

## POSITION STATEMENT

### PREVALENCE OF SUBCLINICAL VARIANT CREUTZFELDT-JAKOB DISEASE INFECTIONS

#### Issue

1. The Department of Health (DH) asked SEAC to advise on whether the results from the National Anonymous Tonsil Archive (NATA) are compatible with those from a completed retrospective survey of appendix tissue<sup>1</sup> and whether the two sets of results can be combined to improve estimates of the prevalence of subclinical variant Creutzfeldt-Jakob Disease (vCJD) infections (those linked to exposure to Bovine Spongiform Encephalopathy (BSE)). SEAC also considered what further work could be undertaken to improve understanding of the prevalence of vCJD infections<sup>2</sup>.

#### Current position

2. Findings from the completed retrospective survey of appendix tissue by Hilton *et al* suggest that there may be considerably more people infected with vCJD than would be predicted from the observed number of clinical vCJD cases alone. Thus, there may be a number of infected people who will either develop the disease in due course (commonly referred to as preclinical infections) or will never develop clinical disease (commonly referred to as subclinical carrier infections). Experimental research in animals supports the view that prion disease infections could persist in a subclinical state<sup>3,4,5,6</sup>. Since it is currently not possible to

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

<sup>2</sup> To address the DH request, SEAC considered the following papers: <http://www.seac.gov.uk/papers/paper100-2.pdf> and <http://www.seac.gov.uk/papers/paper100-6.pdf>

<sup>3</sup> Bishop *et al.* (2006) Predicting susceptibility and incubation time of human-to-human transmission of vCJD. *Lancet Neurology.* 5, 393-398.

<sup>4</sup> Hill *et al.* (2000) Species-barrier-independent prion replication in apparently resistant species. *Proc. Natl. Acad. Sci. USA.* 97, 10248-10253.

<sup>5</sup> 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.

<sup>6</sup> Asante *et al.* (2002) BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. *EMBO J.* 21, 6358-6366.

differentiate preclinical and subclinical carrier infections through testing, and since both are important in considering secondary transmission risks, the two will be considered together in this statement and referred to collectively as subclinical infections. For the purposes of infection control on a precautionary basis, it has to be assumed that all those identified as infected, based on the presence of the abnormal prion protein associated with vCJD, will be infectious<sup>7</sup>. We will here denote the abnormal prion associated with vCJD as PrP<sup>Sc</sup>, the term conventionally used to designate the disease-associated prion protein regardless of animal species or prion strain.

3. It is very important to establish the prevalence of subclinical infections:
  - (a) to assess the risk of disease transmission;
  - (b) to establish the need for, and effectiveness of, the very costly measures that have been introduced on a precautionary basis to reduce the potential risk of transmission of infection from infected people to others; and
  - (c) to determine whether further risk reduction measures are necessary.
4. Current estimates of the prevalence of subclinical infections are based solely on the completed survey of appendix tissues (Hilton *et al*). This survey found that three out of 11,109 appendix samples, collected between 1995 and 2000, were positive for PrP<sup>Sc</sup> using an immunohistochemical (IHC) method. The three positive samples were all from individuals born between 1961 and 1985 (the 1961 to 1985 birth cohort). This birth cohort is presumed to be the most vulnerable to infection because a large proportion of the vCJD cases to date have been from this cohort and because exposure to BSE infected animals peaked between 1989 and 1992.
5. An estimate of the prevalence of subclinical infections between the calendar years 1995 to 2000 based on these data is 237 infections *per* million of the whole UK population (approximately 1 in 4,000 of the population). However, there is considerable uncertainty in this estimate (95% confidence interval, 49-692 infections *per* million). As most of the samples were from the 1961 to 1985 birth cohort, these data provide little information on the prevalence of subclinical infections in other birth cohorts.

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<sup>7</sup> Hill *et al.* (2000) Species-barrier-independent prion replication in apparently resistant species. *Proc. Natl. Acad. Sci. USA.* 97, 10248-10253.

6. The Health Protection Agency (HPA) began NATA in 2003 with the aim of obtaining more precise estimates of the prevalence of subclinical infections by prospectively collecting and analysing around 100,000 pairs of tonsils using a dual enzyme immunoassay (EIA) method, with confirmatory testing by western blot and IHC. By March 2008, no PrP<sup>Sc</sup> positive samples had been found from analysis of nearly 55,000 samples, including about 11,000 samples from the 1961 to 1985 birth cohort. This translates to a prevalence estimate of zero subclinical infections *per* million of the UK population (95% confidence interval, 0-324 infections *per* million) in this birth cohort.
7. Statistical analysis of the data from both the completed appendix survey and NATA show that, because of the wide confidence intervals, the two data sets are not inconsistent with each other. However, should the lack of PrP<sup>Sc</sup>-positive samples from NATA continue, as more samples are collected and analysed, the two data sets will at some stage become discrepant.

