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Prion transmission

Prion excretion and occurrence in the environment

Kevin C. Gough[1,*] and Ben C. Maddison[2]

[1]School of Veterinary Medicine and Science; The University of Nottingham; Sutton Bonington Campus; Sutton Bonington, UK; [2]ADAS UK; Department of Biology; University of Leicester; Leicester, UK

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Prion diseases range from being highly infectious, for example scrapie and CWD, which show facile transmission between susceptible individuals, to showing negligible horizontal transmission, such as BSE and CJD, which are spread via food or iatrogenically, respectively. Scrapie and CWD display considerable in vivo dissemination, with PrP^{Sc} and infectivity being found in a range of peripheral tissues. This in vivo dissemination appears to facilitate the recently reported excretion of prion through multiple routes such as from skin, feces, urine, milk, nasal secretions, saliva and placenta. Furthermore, excreted scrapie and CWD agent is detected within environmental samples such as water and on the surfaces of inanimate objects. The cycle of "uptake of prion from the environment—widespread in vivo prion dissemination—prion excretion—prion persistence in the environment" is likely to explain the facile transmission and maintenance of these diseases within wild and farmed populations over many years.

Introduction

Prion diseases are fatal neurological disorders that are thought to be caused by the misfolding of a benign, widely expressed protein (PrP^{C}) into a distinct pathological conformation(s) (PrP^{Sc}) which is regarded as the disease agent.[1] Prion diseases affect a range of important food production species and include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle and chronic wasting disease (CWD) in deer. CWD also affects wild deer and elk populations within North America. Prion diseases are also well documented within human medicine where Creutzfeldt-Jakob disease (CJD) is the most common disorder, affecting around one in a million of the population.[2] One of the most important developments in prion diseases is the recognition that the BSE agent, a prion disease of cattle, can infect multiple species including cats, goats and humans via the food chain.[3-5] In humans, BSE is known to be the causal agent of variant CJD (vCJD), a prion disorder with a novel human pathology.[3] This description of a newly emerging prion disease that is zoonotic completely revolutionized the paradigm of a prion disease being specific to a single species and introduced the specter of the emergence of other animal prion disorders which are transmissible to man.

One fascinating aspect of prion diseases is the very distinct transmissibility of the different disorders. On the one hand, epidemiological data would suggest that BSE and CJD appear to have limited or no direct transmission from one individual to another.[6] And on the other, scrapie and CWD demonstrate facile transmission between animals, resulting in endemic infections within susceptible populations.[6] The molecular mechanisms underpinning these distinct transmissibility traits are largely unknown. However, there have been considerable recent advances in describing the routes of excretion of the prion agent within animals incubating scrapie or CWD, as well as studies describing the presence of prions within environmental samples. This review will consider these new data on potential routes of disease transmission as well as their implications within the context of prion diseases affecting a range of mammalian species.

*Correspondence to: Kevin C. Gough; Email: kevin.gough@nottingham.ac.uk
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In Vivo Dissemination of PrP^{Sc} and Prion Infectivity

It is a prerequisite that the in vivo dissemination of PrP^{Sc} will need to extend to peripheral tissues and secretory organs in order to facilitate prion dissemination into the environment. While considering the routes of secretion of the prion agent it therefore seems pertinent to consider the tissue tropism of PrP^{Sc} and prion infectivity during pathogenesis.

The most likely infection route of the acquired prion diseases is via oral intake and what follows is an accumulation and amplification of prion infectivity in lymphoid tissues associated with the gut. Prion then spreads to other lymphoreticular (LRS) tissues, including spleen, lymph node, tonsil and appendix, and to the enteric nervous system which leads to the eventual spread of prions to the central nervous system (CNS).[7-11] After infection and replication within the CNS there is centrifugal spread of prions via the peripheral nervous system to other tissues and sites of secondary prion replication. This broad descriptive picture of infection is typical of ovine scrapie, CWD, human vCJD and experimental ovine BSE. In these diseases there is a prolonged involvement of prion replication within the lymphoid tissues throughout disease incubation.

