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Review article

Approaches to investigating transmission of spongiform encephalopathies in domestic animals using BSE as an example

Marion Mathieson SIMMONS[1]*, John SPIROPOULOS[1], Stephen Anthony Charles HAWKINS[1], Susan Jane BELLWORTHY[1], Susan Carol TONGUE[2]

[1] Pathology Department, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK
[2] Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK

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Abstract – Bovine spongiform encephalopathy was a novel spongiform encephalopathy, in an hitherto unaffected species, that had characteristics of a point source epidemic, with an agent that could have been incorporated into a wide variety of feedstuffs and iatrogenically administered to naïve populations, and there was early evidence that it was not restricted to bovines. It was vital to establish, albeit experimentally, which other species might be affected, and whether the epidemic could be maintained by natural transmission, if the source was removed. In contrast, scrapie has been endemic throughout Great Britain for centuries, is maintained naturally (even if we don't know exactly how) and has a known host range. The principles, process and integration of evidence from different types of studies, however, are similar for both of these transmissible spongiform encephalopathies (TSE) and can be applied to any emerging or suspected spongiform encephalopathy. This review discusses the experimental approaches used to determine TSE transmissibility and infectivity and how they relate to natural disease and control measures.

TSE / transmission / natural / experimental / domestic animals

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* Corresponding author:
m.m.simmons@vla.defra.gsi.gov.uk

1. INTRODUCTION

1.1. Spongiform encephalopathies of animals

The spongiform encephalopathies of animals include scrapie, chronic wasting disease (CWD), transmissible mink encephalopathy (TME), bovine spongiform encephalopathy (BSE), feline spongiform encephalopathy (FSE), the spongiform encephalopathies seen in non-domestic captive ungulate species such as eland, oryx and greater kudu, and captive ostriches [85–87]. Spongiform change can also be seen in other diseases, such as rabies, other viral diseases [14, 29, 88], and hepatic encephalopathies. They may be encountered as a genetic or congenital problem [62, 63, 102], as an incidental finding in normal sheep [126], or even as an artefact [108].

However, the only observed natural animal-to-animal transmission of a spongiform encephalopathy occurs in ruminants: scrapie in small ruminant species, CWD in deer and elk, and possibly BSE in small ruminants (although this latter example has only been observed in an experimental flock [8]). Natural spongiform encephalopathies in other species, including humans, are either genetic in origin (e.g. Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia) or have been linked predominantly with an idiopathic transmission mechanism i.e. exposure to contaminated feedstuffs (TME, BSE, FSE, and kuru in humans). There is no recorded occurrence of spongiform encephalopathies being able to transmit effectively within non-ruminant species.

The naturally occurring transmissible spongiform encephalopathies (TSE) are invariably fatal, have long incubation periods and provoke no overt immune response in the host. In some, such as scrapie, there are known genetic effects on whether exposure leads to the development of clinical disease [98, 100]. Additional factors that may affect host susceptibility have been proposed [25, 45, 93] and there could be other unconfirmed, or even as yet unidentified, factors that might affect host susceptibility.

1.2. Aim and objectives

An integral part of the classification of spongiform encephalopathies is whether they are transmissible or not. If it is possible to experimentally transmit "to pass or hand on" [4] i.e. transfer the disease, then it has the potential to be naturally infectious. An infectious disease is one that is due to the "transmission of a specific agent, or its toxic products from an infected person, animal, or reservoir to a susceptible host, either directly or indirectly through an intermediate plant or animal host, vector, or the inanimate environment" [71]. This has implications for disease control strategies; different approaches will be needed if there is an infectious component than if the disease was purely due to a nutritional or genetic cause. It should also be noted that an infectious disease may not be contagious – where contagious is defined as "the communication of disease by direct or indirect contact" i.e. it is communicable to other individuals [3].

Experimental approaches to the investigation of whether transmission occurs have become more sophisticated since the start of the 20th century when Cuillé and Chelle first achieved experimental transmission of sheep scrapie via the conjunctival route in France in 1936 [17, 18]. This experimental evidence of transmissibility was confirmed, somewhat unintentionally, by the iatrogenic transmission of scrapie from sheep to sheep via the medium of

a louping ill vaccine, which led to outbreaks in Great Britain during the 1930s [41].

In this review on the transmission of TSE in animals our first objective is to illustrate the route to the designation of a spongiform encephalopathy as "transmissible", through the example of BSE in the 20th century. The knowledge that a spongiform encephalopathy is transmissible then leads to the question of the relevance of experimental findings to the field situation, where the required outcomes are public health protection, disease control and, ultimately, disease eradication. This then is our second objective; to put transmissibility into a "real-world" context. Scrapie and BSE are our main examples, with other TSE of animals referred to where appropriate. We also aim to briefly highlight some of the challenges and unanswered (or unanswerable) questions that are inevitably raised when a novel spongiform encephalopathy is encountered, and its ability to transmit is investigated.