### **Appendix and tonsil testing**

8. Extensive evaluation of the analytical tests used in the appendix survey<sup>8</sup> and NATA<sup>9</sup> suggests they are each reliable for the detection of PrP<sup>Sc</sup> in these tissues, albeit dependent on the concentration of PrP<sup>Sc</sup> in these tissues. There is no reason to suspect that the three PrP<sup>Sc</sup>-positive appendix samples identified by IHC in the completed appendix survey are false positive results, or that the dual EIA is failing to identify tonsil samples with detectable levels of PrP<sup>Sc</sup>.
9. It is known that human prion diseases have long incubation periods, sometimes running for decades. The pathogenesis of vCJD and the accumulation of PrP<sup>Sc</sup> in specific tissues during the incubation period are not well understood. It is not known at what stage of the incubation period PrP<sup>Sc</sup> accumulates to detectable levels in either appendix or tonsil tissue prior to the onset of clinical disease, although it was possible to detect PrP<sup>Sc</sup> in appendix tissue removed from two patients who later developed clinical disease<sup>10</sup>. Given that the collection of tonsils for NATA is occurring later than the collection of appendix samples by Hilton *et al*, it is conceivable

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<sup>8</sup> Hilton *et al.* (2004) Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. *J. Clin. Pathol.* 57, 300-302.

<sup>9</sup> SEAC 99 Minutes, paragraphs 49-54. <http://www.seac.gov.uk/minutes/99.pdf>

<sup>10</sup> Hilton *et al.* (1998) Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. *Lancet.* 352, 703-704.

that tonsils are being collected from infected individuals further into the incubation period than is the case for those individuals whose appendices were tested by Hilton *et al.* Research in animals provides some indication of the timing of PrP<sup>Sc</sup> accumulation in lymphoid tissues of the upper and lower gastrointestinal tract, but these data cannot be extrapolated readily, and with confidence, to the human situation. Therefore, the capacity of either tonsil or appendix testing to identify people with subclinical infections may never be well understood.

10. Furthermore, whilst detection of PrP<sup>Sc</sup> is considered to be an indication of infection, samples testing positive for PrP<sup>Sc</sup> must be inoculated into an appropriate animal model in order to confirm the presence of a transmissible infection. Mouse bioassays are therefore underway using material from two of the three PrP<sup>Sc</sup>-positive samples from the appendix survey. However, only very small quantities of material suitable for bioassay can be obtained from these samples, and negative results in these assays will not in themselves be a reliable indication of the absence of infectivity<sup>11</sup>.
11. In addition, it is possible that genetic differences between people may alter the pathogenesis of vCJD such that the timing and rate of PrP<sup>Sc</sup> accumulation in appendix and tonsil tissues may differ between individuals. Indeed, genetic differences may even determine the extent of lymphoreticular pathogenesis. This might be one possible explanation for the fact that two out of the three PrP<sup>Sc</sup> positive appendix samples identified by Hilton *et al* were from individuals of the VV genotype<sup>12</sup>.
12. It is also possible that infection arising from exposure to BSE could cause more than one type of prion disease<sup>13,14</sup>. Strains other than that which results in vCJD, if they exist, may have markedly different pathogenesis, tissue distributions and structural forms of the abnormal prion protein. If that is the case they may not be detected readily by the appendix and/or tonsil testing approaches. Therefore, the capability of either appendix or tonsil testing to identify all, and not just a proportion, of BSE-associated infections is uncertain.

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<sup>11</sup> Barron *et al.* (2007) High titres of transmissible spongiform encephalopathy infectivity associated with extremely low levels of PrP<sup>Sc</sup> in vivo. *J. Biol.Chem.* 282, 35878-35886.

<sup>12</sup> 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.

<sup>13</sup> Asante *et al.* (2002) BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. *EMBO J.* 21, 6358-6366.

<sup>14</sup> Asante *et al.* (2006) Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. *Proc. Natl. Acad. Sci. USA.* 103, 10759-10764.

