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A review of cleaning and disinfection guidelines and recommendations following an outbreak of classical scrapie

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ABSTRACT

Classical scrapie is a prion disease of small ruminants, the infectious agent of which has been shown to be extremely persistent in the environment. Cleaning and disinfection (C&D) after a scrapie outbreak is currently recommended by many governments' veterinary advisors and implemented in most farms affected. Yet, the effectiveness of these procedures remains unclear. The aim of this study was to review existing literature and guidelines regarding farm C&D protocols following classical scrapie outbreaks and assess their effectiveness and the challenges that translation of policy and legislative requirements present at a practical level.

A review of the literature was conducted to identify the on-farm C&D protocols used following outbreaks of scrapie, assess those materials with high risk for persistence of the scrapie agent on farms, and review the existing evidence of the effectiveness of recommended C&D protocols. An expert workshop was also organised in Great Britain (GB) to assess: the decision-making process used when implementing C&D protocols on GB farms, the experts' perceptions on the effectiveness of these protocols and changes needed, and their views on potential recommendations for policy and research.

Outputs of the literature review revealed that the current recommended protocol for C&D [1 h treatment with sodium hypochlorite containing 20,000 ppm free chlorine or 2 M sodium hydroxide (NaOH)] is based on laboratory experiments. Only four field farm experiments have been conducted, indicating a lack of data on effectiveness of C&D protocols on farms by the re-occurrence of scrapie infection post re-stocking. Recommendations related to the control of outdoor environment, which are difficult and expensive to implement, vary between countries. The expert workshop concluded that there are no practical, cost-effective C&D alternatives to be considered at this time, with control therefore based on C&D only in combination with additional time

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restrictions on re-stocking and replacement with non-susceptible livestock or more genetically resistant types, where available. Participants agreed that C&D should still be completed on scrapie affected farms, as it is considered to be "good disease practice" and likely to reduce the levels of the prion protein. Participants felt that any additional protocols developed should not be "too prescriptive" (should not be written down in specific policies) because of significant variation in farm types, farm equipment and installations. Under this scenario, control of classical scrapie on farms should be designed with a level of C&D in combination with re-stocking temporal ban and replacement with livestock of limited susceptibility.

1. Introduction

Scrapie is a disease affecting sheep and goats and is part of a group of neurodegenerative disorders termed transmissible spongiform encephalopathies (TSEs). The abnormal form of the prion protein (PrP^{Sc}) is considered a standard biochemical marker due to its characteristic accumulation combined with infectivity (Prusiner, 1982). It became a notifiable disease in the United Kingdom (UK) on January 1st 1993, as required by Council Directive 91/68/EEC. Legal controls in this country are currently in place for farms where classical scrapie animals have been detected. These include (1) the safe disposal of all scrapie positive cases, (2) the monitoring of fallen stock and slaughtered animals for a period of two years, or, alternatively, the entire flock/herd cull with subsequent cleaning and disinfection (C&D), and (3) movement restrictions of animals. For atypical scrapie, C&D may be less relevant as it is unknown whether environmental transmission occurs with this strain which is currently only thought to occur spontaneously in older sheep or goats (Fediaevsky et al., 2010).

In Great Britain (GB), the replacement of susceptible sheep with genetically resistant animals was subsidised, until the National Scrapie Plan (NSP) was terminated in 2012. Currently, the GB government pays for a maximum of 50 animals to be genotyped in case of an outbreak and genotyping is currently still available from the Animal and Plant Health Agency (APHA) and private companies when paid for by the livestock owner.

For animal carcasses, according to European commission regulations (EC) No 1069/2009, entire bodies and all body parts, including hides and skins, of sheep or goats suspected of being infected by a TSE agent (in accordance with Regulation (EC) No 999/2001) or in which the presence of a TSE agent has been officially confirmed, and of those sheep and goats killed in the context of TSE eradication measures, must be disposed of as Category 1 animal by-products. They must be collected by an approved collector with the carcase subsequently requiring disposal by rendering and/or incineration in authorised facilities (Adkin et al., 2014). Disposal methods must involve extreme conditions to inactivate TSEs, including high temperatures (e.g. pressure processing; incineration) and/or extreme pH (e.g. alkaline hydrolysis; lactic acid fermentation) (Adkin et al., 2014). This is due to the highly resistant nature of TSE agents.

For farm premises, the EU regulation lays down rules for the prevention, control and eradication of certain TSEs (Regulation (EC) No 999/2001), but it does not specify a particular decontamination measure or protocol to be used when an outbreak of scrapie is diagnosed. There is only a brief recommendation in Annex VII stating that caprine animals can be introduced "*provided that a cleaning and disinfection of all animal housing on the premises has been carried out following destocking*". A review of the scrapie situation in the European Union (EU) after 10 years of monitoring and control in sheep and goats (EFSA BIOHAZ Panel, 2014) refers to C&D as an 'additional measure' along with grazing restrictions and compulsory enrolment in a national breeding programme or qualification scheme. Within the European Union, only Austria, Denmark, Finland, Germany, Greece, Hungary, Romania, Slovakia, Slovenia, Spain and Sweden carried out disinfection of premises as additional scrapie eradication measure.

In a recent review of the difficulty in disinfecting prions, Acin (Acin, 2015) highlighted the lack of any published official C&D procedure or recommendation defining a method of scrapie decontamination that has been backed by a recognised international organisation. Yet C&D is practiced in many countries and remains a legal requirement in GB following an outbreak of classical scrapie in circumstances where risk assessment deems it to be necessary.

