relative timing of PrP^{vCJD} accumulation in appendix and tonsil tissue during the vCJD incubation period.
 - relative sensitivities of the analytical assays used to detect PrP^{vCJD} in appendix and tonsil samples.
 - potential effect of genotype on the pathogenesis of vCJD and how it may influence the accumulation of PrP^{vCJD} in appendix and tonsil tissues.
11. Additional considerations would include the:
 - relative calendar timing of tissue removal since this determines how far into the incubation period infections may have progressed and therefore the extent to which PrP^{vCJD} may have accumulated in the tissues of individuals infected as a result of the BSE epidemic.

- birth cohort of the individuals from whom the tissues were removed since this determines the likely exposure, and possibly susceptibility, if this is related to age, to BSE.

PrP^{vCJD} accumulation in appendix and tonsil tissue

12. Interpretation of the data from analyses of appendix and tonsil samples is influenced by assumptions made about the timing of PrP^{vCJD} accumulation in these tissues. If PrP^{vCJD} accumulation in these tissues occurs late in the incubation period, either close to, or during, the clinical stage of the disease, these testing approaches would be insensitive to subclinical vCJD detection. Consequently, they would underestimate the true prevalence of subclinical vCJD. In contrast, should PrP^{vCJD} accumulation in these tissues occur soon after infection, the testing approaches might detect the majority of infections, providing reliable data to estimate the prevalence of subclinical vCJD.
13. If there is a substantial difference in the timing and rate of PrP^{vCJD} accumulation in tonsil and appendix tissue during the vCJD incubation period then the sensitivity of each approach, and the number of subclinical infections that might be identified by each approach, would be different. However, there are no human data which would allow a comparison of the relative timing of involvement of the appendix and tonsil.

Appendix testing

14. Three studies that examined the accumulation of PrP^{vCJD} in appendix tissue from clinical cases of vCJD showed PrP^{vCJD} to be present in 6/6⁴, 0/1⁵ and 1/4⁶ cases. PrP^{vCJD} was not found in the appendix of the case of subclinical vCJD infection associated with blood transfusion of MV genotype.⁷ Thus, it is possible that the tissue accumulation of PrP^{vCJD} may be influenced by prion protein gene codon 129 genotype (although, it is possible that the tissue distribution of PrP^{vCJD} in this case may have been, in part, influenced by the route of transmission). These data suggest that the extent of PrP^{vCJD} accumulation in appendix tissue could be variable.
15. PrP^{vCJD} was detected in appendices removed from two vCJD cases 8 months and 2 years prior to the onset of clinical disease^{1,8}. PrP^{vCJD} was not

⁴ Head *et al.* (2004) Peripheral tissue involvement in sporadic, iatrogenic and variant Creutzfeldt-Jakob disease. *Am. J. Pathol.* 164, 143-153.

⁵ Wadsworth *et al.* (2001) Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet.* 358, 171-180.

⁶ Joiner *et al.* (2002) Irregular presence of abnormal prion protein in appendix in variant Creutzfeldt-Jakob disease. *J. Neurol. Neurosurg. Psychiatry.* 73, 597-598.

⁷ Peden *et al.* (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet.* 364, 527-529.

⁸ Hilton *et al.* (1998) Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. *Lancet.* 352, 703-704.

detected in an appendix removed from another vCJD case, 10 years prior to the onset of clinical disease. However in this case the patient may have not yet been infected or else have been in the early stages of infection. In addition, PrP^{vCJD} was not found in the appendix of the case of subclinical vCJD associated with blood transfusion⁹. Three PrP^{vCJD} positive appendix samples were found by Hilton *et al* in the survey of stored appendectomy samples. No clinical cases of vCJD have yet been linked indirectly to these anonymous samples. Therefore, it would appear that PrP^{vCJD} accumulates in appendix tissue some time before the onset, if ever, of clinical disease. However, a more precise timing for PrP^{vCJD} accumulation in appendix tissue during the vCJD incubation period has not been established.