The involvement of lymphoid tissue in prion pathogenesis is dictated by the prion strain and the host species. This is best illustrated with BSE infection of cattle, where after oral inoculation there appears to be a minimal involvement of the LRS and a restriction

of infectivity largely to the CNS.[12-14] This is at odds when the same agent infects other species, such as experimental ovine BSE and human vCJD, where infectivity shows high levels of lymphoreticular involvement, more akin to ovine scrapie. In addition, as well as host species and prion strain, the *PRNP* genotype of the host can also dictate the tissue distribution of prions. For instance, for scrapie-infected sheep with the *PRNP* genotype coding for PrP with amino acids $A_{136}R_{154}R_{171}/V_{136}R_{154}Q_{171}$ (ARR/VRQ), CNS infection was reported without prior infection of the lymphoid tissue,[15] whereas infected VRQ/VRQ animals are typified by extensive lymphoreticular involvement.

The spread of prions from sites of prion replication to other areas in the body by hematogenous dissemination also occurs, most likely due to the presence of free-ranging lymphoid cells within blood. Several studies have demonstrated that prions are present in the blood of sheep infected with scrapie and BSE,[16,17] deer incubating CWD,[18,19] and within the blood of patients with preclinical vCJD.[20]

Where prion disease does result in widespread tissue distribution of infectious agent, it is likely that mucosal tissues, skin and secretory organs will contain prion. The presence of prion at such sites is discussed in detail within the following sections.

Excretion of Prions

It is only very recently that the excretion of prions has been reported. Such investigations utilize methodologies that are exquisitely sensitive in their detection of PrP^{Sc} or prion infectivity. Rodent bioassay represents the most widely applied and sensitive method for measuring prion infectivity and the advent of transgenic animals expressing the *PRNP* gene homologous to the infecting PrP^{Sc} primary amino acid sequence has increased the application of this method further.[21-28] One of the most sensitive rodent bioassays has been reported to detect a brain dilution equivalent to 107,000 monomeric PrP^{Sc} molecules.[29] Remarkably, the technique serial protein misfolding cyclic amplification (sPMCA) is even more sensitive than transgenic mouse bioassay. PMCA was pioneered by Soto and colleagues[30] in a rodent model system of scrapie and is an in vitro technique that amplifies trace amounts of PrP^{Sc} within a test sample during iterative rounds of sonication and incubation at 37°C. This technique can detect PrP^{Sc} down to 26 monomeric PrP^{Sc} molecules[29] and is applicable to the high level amplification of natural sources of PrP^{Sc} including ovine scrapie, bovine BSE, human CJD and cervid CWD.[31-34]

Considering the in vivo dissemination of the prion agent for each prion strain/host combination, the prion diseases most likely to excrete prions are ovine scrapie, cervid CWD, experimental ovine BSE and human vCJD. To date, studies investigating the shedding of the prion agent have focused on ovine scrapie and cervid CWD and the excretion routes studied have included via urine, feces, milk, saliva, skin and within parturient material (**Table 1**).