1.3. Definitions

- PrP^{Sc}: "Prion protein". An abnormal isoform of a naturally occurring host protein (PrP^{C}) which is resistant to proteolysis.
- End-stage/clinical disease: presence of clinical signs and PrP^{Sc} in brainstem and/or lymphoreticular system (LRS).
- Positive animal: PrP^{Sc} detectable, regardless of location (i.e. central nervous system (CNS), peripheral nervous system, lymphoreticular system) or clinical status.
- Exposed animal: known challenge with positive material, or contact with positive animals or a contaminated environment. May or may not also be in one of the categories above.
- Negative animal: no detectable PrP^{Sc} in any tissue tested (must include CNS (if animal dead) and/or LRS).
- Negative control: animal from a flock or farm with good records, no recorded TSE and a feeding history which does not include meat and bone meal supplements.
- Vertical transmission: transmission from one generation to the next via the germline or in utero [11].
- Horizontal transmission: lateral spread to others in the same group and at the same time; spread to contemporaries [11].
- Maternal transmission: there is some difficulty in separating possible horizontal and vertical components to transmission involved with the dam-offspring relationship, and so the term "maternal transmission" is often used in discussion of the transmission of scrapie, maternal transmission being defined as transmission before or immediately after birth.

2. CONFIRMATION OF DISEASE AND/OR INFECTION

The absolute nature of the infectious agent poses a unique challenge and is still a contentious issue. Accumulations of disease-specific prion protein (PrP^{Sc}) in the CNS can be demonstrated in all cases of clinical disease, so the detection of PrP^{Sc} is now used to confirm the disease status of a clinically suspect case at post-mortem [76]. PrP^{Sc} accumulations in a variety of tissues can also be seen in the absence of clinical signs and the demonstration of their presence is generally considered as evidence of exposure and infection. However, such PrP^{Sc} accumulation occurs relatively late in the incubation period of the disease [6, 117], so this reliance on the presence of PrP^{Sc} limits in vivo diagnosis of disease, and surveillance for evidence of exposure or infection, with current diagnostic tools [76]. The currently accepted paradigm is that accumulations of PrP^{Sc} are not only associated with disease, but are also associated with transmission and infectivity [92]. Whether it is the sole infectious component is still a subject of some dispute. Firstly, naturally occurring PrP^{Sc}, when used for transmission experiments, is inevitably contained in a suspension of the tissue in which it originated, and therefore the existence of another factor, or factors, coexisting with PrP^{Sc}, and responsible for infectivity cannot be unequivocally excluded. Secondly, disease has been experimentally produced by tissue suspensions from potentially infected animals in which no PrP^{Sc} was demonstrable with current diagnostic tools [69]. However, in order to investigate

transmission of spongiform encephalopathies, all studies currently use the presence of PrP^{Sc} as a confirmatory marker of disease or exposure/infection.

In experimental studies of TSE, the prolonged incubation periods and the availability of resources coupled with welfare considerations may not allow for individual animals to be followed up to the ultimate fatal endpoint. For this reason there is a lexicon of terms that are applied both in experimental studies and surveillance (see Section 1.3.).

3. THE SEARCH FOR EVIDENCE OF TRANSMISSION OF BOVINE SPONGIFORM ENCEPHALOPATHY

Experimental transmission studies in a wide range of recipient species have established that many species are susceptible to parenteral exposure with positive tissue from TSE cases under experimental circumstances (e.g. cattle, sheep, goats, cats, mink, deer, elk, exotic ungulates, primates, laboratory rodents). Detailed reviews of these transmissions have been published recently [52] and will not be repeated here.

3.1. Bovine spongiform encephalopathy – a TSE?

Following the identification of BSE in cattle [107] and its epidemiological link to contaminated feed [118, 119], the major transmission questions to be addressed, as in any other new disease, were:

- Can it be transmitted?
- Who or what can it be transmitted to in order to determine the potential host range, which food animal species are susceptible, and if there is a public health risk?
- How much is required to achieve transmission/infection, to define infectious dose and host susceptibility?

Then, if and when transmission is achieved:

- What is the pathogenesis of the resulting disease, what is the earliest time at which evidence of exposure can be detected and in which tissue(s)?
- What are the possible routes and mechanisms of transmission under natural as well as experimental conditions?
- What is the relative importance of identified routes and mechanisms in the transmission of the disease under natural conditions in the original host and other species?

Only then can fully effective steps be taken to intervene and minimise any risks to public or animal health that may arise.