Tonsil testing

16. PrP^{vCJD} is consistently detected in the tonsil tissue of clinical vCJD cases^{10,11,12,13,14}, including a case presumed to have been infected via blood transfusion¹⁵. However, PrP^{vCJD} was not found in the tonsils of the case of subclinical vCJD associated with blood transfusion⁷. There are no data on PrP^{vCJD} accumulation in tonsil tissue of other individuals asymptomatic for vCJD infection. Thus, the timing of PrP^{vCJD} accumulation in human tonsil tissue during the vCJD incubation is unknown.
17. Data from published experimental studies in animals that examined PrP^{TSE} accumulation in tonsil tissue during the preclinical stage of the incubation period of TSEs following oral inoculation are summarised in table 2 (Annex 3). Additional unpublished data provided in confidence about PrP^{vCJD} accumulation in inguinal lymph nodes of cynomolgus macaques and PrP^{BSE} accumulation in lingual tonsils of mice is given in table 3 (Annex 4). Although the data are limited, these studies clearly show that PrP^{TSE} accumulates in tonsil tissue during the subclinical stage of the incubation period of BSE in sheep, mice and lemurs, of classical scrapie in sheep and of CWD in deer. Although the precise timing of PrP^{TSE} accumulation cannot be derived from these studies, it appears from these data that

⁹ Peden *et al.* (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet.* 364, 527-529.

¹⁰ Hill *et al.* (1999) Investigation of variant Creutzfeldt-Jakob disease and other human prion disease with tonsil biopsy samples. *Lancet.* 353, 183-189.

¹¹ Bruce *et al.* (2001) Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. *Lancet.* 358, 208-209.

¹² Wadsworth *et al.* (2001) Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet.* 358, 171-180.

¹³ Head *et al.* (2004) Peripheral tissue involvement in sporadic, iatrogenic and variant Creutzfeldt-Jakob disease. *Am. J. Pathol.* 164, 143-153.

¹⁴ Ritchie *et al.* (2004) Advances in the detection of prion protein in peripheral tissues of variant Creutzfeldt-Jakob disease patients using paraffin-embedded tissue blotting. *Neuropathol. Appl. Neurobiol.* 30, 360-368.

¹⁵ Wroe *et al.* (2006) Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. *Lancet.* 368, 2061-2067.

PrP^{TSE} accumulation may not be a late event in the incubation period of these TSEs in these species.

18. The pathogenesis of BSE and classical scrapie in sheep and BSE in non-human primates is generally considered to be a reasonable model for the pathogenesis of vCJD in humans. Thus, it may be reasonable to assume that PrP^{vCJD} also accumulates in tonsils some time prior to the onset of clinical vCJD in humans.

Analytical sensitivity of appendix and tonsil assays

19. The relative analytical sensitivities of the dual enzyme-linked immunoassay (EIA) method used to screen NATA samples and the immunohistochemical (IHC) method used by Hilton *et al* to screen appendix samples have not been established. A study is underway to reanalyse 10 000 NATA samples (all from the 1961 to 1985 birth cohort) by the IHC method used by Hilton *et al*. It is anticipated that these analyses will be completed by the end of 2008.
20. The dual EIA has been demonstrated to be capable of detecting PrP^{vCJD} in tonsil tissue at a 100-1000 fold dilution of that present at the clinical stage of disease. As IHC cannot be applied to a dilution series the sensitivity of the dual EIA and IHC cannot be compared directly. Given the performance of the dual EIA, a reasonable simple working hypothesis may be that the dual EIA method is at least as sensitive as the IHC method. The positive control tonsil samples from clinical vCJD cases provided by NCJDSU that were used to make up dilution series were PrP^{vCJD} positive when tested by IHC by NCJDSU. It is not known whether IHC analysis was conducted on the tonsils supplied by the MRC-PU. An overview of the sensitivity of the EIAs used with animal and human TSE samples provided by the Health Protection Agency (HPA) is at Annex 5.