Excretion of Prions within Urine

The detection of PrP^{Sc} or prion infectivity within urine has been shown unequivocally using rodent bioassay and sPMCA. Seeger and colleagues (2005) demonstrated that mice, during both the preclinical and clinical stages of scrapie and when coincidental with lymphocytic nephritis, secreted scrapie infectivity within their urine and concluded that chronic inflammatory disease of the kidney within prion-infected individuals leads to prionuria.[35] These data were later supported by the demonstration of amplifiable prion in the urine of scrapie infected hamsters at the terminal stages of disease.[36] PrP^{Sc} was detected within a fraction of urine collected by filtration, indicating that PrP^{Sc} may be associated with cells such as leukocytes or the epithelial cells of renal tubules. Importantly, this study also demonstrated the presence of PrP^{Sc} within urine from animals with no inflammation of the kidneys indicating that this is not a prerequisite for prion excretion. Such a conclusion is further supported by analogous studies that found infectivity[37] or amplifiable PrP^{Sc},[38] in the urine of hamsters infected with scrapie where the animals did not display any inflammation of the secretory organs. The study by Gregori and coworkers demonstrated the presence of prion infectivity in urine from clinical animals and also demonstrated the presence of scrapie-infectivity within urinary bladder and kidney tissues.[37] Deposition of prion has also been detected within renal tissues of both scrapie-infected sheep[39] and CWD-infected deer,[40] indicating that analogous secretion may be occurring with prion infections of natural hosts. Indeed, deer with clinical CWD and mild to moderate nephritis produced urine containing sPMCA-detectable PrP^{Sc} and CWD-infectivity.[41]

Overall, data demonstrates the secretion of prion within the urine of animals in the terminal stages of prion disease at levels 10-fold lower[38] or similar to[37] that found in plasma; the latter estimated to be approximately 0.5–5 fg/ml of PrP^{Sc}.[38] While not a prerequisite for prionuria, nephritis may well lead to the increased secretion of prion within urine. In addition, Seeger and colleagues demonstrated that scrapie-infected animals with inflammatory kidney disease excrete prion within urine even within the preclinical stage of scrapie.[35]

Excretion of Prions within Feces

Within scrapie-affected animals the gut-associated lymphoid tissue (GALT) and the enteric nervous system (ENS) are not only implicated in the uptake and early replication of prions after ingestion but are also a site of PrP^{Sc} production and accumulation during later disease progression.[42-44] Accumulation of PrP^{Sc} within the GALT may well lead to transit of the prion into the gut lumen and excretion within feces. Safar and colleagues used a rodent-scrapie model to demonstrate the presence of prions within feces.[45] Conformation-dependent immunoassay (CDI) analysis showed prion within feces from hamsters throughout the incubation of the disease and from terminally sick animals. Interestingly, bioassay analysis of feces demonstrated high titers of infectivity throughout disease incubation following oral challenge but low levels of infectivity following IC and IP challenge. This may indicate that either the oral inoculum can reside and be excreted from the GI tract throughout the incubation of disease and/or that the route of challenge produces very

Table 1. Detection of prion infectivity or PrP^{Sc} within excreta/secreta or within tissues released into the environment

Biological material	Prion strain/host species	Prion detection method	Disease status	Reference
Milk	scrapie/sheep	ovine bioassay	clinical	54
	scrapie/sheep	rodent bioassay	clinical/preclinical	55
	scrapie/sheep	sPMCA	clinical/preclinical	56
Feces	scrapie/hamster	CDI	clinical/preclinical	45
	scrapie/hamster	sPMCA	clinical	42
	CWD/deer	cervid bioassay	clinical/preclinical	46
	scrapie/sheep	sPMCA	clinical/preclinical	48
Urine	scrapie/mouse	rodent bioassay	clinical/preclinical	35
	scrapie/hamster	sPMCA	clinical	36
	scrapie/hamster	rodent bioassay	clinical	37
	scrapie/hamster	sPMCA	clinical	38
	CWD/deer	murine bioassay and sPMCA	clinical	41
Saliva	CWD/deer	cervid bioassay	clinical	18
	CWD/deer	cervid bioassay	preclinical	69
	CWD/deer	murine bioassay	clinical	41
	scrapie/sheep	sPMCA	preclinical	70
Nasal secretions	TME/hamster	rodent bioassay and QUiC[a]	clinical	68
Skin	BSE/kudu	rodent bioassay	clinical	87
	BSE/hamster	Western blot	clinical	88
	scrapie/hamster	Western blot	clinical/preclinical	88
	scrapie/sheep	Western blot	clinical	88
	vCJD/human	Western blot	clinical	89
	CWD/elk	rodent bioassay and sPMCA	clinical	90
Placenta	scrapie/sheep	sheep/goat bioassay	clinical	76
	scrapie/sheep	Western blot	preclinical	105
	scrapie/sheep	Western blot, IHC	clinical/preclinical	78
	scrapie/sheep	ELISA	preclinical	75