It is therefore important that the purpose and the effectiveness of C&D protocols for scrapie on farms is understood in order to maximize its role in disease control and to facilitate communication and effective implementation of these measures with stakeholders.The aims of this study were, therefore, to (1) review the available recommended guidelines provided by different countries or international institutions for the implementation of C&D following an outbreak of scrapie, (2) review what is known on the effectiveness of C&D, (3) assess GB experts' opinion on the practicalities of the implementation of C&D and its effectiveness and (4) collate knowledge gaps, in order to provide recommendations for the use of C&D in national control programs.

2. Methods

For this study a review of the literature and expert opinion consultation were conducted. The literature review aimed to identify current on-farm C&D protocols used following an outbreak of scrapie; to assess the potential high-risk material associated with scrapie survival on farms; and to explore the existing evidence of the effectiveness of recommended C&D protocols. The expert opinion consultation was conducted to assess what scientific and field delivery experts considered the effectiveness of C&D to be, its implementation and where they thought data/knowledge gaps existed which could enhance this effectiveness. The expert opinion was done to obtain the GB experience, as a case study, for the development and implementation of C&D protocols.

2.1. Literature review approach and scope

The literature review was composed of two parts:

(1) A review of the scientific publications: This was completed using a structured rapid review such as that used previously in Knowledge Sharing to inform decision making for policy (Rajic and Young, 2013). The electronic database, Scopus, was searched for the time period 1978–2020. A search in 'Title, Abstract and key words' was conducted in April 2020, using the separate terms defined as "Scrapie AND Cleaning OR Decontamination".

(2) A review of grey literature (research that is unpublished or has been published in non-commercial form) for recommendations or existing protocols for decontamination of scrapie prions from five selected countries (GB, Australia, USA, Norway and Iceland) and international institutions [World Organisation for Animal Health (OIE) and European Union (EU)], for which protocols were publicly available online. Detailed C&D protocols used in the GB were also obtained through contact with the government veterinary officers that were involved in the implementation of these protocols after a scrapie outbreak. For each of the C&D measures identified, the authors' perception on the level of difficulty (low to high) and cost (low to high) of implementing them was indicated.

2.2. Expert opinion workshop to assess GB experience with C&D controls

An expert workshop was conducted on May 10, 2017, at the government Animal & Plant Health Agency (APHA) in Weybridge (UK). The aim was to assess experts' experience and views about the process of implementation of C&D protocols, the effectiveness of these and the existing associated data and knowledge gaps. Scientific experts (n = 10) and operational experts (n = 3) involved in research and management of scrapie outbreaks, respectively, were invited to participate in the study. All the experts consulted were from the APHA and were given a presentation on the results of the literature review, and an introduction on the use of expert opinion prior to the exercise. Experts were required to provide answers to two questions prior to the workshop. A closed question to measure their opinion on the effectiveness of on-farm C&D protocols (from 1, 'Not effective at all', to 5, 'extremely effective') to (a) eliminate scrapie prions, (b) reduce infection pressure and (c) prevent future scrapie cases; and an open question about research needs regarding C&D used to control scrapie at farm level.

The workshop was divided into three parts, where experts were consulted on:

(1) The "development of the protocols". This section aimed at gaining a general understanding of the decision-making process followed to select the actual C&D protocol used in past scrapie outbreaks in GB.
(2) The "effectiveness of C&D". For this, a brief description about the C&D protocol recommended by APHA was presented. The operational experts were asked to identify the barriers for the design and implementation of these protocols. Afterwards, the participants were asked to provide and agree on qualitative estimations of the (a) probability of ineffective implementation, (b) probability of scrapie prion survival immediately after C&D, (c) probability of survival over time after C&D and (d) probability of exposure of new animals to scrapie prions (2 years' time) for different types of material present on farms. Participants were required to classify the probability qualitatively as: very high, high, medium, low, very low and negligible (OIE, 2004). During the discussion, participants were prompted to explain their reason for providing the different estimates or to challenge them.
(3) Potential recommendations for policy, research and for any modification of existing C&D protocols.

The workshop and the interview were audio recorded and all data were transcribed. In addition, a separate interview with an APHA operational staff member with experience in C&D implementation on a goat scrapie farm in GB was conducted. In this interview, the participant was requested to describe the experience of implementing the C&D and provide insight on the farmers' perception and attitude towards C&D of the farm. Thematic qualitative analysis was then performed to identify emerging themes associated with each of the sections described.

3. Results

3.1. Findings from the literature review

Overall, 95 documents were captured using the search term indicated in methods. Five studies related to experiments conducted under field conditions or with materials from naturally contaminated farms with classical scrapie. All studies, were developed by the same research team (Gough et al., 2015; Hawkins et al., 2015; Konold et al., 2015; Gough et al., 2019; Konold et al., 2020). Four studies used the same experimental farm with a high incidence of naturally transmitted scrapie. In Hawkins et al. (2015) study, pens were treated with either 20, 000 ppm available chlorine solution for 1 h followed by two strategies, painting and full re-galvanization or replacement of metalwork (full protocol used by Gough and Hawkins in the experimental farms are shown in Appendix A and B). Scrapie-free sheep of the most susceptible PrP genotype (VRQ/VRQ) were then introduced and reared within these pens and their scrapie status monitored by examination of recto-anal mucosa-associated lymphoid tissue. All animals became infected over an 18-month period. The authors concluded 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"*.

