Persistence of ovine scrapie infectivity in a farm environment following cleaning and decontamination

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Scrapie of sheep/goats and chronic wasting disease of deer/elk are contagious prion diseases where environmental reservoirs are directly implicated in the transmission of disease. In this study, the effectiveness of recommended scrapie farm decontamination regimens was evaluated by a sheep bioassay using buildings naturally contaminated with scrapie. Pens within a farm building were treated with either 20,000 parts per million free chorine solution for one hour or were treated with the same but were followed by painting and full re-galvanisation or replacement of metalwork within the pen. Scrapie susceptible lambs of the PRNP genotype VRQ/VRQ were reared within these pens and their scrapie status was monitored by recto-anal mucosa-associated lymphoid tissue. All animals became infected over an 18-month period, even in the pen that had been subject to the most stringent decontamination process. These data suggest that recommended current guidelines for the decontamination of farm buildings following outbreaks of scrapie do little to reduce the titre of infectious scrapie material and that environmental recontamination could also be an issue associated with these premises.

Introduction
Transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases for which there are no effective treatments or cure. Examples of TSE infections affecting mammalian species include scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, chronic wasting disease (CWD) in deer and elk, and variant Creutzfeldt-Jakob disease and Kuru in human beings. In each case, the aetiologic agent is proposed to be a conformational isomer (PrPSc) of the host-encoded prion protein (PrPC; Prusiner 1998). During a prolonged preclinical phase of disease progression, host PrPC is converted into PrPSc which accumulates, particularly, in the CNS. The conversion of PrPc to PrPSc confers several changes in the biochemical properties of the protein, such as a decreased solubility in detergents and an increase in resistance to proteases and chemical denaturants.

For scrapie and CWD, infectious prions are shed from animals via multiple routes during both the preclinical and clinical stages of disease. For example, sheep infected with the scrapie prion secrete/excrete prion within faeces (Terry and others 2011), saliva (Maddison and others 2010b, Gough and others 2012), urine (Rubenstein and others 2011) and skin (Thomzig and others 2007). Furthermore, parturient material is known to harbour high levels of scrapie infectivity (Pattison and others 1972), and its presence correlates with an increase in the transmission of scrapie during the lambing season (Touzeau and others 2006). For scrapie and CWD, the dissemination of PrPSc coupled with its high stability leads to environmental reservoirs of infectivity. For example, it is known that premises that have housed scrapie-infected animals remain a potential source of infectivity for many years (Georgsson and others 2006) and the authors have demonstrated that scrapie prions can be detected on a range of surfaces within the farm providing likely sources of prion exposure (Maddison and others 2010a). Here, the authors examine the effectiveness of the recommended decontamination method for farm buildings, the use of 20,000 parts per million (ppm) free chlorine, in sodium hypochlorite solution, for one hour. The authors demonstrate that on premises that are affected with sheep scrapie, pens cannot be effectively decontaminated either using the recommended decontamination treatments or using a much more stringent treatment consisting of a complete replacement/re-galvanisation of all metalwork and a complete painting of the pen. Such observations have important implications for the decontamination and restocking of farms following outbreaks of scrapie, and especially in the case of goats, with their lack of scrapie-resistant genotypes.

Materials and methods
Scrapie-affected farm
This study was conducted on an experimental farm with a high incidence of naturally transmitted scrapie. The Animal Health
and Veterinary Laboratories Agency (AHVLA) at Ripley flock was started in 1998 by the purchase of sheep from flocks with cases of scrapie. In the intervening years this flock has contained up to 350 breeding ewes of a mixture of breeds and genotypes of predominantly VRQ, ARQ, AHR and ARR alleles. The breeding policy was to maintain a wide range of susceptible genotypes within the flock and animals were reared in line with common practice within British lowland sheep flocks (Ryder and others 2004). Lambing had been carried out within barns each spring where ewes and lambs were kept for up to a week before going to pasture. The study was carried out within a barn that since 2001 had housed scrapie-exposed sheep at various stages of scrapie disease progression and been used for lambing, blood sampling and semen collection from animals before this study. During this study, the barn did not house any sheep other than those used within the described bioassay for the duration of the experiment. The distances between the four pens were exactly the same. With the barn being a standard livestock building there was natural air circulation, that is, hit and miss boarding from shoulder height and wind break material at either end of the barn, therefore all pens received the same air flow. The biosecurity to all pens was the same during the experiment. During this time the rest of the farm was maintained as a sheep farm and bedding were transported directly to each pen treatment area for each pen and stored in new dedicated bins, within each decontamination area, but outside the pen housing the sheep. Fresh feed and bedding were brought onto the farm as required from a scrapie-free farm. At delivery, feed and bedding were transported directly to each pen treatment area for each pen and stored in new dedicated bins, within each decontaminated area.