[a]QUiC (Quake-induced Conversion) assay affords the high level in vitro amplification of PrP^{Sc}.[104]

distinct prion pathogenesis with regard to the GALT and ENS. However, this study does demonstrate beyond doubt that prion is shed in feces during preclinical and clinical stages of scrapie. An analogous study using lower doses of oral inoculum also demonstrated the fecal shedding of prions during clinical stages of disease by employing sPMCA amplification of prions in fecal extracts.[42] Importantly, these conclusions are also supported by the demonstration that mule deer incubating CWD after oral challenge did not yield prion infectivity within feces within the first 3–4 months after inoculation but feces contained infectivity after 9 months incubation through to clinical disease at 16 to 20 months. Using transgenic mouse bioassay, it was calculated that the levels of infectivity shed through feces over a 10 month period approached that present within a clinically infected whole brain.[46] In addition, white-tailed deer orally inoculated with a mixture of urine and feces from CWD-infected deer were shown to be sub-clinically infected with CWD, again demonstrating that such excreta contain biologically relevant amounts of prion infectivity.[47] With regard to ovine scrapie, naturally infected sheep also excreted prion in their feces during both the preclinical and clinical phases of disease.[48]

Secretion of Prions within Milk

Whether prion infectivity could be contained within milk has been debated for many years and previous bioassay or epidemiology data indicated that ruminant milk did not harbor prions.[49-52] However, several studies in recent years have demonstrated unequivocally the presence of prions within ovine milk from scrapie-infected sheep.

The first data suggesting prions could be secreted in milk was the observation of PrP^{Sc} within the mammary glands of sheep infected concurrently with scrapie and lymphofollicular mastitis.[53] Subsequently, Konold and co-workers looked at the presence of scrapie infectivity in milk by feeding scrapie-free lambs with milk from scrapie-affected ewes.[54] This study demonstrated that the milk harbored infectivity and lambs accumulated PrP^{Sc} within their LRS. Subsequent studies have used sPMCA amplification and transgenic mouse bioassay to assess the presence of prion in milk from animals with pre-clinical disease and with distinct *PRNP* genotypes. Lacroux and co-workers used ovinized mice to measure prion infectivity in immunoprecipitates from ovine milk.[55] They found infectivity in all of the milk fractions

analyzed; the cell pellet, casein whey and cream fractions from both colostrum and milk. It was estimated that a milliliter of whole milk could contain prion infectivity equivalent to that found in 6 μg of posterior brain stem from an animal with clinical scrapie. Furthermore, infectivity was present in the milk of ewes up to 20 months prior to clinical onset of disease and in sheep with either VRQ/VRQ or ARQ/VRQ *PRNP* genotypes. Bioassay data also confirmed the presence of infectivity in milk from ewes with extensive accumulation of PrP^{Sc} within LRS but without ectopic lymphoid follicles or detectable PrP^{Sc} within the mammary gland, therefore demonstrating that such inflammatory conditions and the accumulation of PrP^{Sc} in the mammary gland are not prerequisites for prion secretion. However, it was noted that the presence of follicular mastitis did appear to increase the levels of prion infectivity in milk.

The study by Maddison and coworkers applied sPMCA amplification to prions in milk from sheep with a range of *PRNP* genotypes and with pre-clinical and clinical scrapie.[56] Data demonstrated the presence of PrP^{Sc} in the milk of sheep with a range of *PRNP* genotypes: VRQ/VRQ, ARQ/VRQ, AHQ/VRQ and ARR/VRQ. While the ARQ/VRQ and VRQ/VRQ genotypes are associated with high disease penetrance and widespread in vivo PrP^{Sc} dissemination throughout the LRS, ARR/VRQ and AHQ/VRQ sheep have low clinical disease incidence and limited involvement of the LRS in prion propagation. This study demonstrated that prions are secreted in milk regardless of the accumulation of PrP^{Sc} within the LRS and indicates the secretion of prions in the milk of animals likely to have subclinical disease.