A further study conducted developed an *in vitro* method for modelling scrapie decontamination on the surface of concrete fomites on farms that housed infected animals (Gough et al., 2017). The authors concluded that *"methods currently recommended for prion decontamination result in inadequate reduction of prion seeding activity within this in vitro assay"* and that *"effective treatment was only achieved using repeat dosing of surfaces with 20,000 ppm available chlorine for 4 h"*. This was tested in a farm environment with the use of four applications of 20 000 ppm free chlorine for one hour to livestock barns and concreted areas, and the subsequent use of a serial protein misfolding cyclic amplification (sPMCA) assay for the detection of the scrapie prion. The results showed that the surfaces within the barn were demonstrably free from prion prior to occupancy of the barn with sheep. However, similar to the previous experiment done by Hawkins et al. (2015), PrP^{Sc} was detected in rectal biopsies from 23 out of 24 VRQ/VRQ sheep at 372–687 days post-movement to the disinfected barn (Gough et al., 2019).

It should be noted that sPMCA is at least as sensitive as bioassay and that the observed discrepancy could be explained as a sampling issue where testing of only certain surfaces by sPMCA could miss persistent prions. However when using bioassay, sheep are free to move in all areas of the farm, not only the areas sampled for sPMCA. In addition the infected animal(s) can proliferate and secrete prions in the premises which might increase the infectious load in the farm and subsequent exposure to prions of these animals. Under farm conditions bioassay may therefore be more sensitive than *in vitro* tests which rely on targeted sampling.

Recently, a study was conducted on a large commercial dairy goat farm that suffered a high level incidence of classical scrapie, which prompted the decision for whole herd culling and C&D. The latter consisted of removal of dirt and washing and disinfection of surfaces with sodium hypochlorite (records on concentration and exposure time were not available); removal of all timber and wood material; and removal of soil and application of lime. The new herd of goats entered the premises 4 month after depopulation. The herd was tested 10 years later for evidence of scrapie prion, and no evidence of reinfection was observed (Konold et al., 2020).

3.1.1. Scrapie persistence and risk materials

It is known that the scrapie agent is very robust, and cannot readily be inactivated by standard microbiological disinfection (EFSA BIOHAZ Panel, 2014). Once shed into the environment TSE agents have been shown to resist degradation over long periods in soil (Genovesi et al., 2007; Seidel et al., 2007; Wiggins, 2009; Smith et al., 2011). Several studies have demonstrated the long-term environmental persistence and the residual infectivity of prions (Palsson, 1979; Brown and Gajdusek, 1991; Miller et al., 2004; Georgsson et al., 2006). The earlier field experiments showed that the scrapie agent can persist for at least 3 years in the environment (Palsson, 1979; Brown and Gajdusek, 1991). A later study, however, showed evidence that the agent may persist in the environment for at least 16 years (Georgsson et al., 2006). Specifically, the TSE agent binds strongly to several minerals in the soil and survives for longer periods and hence can potentially be transmitted to new hosts (Johnson et al., 2006; Davies and Brown, 2009). The TSE agent may enter the soil via infected carcasses, meat products, farm effluent or dust (Gale and Stanfield, 2001). In addition, recently Maddison et al. (2015) reported that biological and biochemical properties of the TSE agent that is desorbed from soil can change considerably across the time.

There is also evidence of environmental persistence on farm equipment such as pens and troughs, in addition to pasture (Maddison et al., 2010a). Indeed, horizontal transmission has been documented to occur by indirect contact with contaminated environment both indoors and outdoors: by contact with a metal gate, metal water trough, metal penning and metal fencing, plastic scratching post and wooden fence post, respectively (Maddison et al., 2010a). Persistence of the agent on dust has also been documented and linked to potential cases of reinfection (Gough et al., 2015).

3.1.2. Review of C&D recommendations and protocols used

There is limited guidance on specific protocols to apply C&D for scrapie prevention at farm level (Acin, 2015). Methods for scrapie decontamination recommended by key agencies for public health have previously been reviewed (Acin, 2015). The author argues that recommended best practice for the decontamination are based exclusively on laboratory experiments (Kimberlin et al., 1983; Fichet et al., 2004; Lemmer et al., 2004; Gao et al., 2006; Solassol et al., 2006), and that these have not been tested under outbreak conditions. Under laboratory conditions, the concentration of the disinfectant and the exposure time can be optimised whereas application on farm will be very variable thereby altering the efficacy of the treatment. For example, immediately after the disinfectant is applied on farm it will start evaporating depending on parameters such as temperature, humidity, wind speed and others. Thus exposure of prions on farm to the optimum concentration of disinfectant for the correct amount of time cannot be guaranteed.

Current treatment methodology for the decontamination consists of 1 h treatment with 20,000 ppm free chlorine or 2 M NaOH, such as the protocol used in Hawkins et al. (2015) study. The 2014 EFSA scientific opinion on scrapie detailed the Icelandic experience regarding disinfection measures taken on scrapie farms. This consisted of deep C&D of stables, sheds, barns and equipment with high pressure washing followed by cleaning with 500 ppm of hypochlorite; drying and a final treatment with 300 ppm of iodophor (EFSA BIOHAZ Panel, 2014; Sigurdarson, 1991). However, the effectiveness of this protocol has not yet been demonstrated, as scrapie-free sheep used for restocking became infected on pastures that had been kept free of sheep for up to 3 years. Table 1 shows the type of actions for C&D of farms recommended and/or enforced by different countries, and the authors' perception on the level of difficulty (low to high) and cost (low to high) of implementing. In addition, a summary of the most recent C&D protocol used by APHA and by Hawkins et al. (2015) is shown in Appendix B.

