Environmental Research 151 (2016) 587–594

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

An assessment of the long-term persistence of prion infectivity in aquatic environments

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ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form
26 August 2016
Accepted 27 August 2016

Keywords:
Prion
Scrapie
BSE
Infectivity
Wastewater

ABSTRACT

The environment plays a key role in horizontal transmission of prion diseases, since prions are extremely resistant to classical inactivation procedures. In prior work, we observed the high stability of bovine spongiform encephalopathy (BSE) infectivity when these prions were incubated in aqueous media such as phosphate-buffered saline (PBS) or wastewater for nearly nine months. As a continuation of this experiment, the same samples were maintained in PBS or wastewater for five additional years and residual BSE infectivity was assessed in bovine PrP^{C} transgenic mice. Over this long time period (more than six years), BSE infectivity was reduced by three and one orders of magnitude in wastewater and PBS respectively. To rule out a possible agent specific effect, sheep scrapie prions were subjected to the same experimental protocol, using eight years as the experimental end-point. No significant reduction in scrapie infectivity was observed over the first nine months of wastewater incubation while PBS incubation for eight years only produced a two logarithmic unit reduction in infectivity. By contrast, the dynamics of PrP^{Res} persistence was different, disappearing progressively over the first year. The long persistence of prion infectivity observed in this study for two different agents provides supporting evidence of the assumed high stability of these agents in aquatic environments and that environmental processes or conventional wastewater treatments with low retention times would have little impact on prion infectivity. These results could have great repercussions in terms of risk assessment and safety for animals and human populations.

1. Introduction

One of the most striking features of prions, the causal agents of transmissible spongiform encephalopathies (TSEs), is their resistance to classic inactivation protocols targeted at destroying the nucleic acid components of most known pathogens (Sakudo et al., 2011). In fact, prions are defined as small proteinaceous infectious particles as a result of their resistance to these conventional procedures and of their sensitivity to "protein-directed" inactivation methods (Prusiner, 1982). Currently, there is no gold standard method for prion inactivation and usually combinations of several protocols are required to ensure decontamination. The World Health Organization (WHO) recommends protocols that involve NaOCl (20,000 ppm, 20 °C, 1 h) or NaOH (1 N, 20 °C, 1 h) treatment followed by autoclaving under soaking conditions (134 °C and 3 bar pressure a minimum of 20 min) (WHO, 2000). Other possible treatments are detergents of different nature, treatment with chaotropic salts, phenol, phenolic products or formic acid, or more complex procedures like vaporization or gas plasma sterilization based on hydrogen peroxide (Sakudo et al., 2011). Taking into account the difficulty of prion decontamination, accumulation of prions in the environment is one of the main concerns when dealing with prion diseases. In fact, it is well established that some TSEs like scrapie in sheep and goats or chronic wasting disease (CWD) in cervids are maintained within the corresponding animal populations through natural horizontal transmission because of their persistence in the environment (Gough and Maddison, 2010).

Scrapie was first described in 1732 (Zabel and Reid, 2015) and epidemiologic and experimental studies clearly pointed to environmentally mediated horizontal transmission as a mechanism of disease maintenance (Hoinville, 1996; Brown and Gadjusek, 1991). Remarkably, the disease was reported in a flock after being housed in the same barn as scrapie infected animals 16 years ago

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http://dx.doi.org/10.1016/j.envres.2016.08.031
0013-9351/

(Georgsson et al., 2006). This environmental transmission theory was reinforced when PrP^{Sc} was found to bind to soil materials while retaining its infectivity and enhancing its transmissibility (Johnson et al., 2006, 2007; Seidel et al., 2007). In addition, it has been recently observed that as a consequence of soil binding, PrP^{Sc} can be modified resulting in changes in disease phenotype when transmitted in transgenic mice (Maddison et al., 2015). However, soil is not the only source for horizontal transmission. Currently, there is evidence that PrP^{Sc} persists in fomites found in housing furniture elements through protein misfolding cyclic amplification (PMCA) and can infect healthy animals (Maddison et al., 2010; Hawkins et al., 2015; Konold et al., 2015). Other materials able to retain infectious PrP^{Sc} are class B biosolids (Miles et al., 2011a, 2011b), plants (Pritzkow et al., 2015) and dust (Gough et al., 2015).