Altogether, data demonstrates that prions are secreted in the milk of sheep with clinical, preclinical and very likely subclinical scrapie. This secretion takes place through a prolonged disease incubation period in sheep with multiple *PRNP* genotypes. Prions are secreted in ovine milk regardless of the accumulation of PrP^{Sc} within LRS tissues and, while the coincidence of scrapie with lymphofollicular mastitis may well enhance prion shedding in milk, it is not a prerequisite for this secretion.

The route through which prions are secreted into milk is still unclear. It has been speculated that PrP^{C} is likely to be transported via exocytosis from epithelial cells and via apocrine secretion of milk fat globules.[57] The routes of PrP^{Sc} secretion may be through similar mechanisms. In addition, or alternatively, PrP^{Sc} may be derived from blood. PrP^{Sc} is known to be associated with hematogenic spread during preclinical disease[16,17] and blood components are secreted into milk including macrophages, a $CD68^{+}$ cell type that colocalized with PrP^{Sc} within mammary glands.[53,55] However, the absence of PrP^{Sc} colocalization with $CD68^{+}$ cells in mammary tissue does not preclude prion secretion within milk,[55] perhaps indicating multiple and distinct routes of secretion and that a hematogenic origin of the secreted prions may depend upon/be enhanced by the presence of inflammatory pathology within the mammary gland.

Prions within Saliva or Nasal Secretions

Evidence for the presence of prion infectivity within nasal and oral tissues has been generated over several decades for both rodent models and natural hosts of prion diseases. Using rodent models, prion infectivity was found within gingival tissues,[58,59] the nasal mucosa-associated lymphoid tissues (NALT), the submandibular lymph nodes[60] and within nerve fibers, muscle cells and fungiform papillae of the tongue.[61,62] However, there are considerable discrepancies between studies in terms of the exact cell types that accumulate prion within oral and nasal tissues and this may well reflect differences between the prion strain/host species being investigated.

With natural hosts of prion diseases, tissue homogenates of nasal mucosa from scrapie-affected small ruminants were shown to harbor infectivity[50,63] and prion was present within the papillae of the tongue in sheep with natural scrapie.[64] Furthermore, Vascellari and co-workers (2007) demonstrated that PrP^{Sc} is present within both major (parotid and mandibular) and minor (labial, buccal and palatine) salivary glands; including within epithelial cells and within the lumina of salivary ducts draining saliva into the oral cavity.[65] It is also evident that with both sCJD in humans[66] and ovine scrapie[67] PrP^{Sc} is present within olfactory tissues.

While there is still debate over the location of PrP^{Sc} within oronasal tissues and the routes of centripetal and centrifugal spread of the agent between these tissues and the CNS, overall, data strongly suggest that PrP^{Sc} and infectivity can be secreted into nasal and/or oral secretions. This is likely to occur from a combination of the normal turnover of cells at chemosensory mucosal surfaces and from the secretion of prion through exocrine glands.

Bessen and co-workers (2010) used a rodent transmissible mink encephalopathy (TME) model to demonstrate that following CNS challenge the prion agent was not only found within olfactory sensory epithelium, but was also found within nasal lavages.[68] These data support the secretion of the prion agent into the lumen of the nasal airway. However, given that the effects of route of inoculation, host species and TSE strain on PrP^{Sc} location within the nasal tissues is yet to be defined, prion shedding via nasal secretion within natural prion infections requires confirmation.