3.2. Expert opinion workshop

The emerging themes identified from the expert opinion consultations are shown in Figs. 1 & 2 .

3.2.1. Decision on the implementation of scrapie outbreak controls

The culling of scrapie infected herds or flocks is not mandatory in the EU legislation which provides a range of options which may be implemented differently by each member state. In GB the decision to cull is assessed on a farm-by-farm basis and based on a set of criteria (Fig. 1): the number of test positive cases within the flock, species present (sheep or goats), size of the flock, state of repair of farm housing, degree of record keeping (enabling predictions of control efficacy to be completed), and the motivation of the farm management team in taking corrective actions over the short or long term. For example, there have been three infected goats herds in Great Britain (GB) where these criteria have been applied with the result of the whole-herd cull according to Annex of Regulation 999/2001 (European Parliament and Council of the European Union, 2001) with C&D. In such situations, the lack of evidence on the effectiveness of C&D presented a challenge to communicate such decision to the farmer. In any case, it was always implemented with the consent of the farmers.

3.2.2. Process of development of C&D protocols

The GB C&D protocol has been developed based on literature review and prior field expertise. This protocol has also been developed with experience gained through managing other infectious diseases outbreaks, such as Foot and Mouth Disease. A risk-based approach determines the high-risk areas where the protocol could be applied more strictly (this is based on the protocol used by Hawkins and others (2015), see Appendix B section 2 and 5). Such areas are those where there is considered to be a higher risk of prion contamination such as indoor kidding areas and those areas with a high level of contamination of saliva, milk, faeces or other secretions. However, there are limitations in terms of lack of diagnostic capabilities to measure infectious pressure in different areas of the farm and regarding transmissibility of prion from different areas or materials (such as soil). As the eradication of the TSE agent is not possible according to literature evidence, the protocol aims instead at reducing the infectious pressure. The process is mindful of the possibility of the farm restocking with sheep with resistant genotypes and the needs to maintain a 'trust' with the farmer to ensure cooperation.

Development of the C&D protocol experienced a number of limitations, such as lack of information, knowledge and experience in applying C&D protocols in a scrapie infected farm and their effectiveness (Fig. 1). Some protocols were developed and tested in experimental farms, where environmental conditions are easier to control compared to working farms.

3.2.3. Implementation of C&D

The implementation of C&D in GB places a significant responsibility on the farmer, with government officials in charge of overseeing the activities. The biggest challenge for the implementation of C&D was in the identification and recruitment, by the farmer, of staff to conduct the different tasks. Guaranteeing full compliance was reported as an important challenge. One important recurrent theme associated with the implementation of C&D was the distress that this, in combination with an entire herd cull (in the case of goats and sheep, if requested by the owner), causes to some farmers. Classical scrapie may be perceived as a "stigma" and a "huge burden" to farmers. This was believed to be aggravated by the complexity of classical scrapie cases and the rigid principles of the applicable legislation. It was believed that the stress of this entire experience in combination with the very low incidence of scrapie in GB, may prevent farmers reporting suspect cases of scrapie in the future.

There is some uncertainty in applying the protocols at a 'proportionate' level in an infected farm, and determining the limits of such protocols. The lack of legislation and existence of current knowledge gaps to govern these decisions adds further challenges.

3.2.4. Effectiveness of C&D

Table 2 shows the probability estimates associated with the effectiveness of C&D on different materials based on expert opinion consultation.

When discussing the probability of ineffective implementation, it was considered to be very high in wood, soil and areas where bedding or manure were present due to the fact that organic material interferes with chlorine and its effectiveness. The probability of ineffective implementation was low in metals due to extensive literature on surgical instruments (Edgeworth et al., 2011). However, this low probability was perceived to be dependent on the adequate condition of the metal, and concerns were raised regarding less efficient inactivation of prions on metal surfaces under dry conditions (Secker et al., 2011). Medium probability was assigned for concrete because it was believed that "most farmers don't have a uniform concrete floor". However, it was acknowledged that effectiveness of implementation was also dependent on continuous application of the disinfectant (not applying it a single time) and on adequate conservation of the chlorine (as its concentration reduces over time).

Table 1
Actions for C&D of farms recommended by five selected countries, and their estimation of difficulty and cost.