Decontamination methods
A barn was used containing four pens (at the four corners of the barn) each separated from each other by a minimum of 4 m. Each pen measured 4mx6.4 m. The pens (wall, floor, metalwork) and the pen furniture (hay racks and water trough) were all thoroughly decontaminated during the pen treatments. One pen was left untreated except for brushing out gross debris (pen A). Three other pens were initially pressure washed to remove gross debris (pen B). The three other pens were then treated with sodium hypochlorite solution containing 20,000 ppm free chlorine for one hour before washing with water in accordance with best practice for inactivating surface contaminating prions (pen C). One of the hypochlorite-treated pens then had all moveable metalwork replaced or treated by re-galvanisation, the floor, wall (up to a height of 1.35 m) and every item of immovable steel (gate posts) were then painted in a hard wearing floor paint (pen D). Each pen was accessed via a different entrance leading to a different changing area for each pen with dedicated new clothing, boots and equipment for each pen which were used by all personnel entering the pens to tend the animals (Ryder and others 2009). These changing areas were within the pen decontamination areas, but outside the pen housing the sheep. Fresh feed and bedding were brought onto the farm as required from a scrapie-free farm. At delivery, feed and bedding were transported directly to each pen treatment area for each pen and stored in new dedicated bins, within each decontaminated area.

Sheep bioassay
All procedures were carried out in accordance with the Animal (Scientific Procedures) Act (ASPA) 1986, under licences from the UK Government Home Office. The study was reviewed and approved by the AHVLA Ethical Committee. The study was carried out in facilities licensed under ASPA and owned and managed by AHVLA, an Agency of the Department of Food and Rural Affairs (Defra).

Lambs originated from the AHVLA scrapie-free sheep flock, derived from animals originally imported from New Zealand and maintained in the UK under high levels of biosecurity and which never had any instances of classical scrapie. Three-day-old VRQ/VRQ lambs from this flock were introduced under tight biosecurity conditions to the four pens in groups of five, seven days after the decontamination had been completed. Sheep were fed on a diet of store lamb finisher (Atlees) and hay ad libitum. From six months of age recto-anal mucosa-associated lymphoid tissue (RAMALT) was taken every three months and PrPSc detected by immunohistochemistry as previously described (González and others 2005). Animals that were diagnosed as having preclinical scrapie were removed from the pens and put out into pasture. Sheep remained on pasture until the onset of the clinical signs of scrapie.

Results
A sheep bioassay was used to determine the extent to which contaminating scrapie prions remain after decontamination steps have been taken to remove them from a naturally contaminated farm building. Pens were untreated (pen A), power washed (pen B), power washed followed by treatment with 20,000 ppm free chlorine for one hour (pen C) or power washed, hypochlorite treated and then all surfaces either replaced, re-galvanised or painted (pen D). A bioassay was then carried out by introducing scrapie susceptible lambs into the decontaminated pens and monitoring them by RAMALT testing every three months from six months of age (Fig 1). There was very little difference in the rate at which animals became infected when comparing the untreated pen (pen A) with those that had been power washed or hypochlorite treated. Remarkably, despite the pen D decontamination regimen consisting of a complete replacement of every surface and replacement of re-galvanisation of all metalwork, all five sheep were also scrapie positive by 18 months of age. These data clearly show that despite an exceptionally stringent pen cleaning regimen, there is still enough prion agent within the pen for the infection of sheep. The slower kinetics of the sheep becoming both RAMALT positive, and a longer average time until observation of clinical signs in pen D compared with pens B and C are consistent with a lower infectious dose in pen D after the decontamination regimen.