3.2. Experimental transmission studies

3.2.1. Artificial exposure – artificial routes

Some of these questions were addressed for BSE initially by experimental transmission studies (see Tab. I for details [6, 8, 9, 20, 21, 26, 32, 35, 46, 49, 54, 58–60, 72, 83, 97, 109–114, 116, 117, 124]). In the case of BSE, a sense of urgency accompanied these investigations, partly as a consequence of the subsequent emergence of similar disease in a range of other species [57, 64, 125], and partly because infected animals would have entered the human food chain. Historically the most efficient transmission route to use to provide an indication of potential host range susceptibility was that of intracerebral inoculation (i.c.). Initial studies established that transmission of disease to food animal species using CNS tissues from natural cases of BSE in cattle was possible to cattle, sheep, goats and pigs but not chickens.

Table I summarises the experimental challenges that have been undertaken using cattle BSE as a source, and food animal species as recipients. A similar range of studies could be listed for other donor species/strains (in particular scrapie and CWD), and indeed for BSE challenges into non-food animal recipients, but exhaustively listing these is considered to be beyond the scope of this paper.

3.2.2. Artificial exposure – natural routes

The next stage was to establish if susceptibility could also be demonstrated by more natural routes of infection. The natural route(s) for the transmission of TSE in the field is still not known, but for most experimental purposes the oral route is considered appropriate.

Table I. Food animal species susceptibility to BSE – summary of experimental transmissions from bovine tissue.

Recipient species/ genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Cattle	i.c./i.v.	Brain	0.1/0.5	N/A	16	4–5 months	74–90 weeks	Proof of transmissibility within species[1] [20]. End stage disease looks the same as natural disease regardless of route [110]
Cattle	Oral	Brain	100	$10^{3.46}$	30	4 months	Timed kills	Pathogenesis of BSE in original host [111]. BSE infectivity identified in the CNS, ileum [109] and bone marrow [112] of pre-clinical cases. Endstage disease after experimental challenge is the same as natural disease [46, 110]
Cattle	Oral	Brain	3 × 100, 100, 10, 1, 0.1, 0.01, 0.001	$10^{3.5}$	10–15 per group (total n = 90)	4–6 months	34–98 months	Determination of LD_{50} and minimum infectious dose of BSE in cattle [117]. Establish attack rate for interpretation of pathogenesis study [6]. Establish minimum effective dose for epidemiological modelling. Confirm that experimental endstage disease looks the same regardless of dose and incubation period (Simmons, unpublished data)
Cattle	i.c.	Brain	Log dilutions	N/A	24 (4 per group)	4 months	20–39 months	Comparative titration BSE in cattle and mice showed that cattle approx. 500 times more sensitive than mice (Cattle 10^{6}, mice $10^{3.3}$)[2]
Cattle	i.c.	Range of tissues from initial pathogenesis study time kills	0.1	N/A	325 in groups of 5	4–6 months	23–45 months depending on tissue	In addition to CNS, palatine tonsil [114] and nictitating membrane (Wells, Hawkins, unpublished data) harbour BSE infectivity in cattle. The majority of peripheral tissues assayed were negative (Hawkins, unpublished data)
Cattle	Oral	Brain	100 – 3 × 100	$10^{2.86}$	24	6 months	Time kills	Early pathogenesis and the involvement of Peyer's patches in the distal ileum [97]

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Cattle	Oral	Brain		N/A	56	4–6 months	Time kills (ongoing)	Pathogenesis in original host [49]
Cattle	Oral	Brain	100 or 1	$10^{3.1}$	200	4 months	Timed kills up to a clinical endpoint of 30–78 months	Pathogenesis of BSE in original host. Distribution of tissue infectivity in cattle using a range of statutory screening tests to ensure that SRM controls remain appropriate [6]. Provision of tissue bank (including milk) for future test evaluation. End-stage experimental disease looks like end-stage natural disease (Hawkins and Simmons, unpublished data). No PrP^{Sc} in milk from affected animals [26]
Cattle	Oronasal	Foetal membranes	90 mL oral, 5 mL nasal of a 50% homogenate	N/A	12	2–3 months	Animals survived to 7 years	No demonstrable infectivity in foetal membranes[2] [20]
Cattle	Embryo transfer	Live embryos from clinically affected donors	N/A	N/A	347	Young adult	N/A	BSE cannot be transmitted through embryo transfer [124]
Sheep (positive and negative line Cheviots)	i.c.	Brain	0.5 mL of 10% homogenate	N/A	11	6–18 months	440–994 days	Sheep are susceptible to BSE, including sheep not universally susceptible to scrapie [32]
Sheep (positive and negative line Cheviots)	Oral	Brain	50 mL of 1% homogenate	N/A	12	6–18 months	538–994 days	Sheep are susceptible to BSE by this route [32]
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	20	6 months	664-909 days	Distribution of infectivity in positive sheep [59]. Important for SRM and risk analysis. Verification that the BSE/scrapie discriminatory tests work in the ARQ/ARQ genotype [58]