Areas	Action	Difficulty	Cost	Country
Housing	• Houses burned if C&D is not deemed to be satisfactory possible	High	High	Norway
	• Removal of manure and removal and burning of all wooden materials, and other material that have been directly in contact with the sheep (flooring, wall, drinking basin, etc.)	High	High	Norway
	• Repainting at least the bottom 1.5 m of walls (including windows pane)	Low	Low	Norway
	• All surfaces that cannot be perfectly disinfected and have to be sealed with durable paint on metal and concrete. Creosote on wood *(comment: Alternative to burning of wooden buildings if unfeasible)*	Low	Medium	Iceland
	• Replacement of various materials (doors, windows panes)	Medium	Medium	Norway
	• Fitting new concrete floors	Medium	High	Norway
	• Earth surfaces: Removal of organic material and, when practical, the top 1–2 inches of soil to reduce contamination. Bury or till under the removed material; or, compost the removed material in areas not accessed by domestic or wild ruminants until it can be buried or tilled under.	Medium	Medium	USA
	• Non-earth surfaces (e.g. cement, metal, etc.): Remove all organic material. Bury, incinerate, or compost the removed material in areas not accessed by domestic or wild ruminants and then till under, bury, or incinerate. Clean and wash surfaces and remaining items using hot water and detergent	Medium	Medium	USA
	• Washing down of all buildings to remove gross contamination with organic matter to a height of 2 m until visibly clean. Application of detergent as a degreasing agent. Wash down/rinse with clean water	Low	Medium	GB
	• One year before re-stocking: all buildings have to be washed and disinfected. Emptying the buildings, scraping all floors and walls, opening all walls and ducts and all places where insects or mites could be hidden	Medium	Medium	Iceland
	• Spray of building areas using hypochlorite solution or similar after washing and drying.	Low	Low	Iceland
	• After spraying and drying, building area must be sprayed with iodine	Low	Medium	Iceland
	• Sealing of cleaned and disinfected areas for a year	High	High	Iceland
	• Painting the lower part of outside walls	Low	Low	Norway
Outdoors	• Fitting new fences that have been in contact with sheep	Medium	Medium	Norway
	• Areas where animals commonly gathered scraped and soil buried	High	Medium /High	Iceland
	• Ploughing and/or burning of grass or grazing areas *(comment: Alternative to ban on grazing)*	Medium /High	Medium	Norway
	• Spread manure in well-fenced fields but not on places where water runoff is likely *(comment: Risk of leaking to rivers, etc. Better composting or disinfection)*	Low	Low	Iceland
	• Changing of the upper layer of surrounding unpaved roads	High	High	Norway
	• Grazing areas that cannot be satisfactorily decontaminated must be kept free of sheep and goats for a period of five years	High	High	Norway
	• Two to three year period before re-stocking	High	High	Iceland
	• Four inches of gravel on areas where animals commonly gathered after scraping	Medium	High	Iceland
	• Dry lot areas. Remove manure and top 1−2 inches of soil to reduce contamination. Bury, till under, or compost the removed material in areas not accessed by domestic animals or wildlife	High	Medium /High	USA
	• Pasture areas: 1) when practical, till soil under or do not use area to graze susceptible animals. 2) If this is impractical, do not use the pasture until the animal waste has decomposed and the weather has had an opportunity to dilute any infectivity.	High	Medium/ High	USA
	• Declaration of high risk areas (lambing pens) and low risk areas. Grazing and flock management to avoid high risk areas	Medium	Low	Australia
	• Where property security and management are unsatisfactory, controlled flock depopulation should be considered *(comment: Legal power to enforcement this in extreme situations (no cooperation from farmers)*	Medium	High	Australia
Equipment and materials	• Machinery and manure storage washed and disinfected	Low	Low	GB
	• Physical removal of all bedding and manure	Low	Low	GB
	• Tools (hoof clippers, marking tongs, reusable needles, etc.) disposed of	Low	Medium/ High	Iceland
	• Woodwork that cannot be properly disinfected has to be burned or buried (comment: Environmental pollution. Creosote is an alternative)	Low	Low	Iceland
	• Products assessed as being a significant risk should be disposed of by incineration	Low/ Medium	Medium/ High	Australia
	• Hay, sod, manure, etc. not permitted to move from farm to farm	Low	Low	Iceland
	• Cement, wood, metal and other non-earth surfaces, tools, equipment, instruments, feed, hay, bedding and other materials: remove all organic material and compost or incinerate	Medium	Low	USA
	• Valuable items can be sterilised (134−136 °C) for 10 min, steam sterilisation or disinfection (Comment: Unfeasible for farm items)	High	High	Australia
	• Clean and wash all surfaces, tools, equipment and instruments using hot water and detergent. Allow all surfaces, tools, etc. to dry completely before disinfecting and sanitising with approves disinfectants; incineration, autoclave instruments or disinfectants.	Medium /High	Low	USA
Disinfectant	• Application of a hypochlorite disinfectant. Suitable disinfectant approved under general orders at 2% or to provide 20,000 ppm active chlorine for a minimum of 1 h, for equipment overnight treatment is recommended. Rinsing with clean water to prevent material degradation	Low	Low	GB
	• Reapplication of hypochlorite treatment after a minimum of 7 days in areas of heavy contamination (Items including metal hurdles/ feeding troughs in the parlour or drinking troughs, other equipment from the kidding areas etc. where the level of contact with saliva, milk, faeces and other secretions etc. is highly likely to be high)	Low	Low	GB
	• Deep cleaning and disinfection of stables, sheds, barns and equipment with high pressure washing followed by cleaning with 500 ppm of hypochlorite; drying and a final treatment with 300 ppm of iodophor	Low	Low	Iceland / EFSA
	• Sodium hydroxide, or a sodium hypochlorite solution containing 20,000 ppm available chlorine, for more than one hour at 20 °C.	Low	Low	OIE
		Low	Low	USA

(*continued on next page*)

Table 1 (*continued*)

Areas	Action	Difficulty	Cost	Country
	• To clean dry surfaces, application of 2 percent available chlorine solution [equivalent to about 20,000 p/m; available chlorine: 50 ounces or 6 $^{1}/_{4}$ cups of normal strength (5.25 percent) bleach] to enough water (78 ounces or 9 ¾ cups) to give 1 gallon of solution) at room temperature (at least 65 °F) for 1 h • To clean dry surfaces, application of a 1-molar solution of sodium hydroxide (approximately 4-percent solution (5 ounces sodium hydroxide dissolved in l gallon water)) at room temperature (at least 65 °F) for at least 1 h..	Low	Low	USA

Data from countries were obtained from the following sources: (1) Norway (Alvseike et al., 2006); (2) Iceland: (Sigurdarson, 1991), (3) USA: (LII, 2019), (4) Australia: (AUSVETPLAN, 2000); (5) GB: In house protocol; and (6) EFSA: (EFSA BIOHAZ Panel, 2014).