CWD was first identified in 1967 but was first reported as a syndrome of wasting and progressive neurological disease (Haley and Hoover, 2015), not being classified as a TSE until 1980 (Williams and Young, 1980). This TSE has been found in North America

Table 1
Summary of the prion infectivity persistence studies done in environmental matrices during the past years.

	Matrix	Prion strain/ Isolate	Incubation time Storage conditions	Prion detection method	Infectivity decay	Reference
Solid materials	Soil	Mouse adapted 263k scrapie	3 years Buried in garden	Hamster bioassay	2–3 logs	Brown and Gadjusek, 1991
	Soil minerals	Hamster adapted Hyper TME[a]	Tested after treatment	Hamster bioassay	1 log	Johnson et al., 2006
	Soil	Mouse adapted 263k scrapie	29 months Outdoor lysimeters	Hamster bioassay	None	Seidel et al., 2007
	Soil minerals	Hamster adapted Hyper TME[a]	Tested after treatment	Hamster bioassay	None	Johnson et al., 2007
	Class B biosolids	Mouse adapted RML[b] scrapie	15 days 37 °C	SSCA[c]	2.43 logs	Miles et al., 2011
			10 days 60 °C		3.41 logs	
	Wheat roots and leaves	Mouse adapted 263k scrapie	16 h Rotation at room temperature	Hamster bioassay	None	Pritzkow et al., 2015
	Dust	Sheep scrapie	1 year Environmental conditions	PMCA	ND[e]	Gough et al., 2015
	Fomites	CWD	2.2 years Environmental conditions	Cervid bioassay[d]	ND[e]	Miller et al., 2004
	Fomites	Sheep scrapie	16 years Environmental conditions	Sheep bioassay[d]	ND[e]	Georgsson et al., 2006
	Fomites	CWD	Tested after treatment	Cervid bioassay[d]	ND[e]	Mathiason et al., 2009
	Fomites	Sheep scrapie	20 days	PMCA	ND[e]	Maddison et al., 2010
	Fomites	Sheep scrapie	Tested after treatment	Sheep bioassay[d]	ND[e]	Hawkins et al., 2015
	Fomites	Sheep scrapie	8 weeks	Sheep bioassay[d]	ND[e]	Konold et al., 2015
Aquatic environments	Wastewater	Hamster adapted Hyper TME[a]	20 days 37 °C dark	Hamster bioassay	None	Hinckley et al., 2008
	PBS	BSE	265 days 20 ± 2 °C daylight regime	PrP^{Res} by Western blotting	Negative results[f]	Maluquer de Motes et al., 2008
	Wastewater	Mouse adapted Dawson scrapie	265 days 20 ± 2 °C daylight regime			
	PBS	Mouse adapted RML[b] scrapie	8 weeks 25 °C	SSCA[c]	1.7 logs	Miles et al., 2011
	Wastewater					
	Water with different treatments		8 weeks 37 °C		2.5 logs	
	PBS	BSE	265 days 20 ± 2 °C daylight regime	Bovine PrP Tg mice bioassay	None in PBS 2 logs in wastewater	Maluquer de Motes et al., 2012
	Wastewater		2228 days 20 ± 2 °C daylight regime		1 log in PBS 3 logs in wastewater	Present report
	PBS	Sheep scrapie	265 days 20 ± 2 °C daylight regime	Ovine PrP Tg mice bioassay	None in PBS None in wastewater	
	Wastewater		2890 days 20 ± 2 °C daylight regime		NA[g] in PBS 2 logs in wastewater	

[a] Transmissible mink encephalopathy.
[b] Rocky Mountain Laboratory.
[c] Standard scrapie cell assay.
[d] Transmission in natural farmed conditions.
[e] Prion detection was positive but infectivity decay was not determined because the experimental design.
[f] PrP^{Res} was not detected by Western blotting
[g] Not available.