Potential influence of genotype on PrP^{vCJD} accumulation

21. Two out of the three PrP^{vCJD} positive appendix samples identified by Hilton *et al* were from individuals of the VV genotype¹⁶. As around 10% of population carry this genotype, this finding may indicate that PrP^{vCJD} may accumulate earlier in the incubation period, and/or faster, in the appendix of infected individuals of the VV genotype compared with the MM or MV genotypes.

OTHER TISSUE SURVEYS

¹⁶ Ironside *et al*. (2006) Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study. *BMJ*. 332, 1186-1188.

22. The SEAC Epidemiology Subgroup considered what tissue surveys might, in addition to NATA, lead to better ascertainment of the prevalence of subclinical vCJD. It recommended that a post mortem tissue archive of tissues collected at autopsy and testing blood samples using prototype blood tests be considered and evaluated¹⁷. SEAC endorsed this recommendation¹⁸.
23. The HPA and DH are working to establish an archive of post mortem tissue that would aim to collect 100 000 spleen samples and a smaller number of brain samples from autopsies carried out under the jurisdiction of Coroners. The Coroners Advisory Group of the Ministry of Justice has indicated that proposals for using Coroners officers to obtain consent from the relatives of the deceased to collect and test samples of these tissues for the presence of PrP^{vCJD} are unacceptable to Coroners. The Coroners Advisory Group has suggested a number of alternative mechanisms for obtaining consent, the feasibility of which are currently being explored by DH and the HPA. Should any of these approaches be deemed feasible, the approach most likely to succeed will be tested in a pilot study.
24. HPA, the National Institute for Biological Standards and Control, and NHS Blood and Transplant also continue to investigate the feasibility of using prototype blood assays to test blood samples anonymously. The performance of several prototype blood tests has been evaluated by applying the tests to blinded panels of blood samples. While, as yet, none of these tests have been shown to perform with sufficient sensitivity to be used in a survey of blood samples, the results of using two of the most promising assays on a blinded panel of 'implicated' animal blood specimens are imminent. However it is anticipated that a test of sufficient sensitivity and specificity for a prevalence study will not become available in the short term.
25. DH and HPA are also assessing the possibility of commissioning further surveys. The rationale for this work is the subject of a separate paper prepared by DH (SEAC 100/6), also provided to the Committee. Surveys being considered include:
 - A study of 30,000 archived appendix samples by IHC. The aim would be to collect and test 20 000 samples from the 1961 to 1985 birth cohort and 10 000 samples from the 1941 to 1960 birth cohort. Depending upon the findings, this could provide some assurance about the findings of Hilton *et al*, and in testing a greater number of samples from the 1941 to 60 cohort than Hilton *et al* it should provide a clearer indication of the potential number of infections in that age cohort. In addition, should

¹⁷ SEAC Epidemiology Subgroup (2006) Position statement on the vCJD epidemic. <http://www.seac.gov.uk/statements/state260106subgroup.htm>

¹⁸ SEAC (2006) Response to the SEAC Epidemiology Subgroup position statement on the vCJD epidemic. <http://www.seac.gov.uk/statements/state260106.htm>

PrP^{vCJD} positive samples be found, the study could allow an assessment of whether the genotype distribution of the PrP^{vCJD} positive appendix samples found by Ironside *et al* is a real or chance finding.

- The testing of additional NATA samples by IHC. (The HPA has already commissioned 10,000 NATA samples to be tested by IHC).
- A prospective study of fresh tissue, possibly appendices, or other tissues that are known to accumulate PrP^{vCJD} (see Annex 6) and that may be retrievable.