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Sheep ARQ/ARQ	Oral	Brain	5, 0.5, 0.05, 0.005, 0.0005	$10^{3.97}$	120	3–6 months	Ongoing at VLA	Establishes minimum infectious dose of BSE in sheep by oral route, contributing to epidemiology and risk models. Endstage disease is the same regardless of dose (Bellworthy, Jeffrey, unpublished data)
Sheep ARQ/ARR ARR/ARR	Oral	Brain	5 g	$10^{3.97}$	20 each	6 months	Ongoing at VLA	Are these genotypes susceptible by the oral route? Relevant for genotype-based disease control strategies. What is distribution of infectivity if they are? Is there any evidence of carrier state?
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	30	6 months	569–1 058 days	Provision of material for statutory controls and other requests. Provision of milk from sheep with BSE. Create a BSE affected flock to establish if transmission could occur to in-contact animals and lambs [8]
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	8	2 weeks	535–824 days	Lower age at challenge reduces spread of incubation period, but does not shorten the minimum incubation period (Bellworthy, unpublished data)
Sheep AHQ/AHQ	Oral	Brain	5 g	$10^{3.97}$	5	6 months	568–665 days	Susceptibility and end-stage disease in particular genotype. Relevant for genotype-based disease control strategies. Verification that discriminatory tests work in the genotype. Contribution to BSE "flock" [8]
Sheep VRQ/VRQ	Oral	Brain	5 g	$10^{3.97}$	5	6 months	2 clinical suspects at 1 570 days	Susceptibility of particular genotype. Relevant for genotype-based disease control strategies. Verification that discriminatory tests work in the genotype. Contribution to BSE "flock" [8]
Sheep ARQ/ARQ VRQ/VRQ VRQ/ARQ ARQ/ARR ARR/ARR	i.c.	Brain	0.05 g	N/A	19 (ARQ/ARQ) 10 (VRQ/VRQ) 10 (VRQ/ARQ) N/A (ARQ/ARR) 19 (ARR/ARR)	N/A	495–671 days (ARQ/ARQ) 881–1 092 days (VRQ/ARQ and VRQ/VRQ) 1 008–1 127 days (ARR/ARR)	The ARQ/ARQ, VRQ/VRQ, VRQ/ARQ and ARR/ARR genotypes of sheep are all susceptible to infection with BSE, with shorter incubation period (by this route) in ARQ/ARQ than other genotypes challenged [54]. There were survivors in all genotype groups at the time of publication. Sheep with resistant PrP genotypes are susceptible to BSE [54]. Potentially relevant for genotype-based disease control strategies

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Sheep ARQ/ARQ	Intraperitoneal/ intrasplenic	Brain	N/A	N/A	1 for each route	N/A	672 days and 1 444 days	Widespread peripheral tissue involvement, including muscle [9, 72]
Sheep ARR/ARR	Intraperitoneal/ intrasplenic	Brain	5 mL of 10% homogenate	N/A	1 for each route	N/A	No clinical disease	BSE-related PrP can accumulate in tissues of "scrapie resistant" sheep without any clinical signs. Evidence of potential carrier state? [9, 83]
Goats	i.c.	Brain	0.5 mL of 10% homogenate	N/A	3	4–6 years	506–570 days	Species susceptible [32]. Define end-stage disease [32, 35]
Goats	Oral	Brain	50 mL of 1% homogenate	N/A	3	2–5 years	941–1 501 days	Species susceptible by oral route (one survivor) [32]. Define end-stage disease [32, 35]. Discriminatory tests work in this species [60]
Pigs	i.c./i.v./i.p.	Brain	0.5 mL/1.2 mL/ 8–9 mL	N/A	10	1–2 weeks	69–150 weeks	Species susceptible [21]. Define end-stage disease [113]
Pigs	Oral	Brain	3 × MBM ration equivalent for an 8 week old pig	$10^{2.4}$	10	7–14 weeks	Time kills at 2 and 7 years	Species not susceptible to experimental challenge by this route [113]
Chicken	i.c./i.p.	Brain	50 µL/1 mL of 10% homogenate	N/A	12	1–14 days	N/A Survived up to 5years	Species not susceptible [116]
Chicken	Oral	Brain	5 g on 3 occasion	N/A	11	4–6 weeks	N/A Survived up to 5years	Species not susceptible to experimental challenge by this route [116]
Deer	i.c.	Brain	0.05 g	$10^{3.3}$	6	10–12 months	794–1 060 days (one still alive)	Species susceptible. Define endstage disease (Jeffrey M., personal communication)
Deer	Oral	Brain	25 g	$10^{3.1}$	18	4–6 weeks	Time kills	Is this species susceptible by this route? Ongoing. Negative to date – 4 years post challenge (Jeffrey M., personal communication)

* Mouse (i.c./i.p.) units LD_{50}/g.