Considering the oral secretion of prions, saliva from CWD-infected deer was shown to transmit disease to other susceptible naïve deer when harvested from the animals in both the clinical[18] and preclinical stages[69] of infection, albeit within relatively large volumes of saliva (50 ml). In sheep with preclinical, natural scrapie infections, sPMCA facilitated the detection of PrP^{Sc} within buccal swabs throughout most of the incubation period of the disease with an apparent peak in prion secretion around the mid-term of disease progression.[70] The amounts of prion present in saliva are likely to be low as indicated by CWD-infected saliva producing prolonged incubation periods and incomplete attack rates within the transgenic mouse bioassay.[41]

Secretion of Prions within Parturient Materials

Transmission occurs between scrapie-affected ewes and their lambs[71-73] but data strongly indicates that this is predominantly horizontal transmission[74,75] with lambs exposed to the disease

agent after parturition. It has been understood for many years that placental tissues can harbor prion infectivity[76] and it seems likely that such infectivity will be disseminated during and after parturition when lambs are exposed to placental tissues.

In ruminants, the fetus interacts with the uterus through non-invasive placentation within the uterine lumen. The placenta results from imbrications of fetal chorionic villi with maternal preformed endometrial crypts, and within this structure the uterine cells carry the maternal genotype and the placental chorionic cells carry the fetal genotype. All fetal tissues and fetal annexes were negative for PrP^{Sc}; these included kidney, brain, bladder, spleen and umbilical cord, and amniotic fluid was also free of detectable prion.[75,77-79] With regard to the fetal placental tissues, PrP^{Sc} was present at high concentrations within the cotyledonary tissues.[75,77-80] Andréoletti and coworkers quantified the levels of PrP^{Sc} found within the placental cotyledon and determined that these tissues contained very high levels of prion: just 47 times less than in the obex.[75] Interestingly, the accumulation of PrP^{Sc} within placental tissues appears to be dictated by the *PRNP* genotype of both the ewe and fetus as well as the stage of disease progression within the ewe. The *PRNP* genotype of the conceptus must convey sensitivity to scrapie.[77,78] In addition, scrapie-infected ewes with an ARR/VRQ *PRNP* genotype did not accumulate PrP^{Sc} within the placenta even for fetuses with a scrapie-susceptible *PRNP* genotype.[77] Even where the ewe and fetal *PRNP* genotypes correlated with placental PrP^{Sc} accumulation, this was only detected in ewes where IHC examination of the tonsils yielded PrP^{Sc}.[75] Where placental accumulation of PrP^{Sc} occurs, it has been shown that this can be with ewes in the clinical and preclinical stages of disease.[75,77,80] It therefore appears that in ovine scrapie the placenta harbors very high levels of prion but in utero transmission is not occurring. However, following parturition placental tissue is a likely source of infective agent in the transmission of disease from ewes to lambs.

With regard to other prion diseases, a rodent model for BSE demonstrated that placenta did not contain detectable PrP^{Sc} yet transmission was recorded when mating occurred near the clinical stage of the disease and the doe had detectable PrP^{Sc} within its brain.[51] These data confirm earlier studies within cattle where placenta lacked detectable PrP^{Sc} yet vertical transmission appeared to be occurring.[81,82]

In the human placenta, the fetal epithelium is bathed within maternal blood[83] and it is known that vCJD can be transmitted through blood transfusions.[84] Together this has led to speculation that human prion diseases may be transmitted through the placenta; however, to date there is no evidence of vertical transmission in human vCJD.[85,86]

Skin as a Potential Source of Prion Infectivity

Mammalian skin is composed of different strata and cell types, which are both innervated and carry blood vessels. There is therefore the potential for skin to harbor prions and facilitate its excretion into the environment. Cunningham and coworkers demonstrated the presence of prion infectivity in the skin of BSE-affected kudu.[87] Subsequently, Thomzig et al. used a rodent model to demonstrate that skin-associated prions could be identified from late preclinical stages of disease for both scrapie and BSE,[88] estimating that there is likely to be 5,000–10,000 times less prion in the skin of hamsters challenged with scrapie than in the brains of those same animals. This study also demonstrated the presence of prions in the skin of sheep during the late stages of naturally acquired scrapie where PrP^{Sc} was generally associated with small nerve fibers within the dermis. More recently, PrP^{Sc} was found within the skin of a vCJD patient.[89] In addition, cervine CWD prions have been found within antler velvet,[90] a vascularized skin layer covering the developing antler of male deer, which is shed after the ossification of antlers.