Discussion
Thorough pressure washing of a pen had no effect on the amount of bioavailable scrapie infectivity (pen B). The routine cleaning of prions from within a laboratory, or treatment for a minimum of one hour with 20,000 ppm free chlorine, a method originally based on the use of brain macerates from infected rodents to evaluate the effectiveness of decontamination (Kimberlin and others 1983). Further studies have also investigated the effectiveness of hypochlorite disinfection of metal surfaces to simulate the decontamination of surgical devices within a hospital setting. Such treatments with hypochlorite solution were able to reduce infectivity by 5.5 logs to lower than the sensitivity of the bioassay used (Lemmer and others 2004). Analogous treatment of the pen surfaces did not effectively remove the levels of scrapie infectivity over that of the control pens, indicating that this method of decontamination is not effective within a farm setting. This may be due to the high level of biological matrix that is present upon surfaces within the farm environment, which may reduce the amount of free chlorine available to inactivate any infectious prion. Remarkably 1/5 sheep introduced into pen D had also became scrapie positive within nine months, with all animals in this pen being RAMALT positive by 18 months of age. Pen D was no further away from the control pen (pen A) than any of the other pens within this barn. Localised hot spots of infectivity may be present within scrapie-contaminated environments, but it is unlikely that pen D area had an amount of scrapie contamination that was significantly different than the other areas within this building. Similarly, there were no differences in how the

Footnotes:
1The Animal Health and Veterinary Laboratories agency (AHVLA) now goes by the new name Animal and Plant Health Agency (APHA).

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biosecurity of pen D was maintained, or how this pen was ventilated compared with the other pens. This observation, perhaps, indicates the slower kinetics of disease uptake within this pen and is consistent with a more thorough prion removal and recontamination. These observations may also account for the presence of inadvertent scrapie cases within other studies, where despite stringent biosecurity, control animals have become scrapie positive during challenge studies using barns that also housed scrapie-affected animals (Ryder and others 2009). The bioassay data indicate that the exposure of the sheep to a farm environment after decontamination efforts thought to be effective in removing scrapie is sufficient for the animals to become infected with scrapie. The main exposure routes within this scenario are likely to be via the oral route, during feeding and drinking, and respiratory and conjunctival routes. It has been demonstrated that scrapie infectivity can be efficiently transmitted via the nasal route in sheep (Hamir and others 2008), as is the case for CWD in both murine models and in white-tailed deer (Denkers and others 2010, 2015). Recently, it has also been demonstrated that CWD prions presented as dust when bound to the soil mineral montmorillonite can be infectious via the nasal route (Nichols and others 2013). When considering pens C and D, the actual source of the infectious agent in the pens is not known, it is possible that biologically relevant levels of prion survive on surfaces during the decontamination regimen (pen C). With the use of galvanising and painting (pen D) covering and sealing the surface of the pen, it is possible that scrapie material recontaminated the pens by the movement of infectious prions contained within dusts originating from other parts of the barn that were not decontaminated or from other areas of the farm.

Given that scrapie prions are widespread on the surfaces of affected farms (Maddison and others 2010a), irrespective of the source of the infectious prions in the pens, this study clearly highlights the difficulties that are faced with the effective removal of environmentally associated scrapie infectivity. This is likely to be paralleled in CWD which shows strong similarities to scrapie in terms of both the dissemination of prions into the environment and the facile mode of disease transmission. These data further contribute to the understanding that prion diseases can be highly transmissible between susceptible individuals not just by direct contact but through highly stable environmental reservoirs that are refractory to decontamination.

The presence of these environmentally associated prions in farm buildings make the control of these diseases a considerable challenge, especially in animal species such as goats where there is lack of genetic resistance to scrapie and, therefore, no scope to re-stock farms with animals that are resistant to scrapie.

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FIG 1: Transmission of environmentally acquired scrapie as monitored by recto-anal mucosa-associated lymphoid tissue (RAMAL T). Lambs were housed in four separate pens that had each been treated using four different decontamination regimens. Pens were untreated (pen A), power washed (pen B), power washed followed by treatment with 20,000 ppm free chlorine for one hour (hypochlorite treated; pen C) or power washed, hypochlorite treated and then all surfaces replaced, re-galvanised or painted (pen D). The five sheep in each pen were monitored every three months from six months of age. All sheep in each of the four pens were RAMAL T positive by 18 months of age.

*Mean incubation period for each pen before clinical signs were observed.
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