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Review

Evaluating the Species Barrier

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A Transmissible Spongiform Encephalopathy (TSE) agent from one species generally transmits poorly to a new species, a phenomenon known as the species barrier. However once in the new species it generally but not always adapts and then more readily transmits within the new host. No single test is available to determine accurately the ability of a prion strain to transmit between species. Evaluating the species barrier for any prion strain has to take into consideration as much information as can be gathered for that strain from surveillance and research. The interactions of the agent with a particular host can be measured by *in vivo* and *in vitro* methods and assessing the species barrier needs to make full use of all the tools available. This review will identify the important considerations that need to be made when evaluating the species barrier.

Key words: Prion transmission, PrP, Species barrier, Transgenic models, Transmissible Spongiform Encephalopathies (TSE)

Surveillance

It is important for evaluating risk to animal and human health to be aware of the prion strains that are present within a species and to have a means of detecting novel forms of emerging diseases. Surveillance remains important in determining the extent of prion infection within a species. Animal surveillance in Europe following the outbreak of Bovine Spongiform Encephalopathy (BSE) has determined the extent of the problem and been important for monitoring the decline in BSE following the implementation of the feed bans[1,2]. Moreover animal surveillance in cattle, sheep and goats has allowed the detection of atypical forms of both BSE and scrapie[3,4]. Surveillance has mapped the spread of Chronic Wasting Disease (CWD) from the west to the east of the USA and its spread into Canada[5]. Recent surveillance in Norway has identified the first known cases of CWD in Europe[6,7]. Surveillance in humans has allowed the identification of variant Creutzfeldt-Jakob Disease (vCJD)[8], has assessed variations in sporadic CJD (sCJD) numbers between countries[9] and identified a new form of human prion disease namely Variably Protease-Sensitive Prionopathy (VPSPr)[10]. Ongoing surveillance is required to identify further cases of human prion disease that may occur in different genetic backgrounds, in “at risk” groups or in which diagnosis may be difficult due to confounding factors such as age or other neurodegenerative diseases.

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Abbreviations: BSE: Bovine Spongiform Encephalopathy; CNS: central nervous system; CWD: Chronic Wasting Disease; PMCA: protein misfolding cyclic amplification; RTQuIC; real time quaking induced conversion; sCJD: sporadic Creutzfeldt-Jakob Disease; TSE: Transmissible Spongiform Encephalopathy; vCJD: variant Creutzfeldt-Jakob Disease; VPSPr: Variably Protease-Sensitive Prionopathy

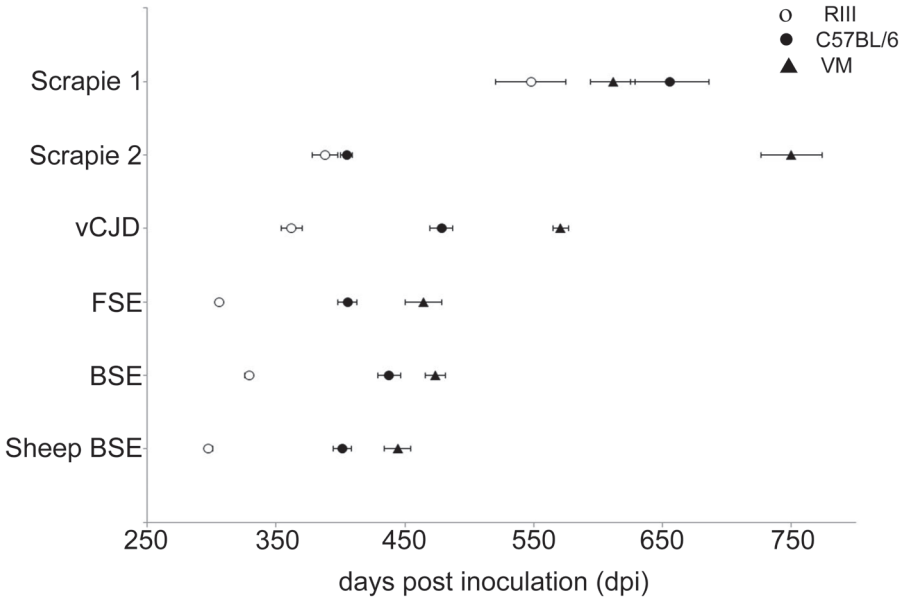

Fig. 1. Incubation period rankings can be a useful tool in assessing TSE strains. Examples of incubation periods from primary passage of experimental sheep BSE, FSE, BSE, vCJD and scrapie in three lines of wild type mice. Abbreviations: BSE, Bovine Spongiform Encephalopathy; FSE, Feline Spongiform Encephalopathy; vCJD, variant Creutzfeldt-Jakob disease.

