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 Endstage disease is the same regardless of dose (Bellworthy, Jeffrey, unpublished data)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age at challenge reduces spread of incubation period, but does not shorten the minimum incubation period (Bellworthy, unpublished data)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 supects 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) 831–1 092 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