[1] Dawson M., Wells G.A., Parker B.N.J., Scott A.C., Transmission studies of BSE in cattle, hamsters, pigs and domestic fowl, in: Bradley R., Savey M., Marchant B. (Eds.), Subacute spongiform encephalopathies, Proc. of a seminar in the CEC Agricultural Research Programme held in Brussels, 12–14 November 1990, Kluwer Academic Publishers, 1991, pp. 25–32.

[2] Hawkins S., Wells G., Austin A. et al., Comparative efficiencies of the bioassay of BSE infectivity in cattle and mice, in: Proc. of the Cambridge Healthtech Institute's 2nd Int. Transmissible Spongiform Encephalopathies Conference, 2–3 October 2000, Alexandria, VA, USA, 2000.

For BSE, epidemiological studies indicated that the oral ingestion of food contaminated with infected ruminant-derived protein, in the form of meat and bone meal (MBM), by cattle was likely to be a major route of transmission [118, 120]. Oral challenge studies showed that transmission of BSE was possible to sheep, goats and cattle by this route [32, 59, 111] and with very low challenge doses [117]. Transmission to pigs or chickens however was not achieved by this experimental route [113, 116].

One difficulty that arises with transmission experiments is the interpretation of a negative result; does it mean that transmission does not occur, or just that in this particular scenario it hasn't? The latter then raises questions as to why it may not have occurred. Is it the dose, route, or are there other factors involved such as species barriers or genetic influences? Given that BSE could be transmitted to pigs by the i.c. route, the absence of BSE transmission to pigs by the oral route indicated that there may be an effective species barrier, but a particular confounding factor in this type of study is that the infectious "dose" of any challenge inoculum is difficult to establish objectively. In most cases, inoculum titre (if known) is quoted as mouse LD50/g, using conventional inbred mice. However, we know that different hosts are differently susceptible [88] and that some TSE isolates do not transmit to particular species (including mice). Any experimentally-established titre is inevitably relative, and not necessarily informative for the recipient species in a particular experiment. Attempts to determine PrP^{Sc}concentration biochemically as a measure of titre are also limited by the assumption that PrP^{Sc} is an accurate and quantifiable marker for infectivity.

Conversely, a positive result in a transmission experiment only means that transmission can occur, not that it *does* in field conditions. It also leads to further questions. One is the relevance of such experiments to the field situation. There are a number of fundamental differences between natural exposure and experimental studies which should not be overlooked when comparing disease models with field cases. In natural disease, the incidence of TSE can be low but in experimental disease the aim is to achieve 100% morbidity, especially if the study contains a time-kill element. Very high doses can be given orally and such experimental exposures result in much higher attack rates than are observed naturally [117]. Time-kill approaches can then be used to study the disease pathogenesis in an experimental model, although it is not known what effect dose may have on pathogenesis. It is reassuring, therefore, that the end-stage disease resulting from such experimental exposure of cattle with BSE is virtually indistinguishable from natural cases in all but morbidity [46].

This experimental approach also assumes an oral route of transmission in the field, which is a reasonable assumption for BSE, given the clear epidemiological links with contaminated feed. However, the infectious material in feed has been subjected to a range of manufacturing processes and heat treatments in the course of MBM production, and experimental studies often use "neat" brain material (untreated) to achieve the best morbidity, since rendering has been shown to reduce titre [27, 91, 94–96]. The possible effects of rendering on the basic biological properties of any given TSE strain are very difficult to define, and almost impossible to control for in any experimental design.

It has also been suggested that age at exposure can affect susceptibility [5], but most experimental designs have focussed on a restricted range of ages at challenge from a logistical point of view.

None of these factors negates the data emerging from such experimental studies, as the studies provide a starting point. Once transmission has been achieved, further experimental protocols can be used to investigate aspects such as minimum effective doses [117], and inoculum can be treated to mimic more closely what is occurring in the field [95]. Data derived from transmission experiments can also be used in risk analyses and mathematical models, both of which may be used to inform the development of appropriate control strategies for TSE in animals, and thus to protect public and animal health. Further studies can also be implemented, as they were with BSE, to investigate hypotheses of the origin of

the disease (for example scrapie to cattle [66]), but countless variables prevent this approach from being comprehensive.