## Combining data from the surveys of appendix and tonsil samples

13. Taken at face value, the NATA data suggest a prevalence of subclinical infections that is appreciably lower than suggested by the data from the completed appendix survey. A range of scenarios could be derived to provide indicative estimates of the prevalence of subclinical infection by combining data from both surveys and making biologically plausible assumptions about the relative sensitivity of the two approaches to detect subclinical infections. Of course, any combination of the two datasets as they currently stand would produce lower estimates than the one based on the appendix survey alone.
14. Given the biological uncertainties about the timing and rate of accumulation of PrP<sup>Sc</sup> in human tonsil and appendix tissue, SEAC does not consider that the data from the appendix survey and NATA can be combined to give a single credible estimate of prevalence of subclinical infections for the purposes of risk management considerations. Therefore, it would be prudent to consider that the estimate based on the appendix survey alone provides a reasonable, pragmatic and precautionary working scenario for the prevalence of subclinical infections. The appendix survey provides evidence for the existence of subclinical infections that there is no reason to disregard, although the various uncertainties indicate that this could be an underestimate, or even an overestimate, of the true prevalence of subclinical infection in the population as a whole.

## Further work

### *Tonsil testing*

15. There is uncertainty about how reliably tonsil testing can detect subclinical infections due to the biological uncertainties described earlier. Nevertheless, SEAC considers that it would be reasonable to continue NATA. This is because the available animal data suggest that accumulation of abnormal prion protein in the tonsil during the subclinical stage of infection is a common, although not uniform, feature of prion disease pathogenesis. It is possible that PrP<sup>Sc</sup>-positive tonsils may yet be found and this would be highly informative.
16. SEAC considers that it would be justified to consider stopping the collection and testing of tonsils from the post-1996 birth cohort. People in this birth cohort would be expected to have had little or

no dietary exposure to BSE. Furthermore, precautionary measures have been taken to protect this birth cohort from blood transfusion associated transmission and the frequency of surgical and invasive dental procedures in this birth cohort is relatively low. Thus, the *a priori* expectation is that the prevalence in this birth cohort is low, and the vCJD case data are consistent with this expectation.

#### *Appendix testing*

17. An initial assessment of the number of available archived appendix samples by the HPA suggests that a further appendix survey could be conducted that would appreciably extend the sample size of the previous survey. Provided appendix samples were collected from the appropriate birth cohorts, in particular the 1941 to 1960 and 1961 to 1985 birth cohorts, and the same analytical methodology as in Hilton *et al* was used to test the samples, the data from a new survey could be combined with the older survey. This would enable the current estimate for the prevalence of subclinical infections derived from appendix studies to be refined, although uncertainties around the reliability of appendix testing to detect subclinical infections would remain. In addition, should PrP<sup>Sc</sup> positive samples be found, a further study could allow an assessment of whether the genotype distribution of the three PrP<sup>Sc</sup> positive appendix samples, as described earlier, is a real or chance finding.

#### *Blood testing*

18. The performance of prototype blood tests, at their current stage of development, suggests that they are not yet sufficiently sensitive and specific for the purposes of an infection prevalence study. And the determination of their specificity is problematic. Furthermore, involvement of blood in the pathogenesis of infection is not well understood so there would be uncertainties about the capability of blood testing to identify subclinical infections, similar to those described above for appendix and tonsil testing. Therefore, testing of blood samples does not currently appear to provide a viable approach to prevalence assessment.

#### *Post mortem tissue archive*

19. As SEAC has previously concluded, the most effective approach to establish the prevalence of subclinical infections is the testing of spleen and, when possible, brain samples collected from Coroners' autopsies. Coroners' autopsies provide the only source of a

sufficiently large number of spleen and brain samples that could be collected within a reasonable time from appropriate birth cohorts. More is known about the involvement of the spleen and brain in the pathogenesis of prion diseases. Therefore, the most reliable estimates of the prevalence of subclinical infection could be derived using this testing approach. Furthermore, as all the prion diseases identified to date involve neuropathological changes, testing brain samples could provide some indication about the possible existence of other strains of BSE-associated infection in humans, if they exist.

20. SEAC strongly urges, as it has done in the past, that the appropriate authorities pursue every avenue to establish such a post mortem tissue archive in order to obtain improved estimates of the prevalence of subclinical infections in humans. This, depending on the outcome, could be valuable information in saving human lives and/or in reducing the considerable cost of precautionary measures currently in place.

***SEAC August 2008***