Skin may therefore represent another route by which prions contaminate the environment. Mechanisms of prion shedding from this organ could include natural sloughing of skin, abrasions of the skin and, in the case of sheep, skin cuts and nicks that are introduced during shearing.

Environmental Sources of Prions

Both cervine CWD and ovine scrapie are transmitted horizontally within animal populations including situations where animal-to-animal contact is avoided,[91] indicating the presence of environmental vectors or reservoirs. In support of this, scrapie infectivity has been shown to persist within a farm environment for 16 years.[92] As detailed above, scrapie and CWD prions are shed from animals in the preclinical and clinical stages of disease. Such dissemination of prions may facilitate horizontal transmission of disease through direct animal-to-animal contact or by contributing to environmental reservoirs of infectivity. Such environmental prions are likely to be stable for many years, however, the location and characteristics of such reservoirs of infectivity is unclear.

It has been hypothesized that soil may harbor prion infectivity. It may be envisaged that as well as the shedding of prions from infected animals onto soil, the burial of farm animals or the natural deposition of wild animal carcasses may contribute to the presence of prions in soil. Various studies have demonstrated the binding and persistence of PrP^{Sc} to soil and soil minerals.[93-97] Importantly, rodent-adapted scrapie remains infectious in bioassays when bound to soil minerals[93] and surprisingly, soil-immobilized prions have enhanced infectivity via the oral route compared to unbound prions.[94] Together, data suggest that soil is likely to be an important environmental reservoir of prion infectivity; however, it should be noted that to date no studies have demonstrated the presence of PrP^{Sc} or prion infectivity in naturally contaminated soils.

While soil remains a strong candidate for an environmental reservoir of prions, PrP^{Sc} or prion infectivity has been found in other fomites. Using a cervine bioassay, CWD infectivity was transmitted within a combination of exposed bedding, water and food of captive animals.[69] CWD PrP^{Sc} was also detected by sPMCA amplification within a single environmental water sample.[98] Ovine scrapie has also been detected on a range of surfaces from a scrapie-infected farm; prion was detected by sPMCA amplification from a range of swabs taken from metal,

Table 2. Levels of scrapie prion detected in excreta/secreta

Biological material	Host species	Prion detection method	Equivalent amount of clinical brainstem (g)	Reference
Skin	hamster	western blot	1–2 x 10^{-4}/g	88
Placenta[a]	sheep	ELISA	2 x 10^{-2}/g	75
Milk	sheep	rodent bioassay	6 x 10^{-6}/ml	55
Feces	sheep	sPMCA	4 x 10^{-9}/g	48
Urine	hamster	sPMCA	1–10 x 10^{-11}/ml	38
	hamster	sPMCA	1 x 10^{-11}/ml	106
Saliva	sheep	sPMCA	2 x 10^{-8}/buccal swab	70

[a]prion was measured within cotyledons.

plastic and wooden surfaces.[99] Together, data indicate that environmental reservoirs of prion exist in multiple locations and are likely to include surfaces, water and soil. Such environmental prions are likely to be present at very low levels and animals would be exposed to these prions on multiple occasions. It remains to be determined whether repeat exposure to such environmental prions would lead to clinical or subclinical disease, but such an outcome would support data from epidemiological and transmission studies.[54,91]

Conclusions

With regard to transmissibility, prion diseases fall into two categories: (1) those that are readily transmitted between susceptible individuals resulting in high disease penetrance within a population, such as scrapie and CWD; (2) those diseases that appear to show limited transmissibility between individuals such as sCJD and cattle BSE and that are transmitted almost exclusively through iatrogenic or foodborne carriage. At present it is unknown which routes of transmission facilitate disease spread in scrapie- and CWD-affected populations, but in recent years there have been considerable advancements in the understanding of prion excretion and maintenance within the environment.

