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Limited efficacy of steam sterilization to inactivate vCJD infectivity

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SUMMARY

Background: The transmission of bovine spongiform encephalopathy (BSE) to humans as variant Creutzfeldt–Jakob Disease (vCJD) raised concerns about potential secondary transmissions due to the resistance of the agents causing transmissible spongiform encephalopathies (TSEs), sometimes known as prions, to commonly used methods of sterilization, notably steam sterilization (or autoclaving). It has been suggested that surgical instruments and other medical devices might retain sufficient infected tissue debris after cleaning and steam sterilization to infect patients on whom they are subsequently used.

Aim: To determine whether concerns about the lack of efficacy of steam sterilization of vCJD were justified.

Methods: The reduction in infectivity of brain macerates of vCJD brain after steam sterilization using the standard temperatures and time recommended for autoclaving in UK hospitals (134–137 °C for 3 min) was measured.

Findings: Reductions in titre of $10^{2.3}$ to $>10^{3.6}$ ID_{50} were found. In three of four samples, infectivity was recovered after steam sterilization.

Conclusion: As noted previously, TSE strains derived from BSE sources appear to be more resistant to steam sterilization and other forms of heat inactivation than other TSE sources.

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Introduction

The transmissible spongiform encephalopathies (TSEs), also known as prions, are renowned for their resistance to heat inactivation and other forms of sterilization that reliably inactivate conventional micro-organisms. The agent causing bovine spongiform encephalopathy (BSE) has shown greater resistance to steam sterilization than other TSE sources. Indeed, the survival of some BSE infectivity during rendering is thought to have been a significant factor in establishing the BSE epizootic.[1] Experiments with mouse-passaged BSE (301V) have shown that it is more resistant to steam sterilization than other TSE sources.[2,3] TSE infectivity can be present in many tissues in the preclinical phase of these diseases.[4] With the discovery of variant Creutzfeldt–Jakob disease (vCJD) in 1996,[5] and its highly probable origin from BSE,[6] concerns were heightened that surgical instruments and other medical devices might remain contaminated with vCJD infectivity after cleaning and steam sterilization. These concerns extend to decontamination methods used more widely in healthcare environments and other situations where TSE infectivity may be present.

The earliest experiments on the effect of heat on TSE infectivity were conducted by Wilson (unpublished 1954, referred to by Stamp *et al.*[7]) using a serially passaged sheep scrapie brain pool and assayed in sheep. Wilson showed that scrapie infectivity survived boiling but not steam sterilization.

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These results were bolstered by Pattison and Millson who showed that goat-passaged scrapie survived boiling for 3 h but not steam sterilization. Two decades later, the effectiveness of steam sterilization began to be investigated more thoroughly.[8] Steam sterilization of a 10% suspension of 263K-infected hamster brain homogenate at 121 °C for 1 h reduced infectivity from $10^{10.1}$ to $10^{2.6}$ median infective dose (ID_{50})/g.[9] There was some survival of infectivity of the mouse-passaged TSE strain 139A after steam sterilization of brain macerates at 126 °C for up to 30 min, but none was detected ($<10^{0.5}$ ID_{50}/0.03 g brain) after longer exposure at 126 °C. In contrast, the mouse-passaged 22A strain survived at higher titres for up to 120 min.[10] The mouse-passaged K. Fu. isolate of CJD was totally inactivated at 115 °C and 130 °C.[11] Heating the homogenate of the 263K/hamster model to 121 °C led to a reduction in infectivity of >6 logs after only 1 min.[12] The 263K/hamster model was steam sterilized at 121 °C for 90 min with a reduction in titre from 9.4 to 3.4 logs (i.e. 6 log reduction), but with some survival.[13] Steam sterilization of guinea-pig-passaged sporadic CJD at 121 °C or 132 °C for 15 min resulted in a reduction in titre of ≤4 log ID_{50}, but a reduction of $\geq 10^5$ ID_{50} was measured after 1 h of exposure. The 263K strain in hamsters showed a reduction in titre of $\geq 10^{8.3}$ ID_{50} after 121 °C for 1 h and $\geq 10^{8.8}$ ID_{50} after 132 °C for 1 h.[14] Overall, there was some evidence that a substantial reduction in titre could be achieved under optimal conditions, although some infectivity survived in some instances.