CARRIER STATE

26. Back-calculation from clinical vCJD cases suggests a much lower prevalence of vCJD in the population than implied by the data from Hilton *et al* (2004). One explanation is that many of those infected enter a carrier state for periods so long that clinical disease does not develop within a normal lifetime. For example, Clarke and Ghani (2005)¹⁹ consider a scenario in which clinical vCJD is confined to just 10% of infected individuals of MM genotype.
27. There is some evidence from experimental studies in mice that suggest primary infections may remain at a subclinical level²⁰ but experimental transmission from these subclinical primary infections can result in clinical disease^{21,22}. There are no data to suggest that such human subclinical carriers of vCJD are of lesser risk of passing on infection to others than infected individuals who will at some point develop clinical disease. Thus, it may be reasonable to assume that all those infected will be infectious.

ADVICE SOUGHT FROM SEAC

28. SEAC is asked to advise on whether and how the numerical results from these studies could be combined. Firstly, advice is therefore sought as to what reasonable assumptions (or ranges of assumptions) can be made about:
- the relative timing of PrP^{vCJD} accumulation in appendix and tonsil tissue during the vCJD incubation period;
 - the relative sensitivities of the analytical assays used to detect PrP^{vCJD} in appendix and tonsil samples as well as the possible

¹⁹ Clarke & Ghani (2005) Projections of future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider genetic susceptibility. *R. J. Soc. Interface*. 2, 19-31.

²⁰ Bishop *et al.* (2006) Predicting susceptibility and incubation time of human-to-human transmission of vCJD. *Lancet Neurology*. 5, 393-398.

²¹ Hill *et al.* (2000) Species-barrier-independent prion replication in apparently resistant species. *Proc. Natl. Acad. Sci. USA*. 97, 10248-10253.

²² Race *et al.* (2001) Long-term subclinical carrier state precedes scrapie replication and adaptation in a resistant species: analogies to bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. *J Virol*. 75, 10106-10112.

effects on sensitivity of using the particular assays on a large-scale;
and

- the potential effect of prion protein gene codon 129 genotype on the pathogenesis of vCJD and how it may influence the relative accumulation of PrP^{vCJD} in appendix and tonsil tissues.

29. Given the committee's views on these topics, advice is sought on the following further questions:

- What research could be commissioned to test the suggested assumptions?
- How can a realistic range of scenarios for the prevalence of vCJD be estimated, given the evidence currently available?
- What further research could significantly improve the estimation of current vCJD prevalence, especially if the planned surveys of post mortem tissues and blood prove to be unfeasible?
- Finally, is it reasonable to assume that all subclinical carriers of vCJD are infective, whether or not they go on to develop clinical disease? What research could be commissioned to test this proposition?

ANNEX 2

Table 1: Data from the completed survey of appendix samples by Hilton *et al* (2004) and data (as of September 2007) from the ongoing NATA study

Birth cohort	Approx age now	Approx age appendix removed	Hilton <i>et al</i> (2004) appendix data	95% CI for number infected in cohort	Approx age tonsil removed	NATA tonsil data	NATA 95% CI	Common 95% CI
1941-1960	47-66	35-58	0 / 574	0 – 6400	-	-	-	0 - 6400
1961-1985	22-46	10-38	3 / 10278	105 – 855	19-46	0 / 9348	0 - 367	105 - 367
1986-1990	17-21	5-13	0 / 394	0 – 9300	14-21	0 / 6928	0 - 532	0 - 532
1991-1995	12-16	-	-	-	9-16	0 / 7517	0 - 484	0 – 484
1996-2000	7-11	-	-	-	4-11	0 / 12594	0 - 292	0 – 292
2001 +	<7	-	-	-	< 7	8735	0 - 422	0 - 422

Table 2: Summary of animal data on PrP^{TSE} accumulation in the tonsil during the subclinical stage of infection²³