3.2.3. Natural transmission

With experimental confirmation that transmission is possible by a particular route, further investigation of the contribution of that route to natural transmission is vital.

For BSE it was clear that the principal driver of the epidemic was the feeding of contaminated MBM [120] – once relevant control measures were introduced the epidemic in Great Britain began to decline [50, 122] – but it was not initially known whether the disease could be sustained within an affected population by other natural or management means.

Evidence from a cohort study did not rule out the possibility of a maternal component to transmission [121]. The risk of developing BSE was slightly increased by being born to a dam approaching the clinical phase of the disease. Whether it represented genetic susceptibility, transmission or a combination of the two could not be determined. However, mathematical modelling indicated that if maternal transmission did occur, then it was highly unlikely to be at a rate that could sustain an epidemic [23]. The route through which such exposure might take place, whether it was due to true vertical transmission, or horizontal transmission through close contact also could not be established from the cohort study. A long-term large-scale experimental study to investigate the possibility of vertical transmission indicated that, "when appropriate sanitary protocols" were followed, "embryos derived from BSE-affected cattle did not transmit the disease" [124].

Ultimately for BSE in cattle the relative importance of the role of feed contaminated with infected MBM was confirmed, and the relative absence of evidence for maternal transmission [23] has enabled effective disease control interventions to be implemented.

4. BSE IN SMALL RUMINANTS

The positive results of oral transmission experiments to sheep and goats [32, 59], and the identification of a single natural case of BSE in a French goat [24] have, however, raised a new challenge: that of BSE in small ruminants. For Great Britain, this raised a concern about the national sheep population. With, hopefully, no naturally-occurring disease to study there remain only three alternatives.

Firstly, to set up small-scale animal experiments (as previously described) to investigate potential routes and mechanisms of transmission; secondly to set-up larger-scale natural transmission investigations, such as an experimental sheep flock; and thirdly, to find an alternative natural disease model that can be studied in the field.

4.1. Direct experimental exposure

Transmission of BSE in small ruminants by blood transfusion has been studied by the first method. Whilst experimental BSE can be transmitted by whole blood transfusion [53], this probably has more relevance in the establishment of a precedent for the protection of public health in the context of human-to-human transmission [1], rather than as a potential iatrogenic route in small ruminants.

4.2. Natural transmission experiments

The second method (the experimental sheep flock) has been used by both the Veterinary Laboratories Agency (VLA) and the Institute for Animal Health Neuropathogenesis Unit (NPU) in Edinburgh. The VLA has an experimental BSE-in-sheep flock in which lambs born to ewes that were orally dosed with 5 g of BSE-positive cattle brain have succumbed to clinical disease [8]. The age at onset for these lambs ranged from 654 to 968 days old. In all cases the birth of the lambs occurred within a few months prior to the onset of clinical disease in their dams. Thus we have evidence of natural transmission of BSE from sheep to sheep, albeit in experimental circumstances. Whether this represents true vertical or perinatal infection cannot be ascertained from this study. A similar but slightly different NPU study [36] did not result in transmission, however it could not be statistically ruled out.

4.3. Alternative disease models

The third method, to find an alternative natural disease model that can be studied in the field, is more problematic. Studies of the natural transmission of the only known naturally-occurring TSE of small ruminants, scrapie, might provide a model for BSE in sheep, should it occur under field conditions. Both scrapie and experimental BSE in sheep have similar clinical signs and they have similar diffuse tissue distributions of PrP^{Sc} [34, 35, 59, 115]. If natural ovine BSE is similar to experimental ovine BSE, then ovine BSE may potentially behave in a similar manner to scrapie as far as routes and mechanisms of transmission are concerned.

4.3.1. Scrapie

This is the most extensively studied TSE model. Several institutions have established, maintained and recorded naturally infected flocks of sheep in order to study various aspects of scrapie, including its transmission. These include the INRA Langlade flock of Romanov sheep, various Institute for Animal Health flocks and the VLA scrapie-affected flock.

Analyses of data collected over more than a decade from the first of these have provided epidemiological evidence for both a maternal and lateral component of transmission [22, 99]. Higher relative risks of clinical scrapie were observed associated with lambing periods. There was also a reduced risk of clinical scrapie in artificially-reared lambs from healthy dams, and an increased risk in maternally-reared lambs from scrapie-affected dams. They proposed that transmission may occur within the first 24 h of life with additional risk for those that then continue to share the maternal environment (all lambs remained on their dams for the first intake of colostrum and then for 24 h).