Strain Identification

Once a new form of disease has been identified it has to be established if this is indeed a new disease or one that has been present for some time. It is also important to establish whether there is any connection between the "new" strain and those already present in the field. Retrospective analysis of atypical scrapie has suggested that this is indeed not a new disease but has been detected in tissue from the 1970's and has most likely come to light through the extensive European animal surveillance programme[11]. Similarly within human prion disease the recently identified VPSPr has now been shown to be present in much earlier cases suggesting it was indeed a previously unrecognised prion disease[12].

Strains can be characterized by both *in vitro* and *in vivo* techniques but it is important to recognize that *in vitro* techniques alone are not sufficient for strain identification. *In vivo* strain identification is undertaken using a validated panel of either wild type or transgenic mice. Following inoculation a number of criteria are measured: the incubation time of disease, the vacuolar pathology and the PrP deposition in the brain at the end point of disease. The importance of the validated panel of mice is that one criteria for assessing strain is the order in which each strain of mice succumbs to disease, namely the ranking of incubation times; this provides important information for assessing the similarity and differences between strains (**Fig. 1**)[13]. Full characterisation of a strain may require a number of sub-passages to overcome the species barrier and to allow stabilization of the strain.

Wild type mouse panels have proven invaluable in identifying the BSE strain and establishing the range of susceptible hosts, i.e. humans, cats, exotic ungulates and primates[13]. They have also determined that a single BSE/vCJD strain is responsible for vCJD cases worldwide[14] and provide a platform for further strain discrimination. *In vivo* strain typing using a variety of models has also identified a number of different strains of scrapie in the field[15,16], and the probability of multiple strains of CWD[17–19]. A similar approach can be taken with panels of mice expressing the human *PRNP* gene. These have proven useful for strain typing human prion agents, identifying new diseases and assessing susceptibility to a particular agent of the different human host genotypes, in particular the *PRNP* codon 129 genotype. Using panels of gene-targeted transgenic mice in which the human *PRNP* gene has replaced the murine gene four strains of sCJD have been defined[20]; VPSPr has been shown to be a unique prion strain[21] and assessment of vCJD infectivity and susceptibility risks has been undertaken[22,23].

There is a multiplicity of models of transgenic mice with *Prnp* genes carrying different mutations and from different species. The transgenic mice range from multiple copies of *Prnp* genes inserted at random into the genome to transgenic mice produced by gene targeting replacing the endogenous murine *Prnp* gene for an alternative *Prnp* gene[22,24].

The differences between these lines should be considered when using them as tools for strain typing and for assessing the species barrier as the level of the PrP protein might alter the susceptibility of a host to a particular agent. The bank vole has also proven over recent years to be a useful model for strain determination due to its apparently universal permissibility to multiple prion strains[25–27]. In addition to traditional strain typing criteria, the glycosylation pattern of PrP^{Sc} can also be used as an indicator of a TSE strain as many but not all strains have a unique profile of di, mono and unglycosylated PrP^{Sc}[28,29]. Different strains may also display different properties when amplified in *in vitro* assay systems such as protein misfolding cyclic amplification (PMCA) and real time quaking induced conversion (RTQuIC). Use of these tools in combination with *in vivo* strain typing can thus provide a powerful method of strain typing.

Host /agent Determinants of Transmission

What defines the ability of a prion strain to transmit within or between species? Some transmit readily within and between species e.g. scrapie and CWD whereas some are very hard to transmit e.g. sCJD and some agents may not transmit at all between individuals. The only known zoonotic prion agent to date is BSE. Understanding the interaction between a host and agent is important in evaluating a species barrier but there are many aspects of this interaction that are not well understood.