TSE strain (route & dose)	Detection method (analysis time points)	Time post inoculation infectivity / PrP ^{Sc} first detected in tonsil	Time to clinical disease	Reference
Cattle				
BSE (oral, 100g brain)	Cattle bioassay on pooled tissue (6, 10, 18, 22, 26, 32 and 36 months post inoculation)	10 months (but only 1/5 bioassay animals at this time point and 0/5 bioassay animals at subsequent time points)	34-40 months	Wells <i>et al</i> (2005) ²⁴
BSE (oral, 100g brain)	Bovine mouse bioassay (20, 24, 27, 30 and 33 months post inoculation)	20 months (but only 1/6 bioassay animals at this and subsequent time points)	Not given	Espinosa <i>et al</i> (2007) ²⁵
Deer				
CWD (oral, 10g brain)	IHC (10, 42, 53, 77, 78 and 80 days post inoculation)	From 78 days post inoculation (one animal culled at each time point)	Not given	Sigurdson <i>et al</i> (1999) ²⁶
Sheep				
Scrapie (animals born and raised within an infected flock)	IHC (every 3 months from 9 months of age)	9 months of age (6/6 VRQ/VRQ animals)	25 months of age	Schreuder <i>et a.</i> (1998) ²⁷
Scrapie (oral, 5g brain via stomach tube)	IHC (1 and 5 weeks, 5 and 11 months post inoculation)	5 weeks (2/3 VQ/VQ recipients given inoculum from VQ/VQ donor) 11 months (2/3 AQ/VQ recipients given inoculum from	Not given	Heggobo <i>et al</i> (2003) ²⁸

²³ Studies were identified using the PubMed search engine and the following search terms some in combination: CJD, Creutzfeldt-Jakob disease, BSE, bovine spongiform encephalopathy, scrapie, pathogenesis, tonsil, tissue distribution.

²⁴ Wells *et al.* (2005) Pathogenesis of experimental bovine spongiform encephalopathy: preclinical infectivity in tonsil and observations on the distribution of lingual tonsil in slaughtered cattle. *Vet. Rec.* 156, 401-407.

²⁵ Espinosa *et al.* (2007) Progression of prion infectivity in asymptomatic cattle after oral bovine spongiform encephalopathy challenge. *J. Gen. Virol.* 88, 1379-1383.

²⁶ Sigurdson *et al.* (1999) Oral transmission and early lymphoid tropism of chronic wasting disease PrP^{res} in mule deer fawns (*Odocoileus hemionus*). 80, 2757-2764.

²⁷ Schreuder *et al.* (1998) Tonsillar biopsy and PrP^{Sc} detection in the preclinical diagnosis of scrapie. *Vet. Rec.* 142, 564-568.

BSE (oral, 5g brain)	IHC (6, 10, 11-20 months post inoculation)	AQ/VQ donor) 11-13 months (6/9 ARQ/ARQ) 16-20 months (3/9 ARQ/ARQ) All animals PrP ^{Sc} negative for tonsil biopsy at 6 and 10 months.	20-24 months	Thuring <i>et al</i> (2005) ²⁹
BSE (oral, 5g brain)	Mouse bioassay and IHC (4, 10, 16 and 22 months)	16 months (4/4 ARQ/ARQ sheep by bioassay, 2/4 ARQ/ARQ sheep by IHC)	From 22 months	Bellworthy <i>et al</i> (2005) ³⁰
BSE (oral, 5g brain)	IHC (6, 9, 12, 15, 17, 19, 21, and 72 months post inoculation)	From 6 months (one animal culled at each time point)	From 20 months	van Keulen <i>et al</i> (2007) ³¹
<u>Non-human primates</u>				
BSE (oral, 0.5 or 1.0g BSE brain)	IHC (5 months)	5 months (1/2 lemurs). PrP ^{Sc} also found in 9/18 asymptomatic captive lemurs that may have ingested BSE contaminated dietary supplements but the timing of exposure is unknown.	Unknown	Bons <i>et al</i> (1999) ³²

²⁸ Heggebo *et al.* (2003) Detection of PrP^{Sc} in lymphoid tissues of lambs experimentally exposed to the scrapie agent. *J. Comp. Path.* 128, 172-181.