The Institute for Animal Health flocks have established that, despite earlier contradictory findings [30, 31, 33], true vertical transmission of ovine scrapie (via the germ-line or in utero) is improbable [36, 37]. A scrapie-free flock has been established by embryo-transfer (ET) from one with a long-standing scrapie problem. The ET-derived flock has remained scrapie-free since its establishment in 1996, even though it has a similar *PrP* genetic profile to the original flock. Of interest to mechanisms of horizontal/lateral transmission is the fact that the "clean" flock was established and maintained in a scrapie naïve environment; a parallel ET-derived flock that was maintained in close proximity to, but separate from, the original scrapie-affected flock did experience clinical scrapie cases [37]. Lateral transmission has also been shown to occur in the absence of lambing [38].

In the VLA flock it has been shown that lateral transmission occurs [84] and that exposure to a contaminated environment only is sufficient to produce disease (Dexter, Tongue, Bellworthy, unpublished data).

These flocks are managed in a way that maintains high frequencies of sheep with PrP genotypes at high risk of developing clinical disease. Thus with a high incidence of clinical disease and high infectious load, they also provide controlled environments in which to study the pathogenesis of naturally acquired disease. They effectively counter the difficulties of studying a disease that occurs at a low flock-level incidence, however it must be recognised that whilst they provide evidence for routes and mechanisms of natural transmission and estimates of transmission parameters, they are probably not representative of any but the most heavily affected (worst-case scenario) commercial flocks. They are also limited in the range of breeds present, and (potentially) in the number of different scrapie isolates/strains present. These flocks may mimic natural exposure, but at a level that no commercial flock-owner would be able to tolerate and remain as an economically viable unit. Because of this the relative importance of different components of transmission may vary in commercial field flocks and therefore intervention measures may have different outcomes. These institutionalised research flocks, therefore, act as an important bridge between the artificial exposure – natural route transmission experiments – and the true field situation.

A variety of experimental studies using the approaches outlined above have provided

evidence for possible routes of transmission of scrapie. PrP^{Sc} has been found in tissues that could be involved in the natural dissemination of the infectious agent i.e., routes that could lead to exit of the infectious agent from the animal, and result in either environmental contamination or direct transmission. These tissues include the lympho-reticular system of the gut [40, 103, 115], chronically inflamed mammary tissue associated with lymphocyctic mastitis [73], kidney tissue [90], salivary glands [104], nictitating membrane [77], and placentae [2, 81, 101].

For the majority of these tissues, evidence of infectivity or the presence of PrP^{Sc} in associated secretions and excretions is still elusive for scrapie in small ruminants. The exception is blood [55]. Although experimental blood transfusions have resulted in clinical scrapie [55], just as with BSE, it is unlikely to play a major role: blood transfusions are not regular occurrences in sheep veterinary practice.

On the other hand, not only has PrP^{Sc} and infectivity been demonstrated in placentae [3, 81, 101], but it has also been shown to produce clinical scrapie when administered orally to sheep [78, 79]. This was proposed by the authors as a mechanism for lateral transmission from ewe to ewe at lambing time. Placenta has also been cited as a possible explanation for some of the epidemiological findings thought to be associated with mechanisms of maternal transmission [74], although much of the epidemiological evidence may also be interpreted as a contribution to transmission via the lateral route, especially that of environmental contamination. For example, there are reduced odds of ever becoming a scrapie-affected flock if the flock sometimes lambs in different places, compared to those flocks that always lamb in the same place [74]; there is decreased risk of disease associated with lambing in individual pens [75], and there were increased odds for scrapie-positive status of a flock that was found to be associated with failure to remove placenta from bedding along with its disposal in compost.

Epidemiological cross-sectional [74, 75] and case-control studies [47, 51, 80] have provided supporting evidence for the role of various allied management practices in the transmission of scrapie in the field. So far they lack the consistency and specifics necessary for the development of appropriate intervention measures. The scrapie literature does however illustrate how the different types of investigations into aspects of transmission, and the different disciplines, are complementary. Experimental studies of transmission routes and epidemiological studies of risk factors are intrinsically linked in a positive feedback loop, each informing the other.

4.3.2. Chronic wasting disease

The other naturally occurring TSE, CWD of deer is probably less relevant as a model for BSE in small ruminants, has been recently reviewed elsewhere [123] and is covered by Sigurdson in this special issue [89].

4.3.3. Other disease models

Host-specific experimental studies in large animals are expensive and do take time to produce results. The former means that they are difficult to fund. The latter means that they may have to be run in parallel with other experiments, often with more start-up assumptions than desirable, rather than in a logical step-wise order following on from previous findings. They are, however, of paramount importance. They provide an opportunity to study the disease in the original host species; they can be comparable across studies, if standardised protocols are used, and they eliminate the noise of variability, the difficulties of loss to follow-up and the potential biases that are experienced with epidemiological studies. To counter the time and resource limitations, other models have been sought.