While the route and dose of inoculum have some influence over the disease process many other aspects are also important. The ability of an agent to replicate in the periphery and to be transported from the periphery to the central nervous system (CNS) will influence its ability to sustain an infection. One of the most studied aspects of host susceptibility is the *Prnp* genotype of the host and polymorphisms controlling susceptibility and resistance has been elucidated for many species[30–32]. *Prnp* sequence similarity between host and agent often leads to high susceptibility and differences lead to enhanced resistance but this is not always a good predictor of transmission potential of a particular agent[33,34]. While some genotypes can provide resistance to one prion strain with a different strain these may be the most susceptible animals. This is particularly well portrayed in sheep with susceptibility and resistance to classical and atypical scrapie where the genotypes leading to resistance with one agent result in susceptibility to the other[35]. In humans, the codon 129 methionine/valine polymorphism in the human *PRNP* gene has been well studied in at both population level[36], in infected humans[32] and in experimental models and has been shown to influence both susceptibility and pathology of disease[22].

Defining Distribution of Infectivity and Routes of Transmission

Possible routes of transmission vary for host agent combinations for example BSE in cattle affects the CNS but shows little involvement of the peripheral tissues. The oral route of transmission through infected meat is therefore considered the most likely manner in which humans acquired vCJD. However when BSE was experimentally transmitted to sheep both CNS and peripheral tissues harbored infectivity[37] and BSE transmission to humans in the form of vCJD has shown a very different distribution of infectivity to that of BSE in cattle[38–41]. This widespread distribution of infectivity in humans has led to transmission of infectivity through blood transfusion which has been demonstrated both experimentally[42–46] and in patients with vCJD[47–49]. Indeed the blood transfusion route appears to be a highly efficient route of prion transmission[46]. CWD in deer appears to have an extensive involvement of peripheral tissues[50–52] and therefore there are many routes by which CWD can be transmitted both within and between species. Understanding the distribution of infectivity is therefore an important prerequisite for defining and testing possible routes of transmission.

Adaptation of a Strain within a Host

Once a prion strain has transmitted across a species barrier it generally adapts to its new host and onward transmission within that host becomes more efficient with a greater number of individuals succumbing to disease and shorter incubation times than those observed in the cross species transmission. The ability of a strain to adapt to a new host can in part be modelled in transgenic mice or *in vitro* conversion assays[53](**Fig. 2**). With multiple lines of mice expressing *Prnp* from different species a single transmission from one species to another can give some indication of the likelihood

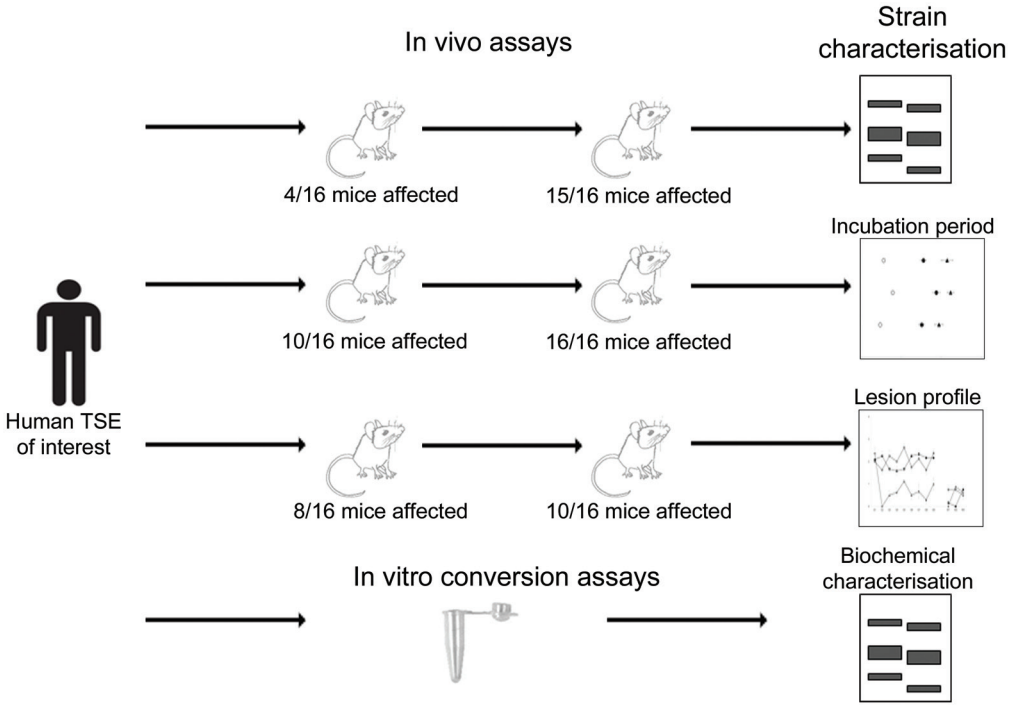

Fig. 2. Assessing adaptation of a TSE agent *in vivo* and *in vitro*. Serial passage through mice demonstrates higher susceptibilities and strain characteristics can be determined. *In vitro* assays can give a biochemical assessment of transmission and adaptation.

of transmission to that species. To assess the adaptation further transmissions in the same line of mice often demonstrate reductions in incubation times and higher susceptibilities. There is one notable exception to this, however, in vCJD which when passed through gene-targeted transgenic mice expressing the human *PRNP* gene shows no adaptation and indeed with passage the numbers of mice showing pathology decreases. This suggests that humans may not be a good host for vCJD (Diack, personal communication).