One source of potential variability in the above experiments was the selection of TSE model. The heat inactivation properties of two TSE strains (22C and 22A) were compared. Despite sharing the same origin, these two strains differed in thermostability.[15,16] As mentioned above, the 22A strain was much more resistant to steam sterilization at 126 °C than the 139A strain, with both strains showing little increase in inactivation after exposure for increasing lengths of time.[10] Five mouse-passaged strains using SV or VM mice, which differ in genotype of the host protein PrP that is associated with the TSE agents, were found to vary in the degree of inactivation after steam sterilization at 126 °C for 30 min. Little effect of the PrP genotype was observed.[3,10] Some infectivity was detected after steam sterilization of three strains: 22A, 301V and 263K. Most infectivity was detected in the 301V samples. Although least infectivity was detected in the 22A samples after steam sterilization, the initial titre was lower so the measurable clearance was less. Statistical analysis showed that there was a significant difference in the reduction in titre with respect to the strain of agent.[2] Recent results show that thermostabilities of TSE models differ over a range of 19 °C, with the BSE-derived strain 301V being the most thermostable.[17]

The first steam sterilization experiment with BSE was performed by Taylor *et al.*[18] They showed about a 3 log reduction in the titre of cattle BSE after steam sterilization of brain macerates at 134 °C for 18 min. Much larger reductions in titre were observed for ME7 and 263K, indicating for the first time that, as suspected, the BSE agent was much more thermostable than other TSE sources. In another experiment, BSE-infected cow brain macerates were steam sterilized at 134 °C for 18 min, resulting in a reduction in titre of $10^{2.5}$ ID_{50}. A second steam sterilization cycle did not reduce the titre any further.[2] In contrast, the titre of the 263K hamster-passaged TSE strain was reduced by $10^{3.9}$ ID_{50} in the first cycle and a further $10^{1.2}$ ID_{50} in the second cycle.[19]

In addition to the effects of the strain of TSE agent, the conditions also varied considerably in the experiments cited above. Notably, the presentation of the sample (whether as homogenate, tissue macerate, whole brain, or dried or fixed tissue) and the sterilization protocol (whether using gravity displacement or porous load cycles, or the use of sealed vials in more basic experiments) are believed to affect the efficiency of steam sterilization.[2]

In order to determine whether concerns about the lack of efficacy of steam sterilization of vCJD were justified, an experiment was performed to determine the degree of inactivation of vCJD following steam sterilization using standard temperatures and time. This study re-examined the UK recommendation for sterilization of surgical instruments by exposure of vCJD brain macerates to 134–137 °C for 3 min in a porous load steam sterilizer. The results were compared with those for BSE and a mouse-passaged BSE source. Undiluted brain macerates were used in order to simulate small lumps of tissue debris that may remain on medical devices. Previous experience has shown that more TSE infectivity tends to survive in macerates than other forms of preparation; therefore, macerates also represent the greatest challenge for steam sterilization.

Methods

Samples from the brains of two patients who died of vCJD were kindly provided by the National Creutzfeldt–Jakob Disease Surveillance Unit, Edinburgh, UK. Brain from a cow which was culled with BSE was kindly provided by the Veterinary Laboratories Agency. Mouse brain from the VM/Dk strain of mouse, infected with the 301V strain of mouse-passaged BSE agent,[20] was provided from laboratory resources.

Brain macerates (50 mg) were placed on the side of glass Griffiths tubes in small lumps with the avoidance of any smearing.[2] The tubes were covered and immediately frozen at −20 °C to limit the degree of dehydration prior to sterilization. The samples were thawed immediately prior to steam sterilization in a British steam sterilizer (British Sterilizer Company, Ilford, UK, Model Sdufa.vac.wj.vp) at 134 °C or 137 °C for 3 min, using a porous load programme. The holding time of 3 min was determined by reference to the thermocouple in the machine vent (i.e. the active chamber discharge) to a tolerance of ±0.2 °C. Temperatures were monitored independently by Scottish Healthcare Supplies using six thermocouples, with some placed in equivalent 50-mg samples of control brain tissue throughout the sterilization chamber. Specifically, the first thermocouple (T1) was placed in the chamber vent, T2 was placed in fresh tissue in an open Petri dish, T3 was placed in fresh tissue in a closed Petri dish, T4 was placed in free space within a closed Petri dish, T5 was placed in free space in an open Petri dish, and T6 was placed in free space in the chamber. Data were recorded using an Anville Series 500 logger (Anville Instruments Ltd, Farnham, Surrey, UK). TSE infectivity was assayed quantitatively in mice using a standard protocol. After steam sterilization of the samples, the macerates which had been hardened by the treatment were released from the side of the tube with minimal tissue remaining, homogenized in nine volumes of physiological saline in the Griffiths tubes, and subjected to 10-fold serial dilution. Selected dilutions were injected into groups of 18 RIII mice for BSE- and vCJD-infected samples, and VM/Dk mice for

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