The role of hamsters, mice, the burgeoning range of murine transgenes and other models such as voles is a large subject in its own right, and is covered by Groschup and Buschmann in this special issue [44] and elsewhere [28, 43]. In the past such models have been useful [12, 13], but they also have limitations. For example, laboratory wild-type mice cannot replace the original donor species due to the species-transmission barrier and to their different biology and physiology compared

to ruminants. The former has been addressed with the advent of transgenic mice, the latter is insurmountable. Even these do not replicate reality, and the interpretation and extrapolation of any results back to the donor/host-species needs to be a considered, objective process. For example, data from different transgenic mouse lines are not directly comparable, even between lines which have a common transgene [16, 105].

5. PUBLIC HEALTH

The ultimate question of whether a TSE has implications for public health – i.e. is transmissible to man – is difficult to address in the absence of transmission experiments on people. The most appropriate alternative is to use non-human primates [48, 67, 68, 70] which have indicated that BSE transmits with a end-stage disease indistinguishable from variant Creutzfeldt-Jacob disease (vCJD). However these experiments are limited by ethical constraints. Here the development of transgenic mice has been of prospective value, but at the same time, can be misleading. For example, mice with a single copy of the human *PrP* gene were not susceptible experimentally to BSE [10] while at the same time, epidemiological and strain-typing studies were producing a very strong body of circumstantial evidence that vCJD was a consequence of BSE infection in man. The inevitable limitation of such transgenic mice is that only one human gene is present in the model, and disease susceptibility and incubation period are inevitably multi-factorial. Transgenic mouse models which overexpress human *PrP* are also available, and they are highly susceptible to BSE [7, 15, 65, 106] but these may not be a true indicator of susceptibility in humans. Detailed discussion of these models is outwith the scope of this paper and is covered in detail by Groschup and Buschmann in this special issue [44].

6. REMAINING CHALLENGES

Many challenges remain even when a spongiform encephalopathy has been identified as transmissible, and when routes and mechanisms have been proposed.

What are the effects of repeated low dose exposure? What happens when there is intercurrent disease? How do *PrP* genetics influence the transmission process? Is any apparent reduction in susceptibility actually an effect of incubation period prolongation to beyond the natural lifespan? What is the implication of carrier state/subclinical disease for disease control and health? How can we detect animals in the early stage of disease incubation – a phase "silent" to current investigative tools?

For BSE and scrapie some of these questions have been addressed partially [39, 42, 45, 49, 56, 61]. It is possible that for novel TSE many of these questions will remain unanswered or unpursued, except by the most determined of researchers after the funding, stimulated by the public health and political aspects of BSE and vCJD, has dwindled.

Perhaps the greatest conundrum for researchers faced with a new TSE in a species, or a TSE in a species in which it has not previously been described, is whether it is "new", or merely "newly observed". This is a particular issue for BSE, should it be found in the sheep population. With much speculation over the years that scrapie could be the origin of BSE, it might not be too surprising if a detailed study of scrapie isolates revealed one with BSE-like characteristics. A number of studies in the UK and elsewhere [19, 66, 82] have taken a direct approach to this question by looking at the experimental phenotype in cattle experimentally challenged with scrapie isolates, but the diversity of scrapie isolates precludes this approach being exhaustive.

Given that no one type of study can provide all the details or all the answers required, and because of the constraints implicit in each type of study, it is important that researchers respect and integrate the work from other areas, are rigorous, do not overestimate their findings despite various pressures to do so, and are honest: both in the presentation of their findings and in the value of the outcomes. Some of those interested in pure science may disparage studies that they deem to be of low scientific merit, but which are actually of high value to those involved in policy and decision-making: equally some work of high scientific merit may

be extremely interesting in its own right, but not actually necessary to advance disease control and protect public health.

7. CONCLUSION

The approaches to the investigation of the transmission of BSE and scrapie, outlined above, differ only slightly. Those differences are due to the nature of the two diseases. BSE was a novel spongiform encephalopathy, in a hitherto unaffected species, that had characteristics of a point source epidemic, with an agent that could have been incorporated into a wide variety of feedstuffs and iatrogenically administered to naïve populations, and there was early evidence that it was not restricted to bovines. It was vital to establish, albeit experimentally, which other species might be affected, and whether the epidemic could be maintained by natural transmission, if the source was removed. In contrast, scrapie has been endemic throughout Great Britain for centuries, is maintained naturally (even if we don't know exactly how) and has a known host range. The principles, process and integration of evidence from different types of studies, however, are similar for both of these TSE and can be applied to any emerging or suspected spongiform encephalopathy.

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