Determining Zoonotic Potential of a prion Agent

To date the only known zoonotic prion agent is BSE, which transmitted to humans as vCJD[54,55]. Other agents such as scrapie have been proposed to be potential zoonotic agents[56] and concern over the possibility of CWD transmitting to humans has been raised, and the potential for this examined both *in vivo*[57–59] and *in vitro*[60,61]. There is no simple method to assess the zoonotic potential of a prion, but, knowledge of surveillance, strain identification and potential routes of transmission are all important in making such an assessment. Moreover, there are a number of *in vivo* and *in vitro* systems that can provide useful information to aid in assessing the potential zoonotic risk. The *in vitro* conversion assays using material containing a form of human PrP as a template for misfolding can provide information of the ability of human PrP to replicate PrP^{Sc} from other species. Indeed the PMCA assay has been used extensively in this respect and results from this assay have suggested no barrier to transmission between BSE and CWD, but scrapie does not appear to readily amplify human PrP[60]. However *in vitro* amplification systems cannot encompass all the elements that make up the species barrier and *in vivo* studies are also an important component of assessing risk. Non-human primates have been used for assessing zoonotic potential but the most common and widely used tool is transgenic mice expressing the human *PRNP* gene with different polymorphisms at codon 129. Many different lines of these transgenic mice exist with different copy numbers and different sites of integration into the mouse genome. BSE has transmitted to some but not all of these mouse lines[22,62] and has been transmitted to macaques[63,64]. Scrapie has also been shown to transmit to mice overexpessing the human PrP gene[56] and has also been demonstrated to transmit to squirrel monkeys and a cynomolgus macaque[65,66]. Conflicting evidence exists for the transmission of CWD to transgenic mice expressing the human *PRNP* gene and it has been shown to transmit to squirrel monkeys but not macaques[67,68]. These results therefore pose a

Table 1. Assessing zoonotic potential of TSE agents

		BSE	Scrapie	CWD	
Overexpressing human PrP transgenic mice	+	+ [62, 69]	+ [56]	+ [70]	-
Gene targeted human PrP transgenic mice	+	− [22]	ND	− [57]	-
Transmission to primates	+	+ [63, 64]	+ [65]	+ [66, 71]	-
In vitro conversion assays using humanised substrate	+	+ [60, 61]	− [60]	+ [60]	-
Likelihood of zoonotic transmission	**Likely**	**Yes**	**?**	**?**	**Unlikely**

There are a number of different ways in which to assess zoonotic potential. Using a combination of evidence from both surveillance and research using *in vivo* and *in vitro* studies gives the best indication of the likelihood of zoonotic transmission. As shown in this table, if all studies give positive (+) results this could be taken as an indication that the risk of zoonotic transmission is high or likely. In contrast, all negative (−) results would indicate that the risk of zoonotic transmission is low or unlikely. Abbreviations: BSE, Bovine Spongiform Encephalopathy; CWD, Chronic Wasting Disease; ND, not done

dilemma since all three agents show positivity in at least two assay systems, which, while not proof that transmission to humans will occur makes it difficult to rule out the possibility of zoonotic transmission.

Summary

The many elements that make up the species barrier are still not fully understood. However, there are many tools within research to examine the possibility of transmission of an agent within and between species. While *in vivo* studies remain an important and vital tool in this assessment, the more recently developed *in vitro* assay systems provide important rapid additional information on the replication ability of the agent with different host species and genotypes with those species. A combination of both surveillance and strain typing can assist in the identification of new strains and compare new strains with old strains. Both *in vivo* and *in vitro* studies allow an assessment of the transmission potential to different species and the influence of the host genotype on host susceptibility. The potential for zoonotic transmission can most fully be assessed using all the tools available and by taking all the evidence from both surveillance and research studies into account (**Table 1**).

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