²⁹ Thuring *et al.* (2005) Immunohistochemical distinction between preclinical bovine spongiform encephalopathy and scrapie infection in sheep. *J. Comp. Pathol.* 132, 59-69.

³⁰ Bellworthy *et al.* (2005) Tissue distribution of bovine spongiform encephalopathy infectivity in Romney sheep up to the onset of clinical disease after oral challenge. *Vet. Rec.* 156, 197-202.

³¹ Van Keulen *et al.* (2007) Pathogenesis of bovine spongiform encephalopathy in sheep. *Arch. Virol.*

³² Bons *et al.* (1999) Natural and experimental oral infection of nonhuman primates by bovine spongiform encephalopathy agents. *Proc. Natl. Acad. Sci. USA.* 96, 4046-4051.

Information Provided by the Health Protection Agency on the Sensitivity of the EIAs used by the NATA with animal and human TSE samples

Tonsil tissue from sheep

As part of the NATA study, unfixed palatine tonsil samples from sheep with scrapie and uninfected controls were obtained from the VLA. From these samples, 12% homogenates were made by same protocol as for human tonsils, and these were tested by both EIAs. Thirty-two scrapie samples were tested and all were reactive in both EIAs, and the ten controls negatives were all unreactive. Some of the scrapie samples could be detected down to a 10^{-4} dilution by EIA and to 10^{-3} by Western blotting.

Tonsil tissue from vCJD patients

Aliquots of tonsil tissue taken at autopsy from six patients who died of vCJD were kindly supplied by the National CJD-SU and the MRC-PU. Twelve percent homogenates were prepared and they were tested by both EIAs after dilution from 10^{-1} to 10^{-5} into negative homogenate. Depending on the quality of the tissue, PrP^{CJD} was detectable down to a dilution of 10^{-3} in the Microsens EIA and 10^{-2} in the Bio-Rad EIA. This is equivalent to 12 µg vCJD tonsil tissue in the Idexx EIA and 480 µg in the Bio-Rad. The amount of PrP^{CJD} detected varied from tonsil homogenate to homogenate as judged by the OD values. This variation may be due to biological differences in some cases, but a significant contributory factor was the quality of the tissue. The samples visibly varied in the amount of germinal centre tissue to connective tissue. Some of the samples giving low OD values probably had little germinal centre tissue present. Western blotting of aliquots of the vCJD samples showed that the expected specific band pattern of PrP^{CJD} was detectable. The sensitivities of the WBs and the EIAs appeared comparable.

Conclusions about sensitivities of the EIAs

- i) The Microsens EIA is more sensitive than the Bio-Rad EIA for detection of PrP^{CJD} in lymphatic tissue.
- ii) The two EIAs should be sufficiently sensitive to detect PrP^{CJD} in tonsils from asymptomatic individuals incubating vCJD, in whom levels of PrP^{CJD} might be expected to be $1/10^{\text{th}}$ to $1/1,000^{\text{th}}$ of those in symptomatic patients.

Data on Tissue Infectivity

(Taken from WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies, WHO, 2006).

Data entries are shown as follows:

- + Presence of infectivity or PrP^{TSE}
- Absence of detectable infectivity or PrP^{TSE}
- NT Not tested
- NA Not applicable
- ? controversial results
- () limited or preliminary data.

Table IA: High-infectivity tissues

CNS tissues that attain a high titre of infectivity in the later stages of TSE and certain tissues anatomically associated with the CNS

Tissues	Human TSEs			
	vCJD		Other TSEs	
	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}
Brain	+	+	+	+
Spinal cord	+	+	+	+
Retina	NT	+	+	+
Optic nerve ²	NT	+	NT	+
Spinal ganglia	+	+	NT	+
Trigeminal ganglia	+	+	NT	+
Pituitary gland ³	NT	+	+	+

Dura mater ³	NT	-	+	-
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Table IB: Lower-infectivity tissues

Peripheral tissues that have tested positive for infectivity and/or PrP^{TSE} in at least one form of TSE