The experts agreed that it was important to separate probability of survival of prion into two time-periods: 'immediately after C&D' and 'over time after C&D'. The probability was believed to reduce over time due to 'variance of weather conditions' (repeated drying and wetting) and the 'dilution effect' due to rain (Maddison et al., 2015; Konold et al., 2015). The dilution effect was an important factor associated with the risk of exposure and was believed to be more important in outdoor environments than in indoor. However, it was also believed that because buildings are likely to be cleaned on a regular basis there might also be a significant dilution effect in an indoor environment. On the other hand, for some farms it was perceived that a layer of bedding and organic material would be created over time and that this potentially creates a physical barrier to the TSE agent.

Farmers' attitude to carrying out the C&D protocols was another factor associated with effectiveness of C&D. However, it was argued that the infrastructure of some farms were impossible to effectively disinfect and that the only control method was to repopulate with resistant genotypes (compulsory measure) or to "demolish and start again". Furthermore, it was mentioned that areas outside of the buildings were not possible to disinfect and that these may re-contaminate the farm eventually.

3.2.5. *Recommendations for future research, implementation and policy*

The workshop identified the lack of approved protocols to undertake C&D in scrapie farms in GB and that the limited knowledge in understanding the effectiveness of C&D protocols remains a problem. However, it was evidently clear that C&D should still be done on scrapie farms as part of a good disease management and to reduce infectious pressure. In addition, it was stated that C&D should always consider animals' genotype that will be used for restocking (EFSA BioHaz Panel et al., 2017; European Parliament and Council of the European Union, 2001).

Participants identified several areas where research is currently needed (Fig. 2). It was stated that research on easy or rapid diagnostic methods that could measure prion load in the environment would be beneficial, especially to allow identification of those high-risk areas. Testing the effectiveness of different C&D protocols over time in soil and other materials in real farm scenarios and in experimental farms were recommended. It was suggested that studies could be conducted on farms that were depopulated because of scrapie in the past and test if the prion protein remains. Assessing the time of survival (or the half-life period) of the scrapie prion protein on farms was also identified as a research requirement. However, it was perceived that some experiments, such as bioassays, are extremely expensive and would likely never be conducted unless a new outbreak of a zoonotic TSE occurs.

4. Discussion

This study set out to review the existing recommended guidelines for C&D on farms following an outbreak of classical scrapie using a review of available literature and an expert opinion workshop. Literature has been published on the environmental persistence of TSE agents on various surface types and materials from which horizontal transmission can then occur. Studies on the effectiveness of C&D were more limited especially in the farm environment where it was concluded that C&D did little to reduce the titre of infectious scrapie material (Hawkins et al., 2015; Konold et al., 2015).

A considerable reduction of infectivity is achieved by cleaning, which involves removal of waste, dust and loose objects (dry cleaning), wetting of surfaces with water with or without detergent (wet cleaning), followed by complete drying before disinfectants are applied (Gosling, 2018). This protocol was adopted by several countries prior to disinfection (see Table 1). Whilst this is likely to remove some prion activity and may make prions more accessible for subsequent disinfectants, it will not be able to reduce it to the high level of more than 90 % reported for bacteria (Fotheringham, 1995) due to the general resistance of prions to chemical inactivation. Indeed, implementation of cleaning, even in combination with disinfection, did not prevent re-infection with the scrapie agent (Hawkins et al., 2015).

Specific guidance on protocols for C&D after a scrapie outbreak by different agencies were limited, as previously found by Acin (2015). However, the list of recommendations presented here show that some variation exists between countries and agencies. Many of these recommendations, in particular those related to the control of outdoor environment, could be considered as difficult and expensive to implement. These measures, if enforced without any economical support, could represent an important shock to these type of farmers, whose business income are amongst the lowest in the agricultural sector (DEFRA, 2019). Furthermore, some of the measures, such as exposure to chlorine from the use of hypochlorite, can represent environmental concerns and a threat to human health (ATSDR, 2010; Luo et al., 2014). However, expert opinion workshop highlighted that development of protocols were limited by lack of information and experience in applying C&D on scrapie infected farms and their effectiveness. It was concluded that there are currently no alternatives for C&D protocols used in GB on scrapie infected farms to be considered at this time. Indeed, most of the C&D protocols employed by different countries were based on the same limited experimental evidence and strongly recommend the use of a 1 h treatment with 20,000 ppm free chlorine or with 2 M sodium hydroxide. There is an evident gap in validation of these methods to ensure the safety and reproducibility of prion decontamination at farm level. In addition, the workshop participants stated that the current protocol was evaluated for an "experimental farm" using sheep of highly susceptible genotypes only, and there remained the problem of how to modify the protocol to be implemented in "real farm scenarios" where there is likely a mixture of animals with different genetic susceptibility. In spite of being knowingly ineffective, the experts agreed that C&D should still be done on scrapie farms because it was a "good disease practice" and helped reduce infectious pressure. Furthermore experts felt that the protocol should not be too prescriptive (should not be written down in the legislation) because of differences in farm types. Yet, given the lack of field data, there is a need for more countries to publish their scrapie C&D protocols and experiences, so that a larger body of evidence on potential effectiveness can be obtained.

