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研究報告の概要

本稿では，伝染性海綿状脳症（TSE）の伝播性を調べるための実験的アプローチ法を要約し，実験における所見と自然発生する TSE〔主にウシ海綿状脳症（BSE）及びスクレイピー〕との関連性を考察している。BSE はこれまで影響を受けなかった動物種における新規の海綿状脳症であり，点感染源の特徴を有する。本実験では，人工的感染経路（脳内接種）及び自然感染経路（経口）を用いて伝播の効率ならびに宿主の感受性を，特に食用動物種に焦点を当てて特定した。実験的伝播が認められても，曝露時の動物の年齢等，種々のパラメータの影響を受けることから結果の解釈は常に困難であった。しかしながら，ヒツジでは，BSE 陽性ウシの脳を経口投与した雌ヒツジから，その仔ヒツジへの BSE 伝播が示された。

これとは対照的に，スクレイピーは英国で数世紀にわたりヒツジ個体群において地域固有のものであった。それにもかかわらず，スクレイピーの真の垂直（子宮内）伝播は確認されておらず，一方で水平伝播が確認されている。すなわち，疾患を引き起こすには汚染された環境に曝露するだけで十分であると考えられる。特に胎盤はスクレイピーの自然伝播の原因とされており，感染性の PrP^{Sc} プリオンを含むことが立証されている。現時点では多くの疑問が依然として解明されておらず，結論として著者らは，様々な分野の研究者らに対して TSE の特性をより理解するため協力及び支援を強く呼び掛けている。

報告企業の意見	今後の対応
本概論は，TSE 研究の複雑さを明らかにしており，反芻動物でない，生物学上遠隔種のトランスジェニックマウスを用いた研究であっても，全ての研究結果は有益であり，疾患管理の向上及び公衆衛生を守る上で役立つであろう。	ヒトに影響するプリオン関連疾患伝播のメカニズムの更なる理解に関連した調査の情報を収集する以外，現時点で新たな安全対策上の措置を講じる必要はないと考える。

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Review article

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

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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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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 end-point 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]