From studying experimental models as well as natural infections, it has been revealed that scrapie and CWD agents are excreted/secreted in parturient-associated biological materials, namely milk and placenta. The latter contains extremely high levels of prion infectivity[75] and seems an obvious source of postpartum exposure of lambs and flock mates. Milk has also been proven to contain biologically relevant levels of disease agent with scrapie being transmitted readily within this matrix from ewe to lamb.[54] Furthermore, transmission via milk resulted in further horizontal transmission between flock mates indicating the excretion of prion infectivity from animals very early in disease incubation. Indeed, it has also been shown that the scrapie and CWD prions are excreted in urine, feces and saliva and are likely to be excreted from skin. While levels of prion within these excreta/secreta are very low, they are produced throughout long periods of preclinical disease as well as clinical disease. Furthermore, the levels of prion in such materials are likely to be increased by concurrent inflammatory conditions affecting the relevant secretory organ or site. Such dissemination of prion into the environment is very likely to facilitate the repeat exposure of flockmates to low levels of the disease agent, possibly over years. At present it is not clear what the effects are of repeated challenge with a prion agent. Using distinct rodent scrapie models and inoculation routes, two studies have reported contrasting results. One study indicated that repeat exposure reduced risk compared to a single challenge with the cumulative dose;[100,101] in contrast, a second study found that repeated doses of low infectivity produced much higher incidences of disease than a single challenge with the same cumulative dose.[102] Given the unequivocal data showing that scrapie and CWD prions are secreted at low levels from multiple routes it seems imperative that the effects of repeat exposures to low levels of prion are investigated further. Given the results with scrapie-contaminated milk and CWD-contaminated saliva, it seems very likely that these low levels of prion in different secreta/excreta are capable of transmitting disease upon prolonged exposure, either through direct animal-to-animal contact or through environmental reservoirs of infectivity.

As discussed, what sets scrapie and CWD apart from sCJD and BSE are their widespread in vivo dissemination of the disease agent, a trait that may well dictate excretion/secretion of prion and therefore the facile transmission of disease. As such, while BSE is highly infectious via the oral route in cattle,[103] a lack of the BSE agent in secreta and excreta, even within placental tissues,[81,82] would appear to block the horizontal transmission of BSE infectivity within a herd. Similarly, sCJD would be highly unlikely to be naturally secreted and therefore transmitted via the environment. However, it should be noted that there are no reported studies that have used the highly sensitive methodologies of transgenic mouse bioassay or sPMCA to analyze sCJD and cattle BSE secreta/excreta to test these hypotheses directly. It is also worth considering that vCJD has similar in vivo prion dissemination compared to scrapie and CWD.[89] It may therefore be argued that prion in this disorder is likely to be excreted through similar mechanisms. Again, this remains to be tested and secretion of vCJD prions would have obvious, worrying implications regarding the spread of infectious human prions.

The demonstrations of prion shedding into the environment have considerable implications for the understanding of transmission routes for prion diseases in farmed and wild animal populations. However, the relative contribution of different prion excretion routes to the horizontal transmission of disease remains to be established and the first step in this investigation

would be the determination of relative infectivity titers of prion produced from each excretion route within experimental models or natural infections. Comparison of levels of scrapie infectivity or PrP^{Sc} across different studies allows a crude estimation of comparative levels (**Table 2**) and indicates that urine, feces and saliva contain considerably less prion than milk and skin, with placenta containing extremely high levels of prion. Furthermore, it is imperative to further determine the effects of multiple exposures to low levels of prion via the oral route. However, even given these limitations, the discovery of multiple routes of prion excretion from scrapie- and CWD-infected animals is already informing control strategies to reduce horizontal transmission of animal diseases and may also inform risk analyses with regard to human exposure to animal prions.

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