Tissues	Human TSEs			
	vCJD		Other TSEs	
	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}
Peripheral nerves	+	+	(-)	+
Enteric plexuses ⁴	NT	+	NT	(-)
Spleen	+	+	+	+
Lymph Nodes	+	+	+	-
Tonsil	+	+	NT	-
Nictitating membrane	NA	NA	NA	NA
Thymus	NT	+	NT	-
Esophagus	NT	-	NT	-
Fore-stomach ⁵	NA	NA	NA	NA
Stomach/abomasum	NT	-	NT	NT
Duodenum	NT	-	NT	NT
Jejunum ⁶	NT	+	NT	-
Ileum ^{6,7}	NT	+	NT	-
Large intestine ⁶	+	+	NT	-
Appendix	-	+	NT	-
Placenta ⁸	NT	-	(+)	-
Lung	NT	-	+	-
Liver	NT	-	+	-

Kidney	NT	-	+	-
Adrenal	NT	+	-	-
Pancreas	NT	-	NT	-
Bone Marrow	-	-	(-)	-
Skeletal Muscle ⁹	NT	+	(-)	+
Tongue ¹⁰	NT	-	NT	-
Blood Vessels	NT	+	NT	+
Nasal Mucosa ¹¹	NT	NT	NT	+
Salivary gland	NT	-	NT	NT
Cornea ¹²	NT	-	+	-
CSF	-	-	+	-
Blood ¹³	+	?	-	?

Table IC: Tissues with no detectable infectivity

Tissues that have been examined for infectivity and/or PrP^{TSE} with negative results

Tissues	Human TSEs			
	vCJD		Other TSEs	
	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}
Testis	NT	-	(-)	-
Prostate/ Epididymis/ Seminal vesicle	NT	-	(-)	-
Semen	NT	-	(-)	-
Ovary	NT	-	NT	-
Uterus (non-gravid)	NT	-	NT	-
Placenta fluids	NT	NT	(-)	NT
Fetus ¹⁴	NT	NT	NT	NT
Embryos ¹⁴	NT	NT	NT	NT

Bone	NT	NT	NT	NT
Heart/pericardium	NT	-	-	-
Tendon	NT	NT	NT	NT
Gingival tissue	NT	-	-	-
Dental pulp	NT	-	NT	-
Trachea	NT	-	NT	-
Skin	NT	-	NT	-
Adipose tissue	NT	-	(-)	-
Thyroid gland	NT	-	(-)	-
Mammary gland/udder	NT	NT	NT	NT
Milk ¹⁵	NT	NT	(-)	NT
Colostrum ¹⁶	NT	NT	(-)	NT
Cord blood ¹⁷	NT	NT	(-)	NT
Saliva	NT	-	-	NT
Sweat	NT	NT	-	NT
Tears	NY	NT	-	NT
Nasal Mucus	NT	-	-	NT
Bile	NT	NT	NT	NT
Urine ^{16, 17}	NT	NT	-	-
Faeces	NT	NT	-	NT

Footnotes

1. Infectivity bioassays of human tissues have been conducted in either primates or mice (or both); bioassays of cattle tissues have been conducted in either cattle or mice (or both); and most bioassays of sheep and/or goat tissues have been conducted only in mice. In regard to sheep and goats, not all results are consistent for both species.
2. In experimental models of TSE, the optic nerve has been shown to be a route of neuroinvasion and contains high titres of infectivity.