Participants in the workshop identified several areas where research is currently needed. Firstly, the importance of knowing the prion survival over time after C&D was highlighted as a priority. In general, participants were confident that the risk of exposure to prion by new

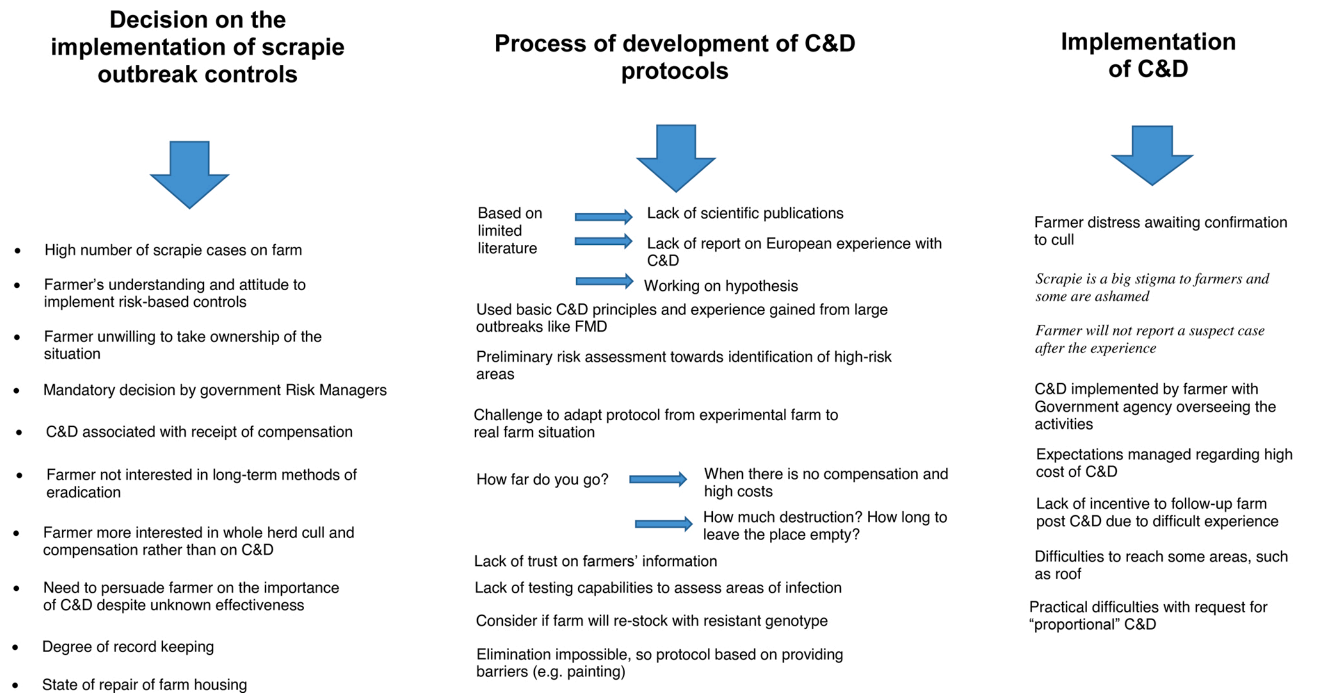

Fig. 1. Themes associated with decisions regarding implementing C&D in scrapie farms.