3. No experimental data about infectivity in human pituitary gland or dura mater have been reported, but cadaveric dura mater allograft patches, and growth hormone derived from cadaveric pituitaries have transmitted disease to hundreds of people and therefore must be included in the category of high-risk tissues.
4. In cattle, PrP^{TSE} was limited to enteric plexus in the distal ileum.
5. Ruminant fore-stomachs (reticulum, rumen, and omasum) are widely consumed, as is the true stomach (abomasum). The abomasum of cattle (and sometimes sheep) is also a source of rennet.
6. In vCJD, transmission to mice has so far been limited to rectal tissue, and PrP^{TSE} was detected only in gut-associated lymphoid and nervous tissue (mucosa, muscle, and serosa were negative). In goats, PrP^{TSE} was also limited to gut-associated lymphoid and nervous tissue [Andreoletti, unpublished data].
7. In cattle and sheep, only the distal ileum has been bioassayed for infectivity.
8. A single report of transmission of CJD infectivity from human placenta has never been confirmed and is considered improbable.
9. Muscle homogenates have not transmitted disease to primates from humans with sCJD, or to cattle from cattle with BSE. However, intracerebral inoculation of a semitendinosus muscle homogenate (including nervous and lymphatic elements) from a single cow with BSE has transmitted disease to PrP over-expressing transgenic mice at a rate indicative of only trace levels of infectivity. Also, recent published and unpublished studies have reported the presence of PrP^{TSE} in skeletal muscle in experimental rodent models of scrapie and vCJD, in experimental and natural infections of sheep and goats, in sheep orally dosed with BSE [Andreoletti, unpublished data], and in humans with sCJD, iCJD and vCJD. Bioassays to determine whether PrP^{TSE} is associated with transmissibility in these experimental or natural infections are in progress.
10. In cattle, infectivity bioassay was negative, but the presence of PrP^{TSE} in palatine tonsil has raised concern about possible infectivity in lingual tonsillar tissue at the base of the tongue that may not be removed at slaughter.
11. In sCJD, PrP^{TSE} is limited to olfactory mucosa.
12. Because only one or two cases of CJD have been plausibly attributed to corneal transplants among hundreds of thousands of recipients, cornea is categorised as a lower-risk tissue; other anterior chamber tissues (lens, aqueous humor, iris, conjunctiva) have been tested with a negative result both in vCJD and other human TSEs, and there is no epidemiological evidence that they have been associated with

iatrogenic disease transmission.

13. A wealth of data from studies of blood infectivity in experimental animal models of TSE has been extended by recent studies documenting infectivity in the blood of sheep with naturally occurring scrapie, and, from epidemiological observations, three blood-associated vCJD transmissions in humans. Blood has not been shown to transmit disease from patients with any other form of TSE, or from cattle with BSE, including fetal calf blood. However, several laboratories using new, highly sensitive methods to detect PrP^{TSE} claim success in studies of plasma and/or buff y coat in a variety of animal and human TSEs. Because the tests are all in a preliminary stage of development, and do not yet include results on blinded testing of specimens from naturally infected humans or animals, the Consultation felt that it was still too early to evaluate the validity of these tests with sufficient confidence to permit either a negative or positive conclusion.

14. Embryos from BSE-affected cattle have not transmitted disease to mice, but no infectivity measurements have been made with fetal calf tissues other than blood (negative mouse bioassay). Calves born of dams that received embryos from BSE-affected cattle have survived for observations periods of up to seven years, and examination of the brains of both the unaffected dams and their off spring revealed no spongiform encephalopathy or PrP^{TSE}.

15. Evidence that infectivity is not present in milk includes temporo-spatial epidemiologic observations failing to detect maternal transmission; clinical observations of over a hundred calves nursed by infected cows that have not developed BSE; and experimental observations that milk from infected cows has not transmitted disease when administered intracerebrally or orally to mice. Also, PrP^{TSE} has not been detected in milk from cattle incubating BSE following experimental oral challenge.

16. Early reports of transmission of CJD infectivity from human cord blood, colostrum, and urine have never been confirmed and are considered improbable. A recent bioassay in PrP over-expressing transgenic mice of colostrum from a cow with BSE gave a negative result; and PrP^{TSE} has not been detected in colostrum from cattle incubating BSE following experimental oral challenge.

17. IgG short chains mimicking the Western blot behaviour of PrP^{TSE} have been identified in the urine of sporadic, variant, and familial CJD patients.