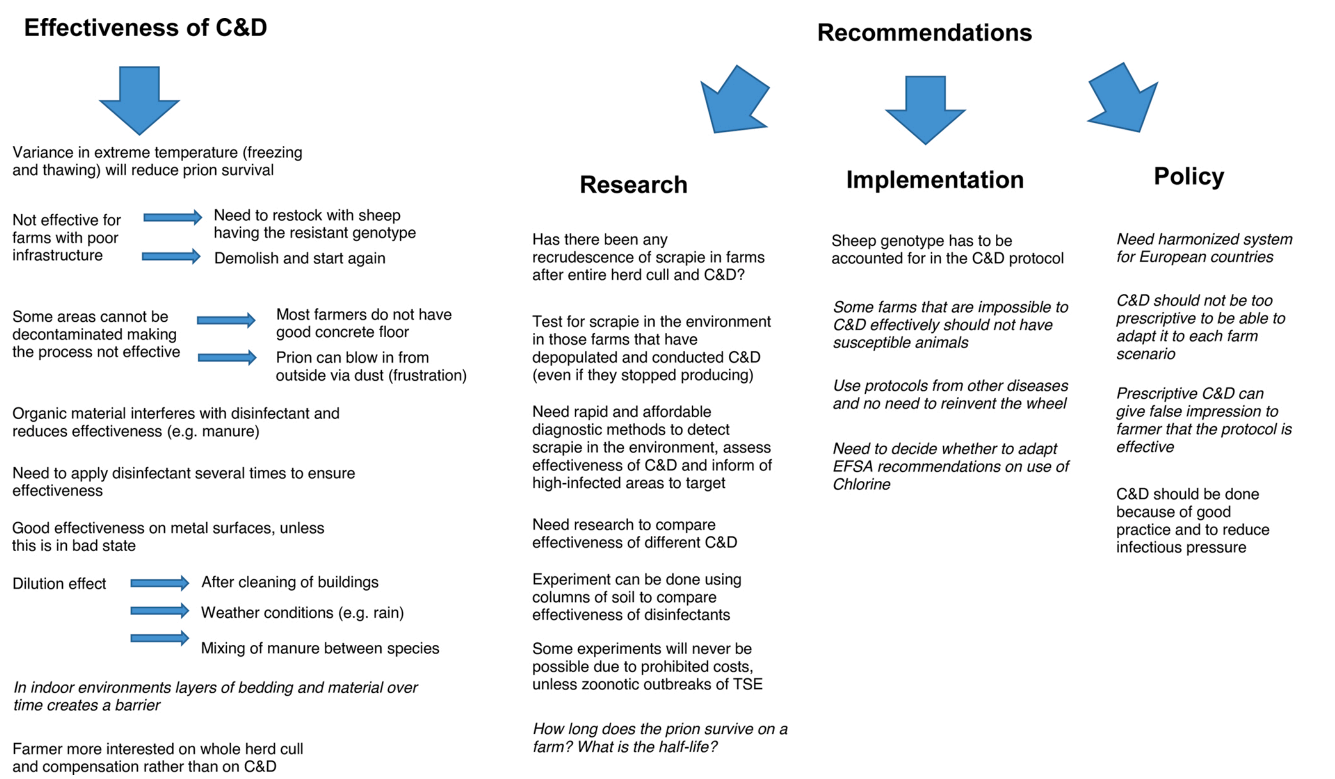

Fig. 2. Themes associated with C&D effectiveness and key recommendations.

animals in 2 years' time after C&D may decline due the weathering process reducing the infectivity (Konold et al., 2015). The unusual resistance of prions to thermal inactivation or disinfectants commonly used against pathogens, such as alkylating agents (formalin), some halogens (iodine), detergents [sodium dodecyl sulphate SDS)], organic solvents (ethanol) and oxidizing agents (hydrogen peroxide) when used on their own (Taylor, 2000) poses a serious threat to the control of infection in farms. Although more recent studies indicate that there may be other potent disinfectants, such as hypochlorous acid (Hughson et al., 2016) or CAC-717 (Sakudo et al., 2020), or combinations of disinfectants (0.2 % SDS and 0.3 % NaOH in 20 % *n*-propanol) (Beekes et al., 2010), more validation data are required and practicability has to be considered when applied to farms rather than steel instruments. At farm level, it is known that there are many fomites which are capable of

Table 2
Probability estimates associated with the effectiveness of C&D on scrapie affected farms.

	Probability of ineffective implementation of C&D	Probability of survival of TSE agent immediately after C&D	Probability of survival of TSE agent over time after C&D	Risk of exposure to TSE agent by new animals in 2 years' time
Wood	Very high	Very high	Low if outdoor, otherwise High-Medium	Destroyed – negligible
Metal	Low	Low	Low	Low
Concrete	Medium	High-Medium	Low if outdoor, High-Medium if indoor	Low-Very low outdoor, Medium-Low indoor
Soil /pasture	Very high	Very high	Low	Low-Very low
Manure/ bed	Very high	High	Medium	Medium-Negligible

contributing to disease transmission (Maddison et al., 2010a). It has been shown that prions can bind and be released from stainless steel, aluminium, polypropylene, glass, cement, wood and rocks, and that hamsters exposed to contaminated wood, polypropylene and cement succumb to a TSE with a 100 % attack rate (Pritzkow et al., 2018). To this, we must add that scrapie may persist in the environment for at least 16 years (Georgsson et al., 2006), changing its biological and biochemical properties across the time when it is in soil (Maddison et al., 2015). Fertilising soil with humus may reduce prion infectivity as shown for chronic wasting disease prions due to the active component humic acid (Kuznetsova et al., 2018) but it is not known whether this also applies to scrapie since persistence of prions appears to be strain dependent (Maddison et al., 2010b).

Participants suggested that studies could be conducted on farms that were depopulated because of scrapie in the past to assess if the prion protein remains. However, it was perceived that some experiments such as bioassays are time-consuming and extremely expensive and that there are limitations in terms of lack of fast, cheap sensitive diagnostic tests to measure infectious pressure in different areas and regarding transmissibility of prions from different areas or materials. It is important to note that sPMCA and real-time quaking-induced conversion (RT-QuIC) could be used to measure prion load in the environment. These are rapid and ultrasensitive methods, which will facilitate future development and validation of decontamination procedures (Rubenstein et al., 2011; Konold et al., 2015; Belondrade et al., 2016) but require specialist equipment and validation themselves to determine diagnostic sensitivity. It should be reiterated here that such techniques are subject to sampling conditions and whilst a positive test will indicate the presence of prions a negative test will only indicate the lack of prions in that particular test sample and cannot be taken as indicative of the rest of the farm premises.

In summary, the current guidelines for C&D of farms after a scrapie outbreak are based on experimental data and have not been fully validated with environmental realism. Literature demonstrates the difficulty in removing scrapie infectivity from the farm environment and that genetically susceptible sheep can become infected within 18 months after C&D. The current reported incidence of classical scrapie in sheep flocks and goat herds is low and alternative forms of control exist with selection of resistant genotypes. The challenges in translating policy and legislative requirements at an applied level emphasise the need for further research into practical and effective prion decontamination methods, also using novel disinfectants that may be less corrosive and less harmful to the environment.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2021.105388.

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