EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

OPINION ON

THE SAFETY OF RUMINANT BLOOD WITH RESPECT TO TSE RISKS

ADOPTED BY THE

SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 13-14 APRIL 2000

OPINION

Animal (incl. ruminant) blood is currently legally fed to animals (including ruminants) after very gentle processes, spread on pasture as fertiliser, or used for other products that may reach man or animals (incl. ruminants). There is some concern that animal TSEs might be spread by these means or through specific blood components or blood based products that are still permitted to enter the market. It is therefore necessary to establish if animal TSEs can be transferred via blood and the SSC was requested to:

A. Assess for ruminant blood in general and if possible for each component the risk that it could harbour the BSE (TSE) agent and hence transfer the disease.

B. Identify the main ruminant blood-based products that are currently on the market, including products containing ruminant blood or those in which ruminant blood is used in their manufacture.

C. Assess for each product, the function of the blood component included and the risk that that product could transfer BSE (TSE) to ruminants.

D. Outline possible measures that could mitigate any identified risk, as far as possible together with a (qualitative) assessment of the potential impact of the measure on the risk.

In order to answer these questions, a Working Group was created which delivered a detailed report upon which this opinion is based (see attachment). On the basis of this report, the SSC elaborated the following summary account, conclusions and recommendations.

Remark: The present opinion does not address the possible risks resulting from the exposure of workers to contaminated material. The SSC is presently preparing *Notes on the safe handling, transport and storage of MBM and other bovine derived materials which may be contaminated with BSE or other pathogens*.

REGARDING EXPERIMENTAL STUDIES ON TSE INFECTIVITY IN BLOOD AND ITS COMPONENTS.

The majority of assays of infectivity in blood have been carried out in animals or humans with clinically overt TSE and in consequence there remains substantial ignorance about the early pathogenetic involvement of the blood, especially in the naturally occurring

diseases. In BSE, transmission has not been achieved in the majority of studies either in natural and experimental disease, although recent investigations indicate this possibility. In experimental scrapie, blood components obtained during both the pre-clinical and clinical stages of disease from rodents, have revealed the presence of the infectious agent. The results reviewed in the Working Group Report are consistent with recent data in mice infected with mouse-adapted human TSE, and in hamsters infected with hamster-adapted scrapie.

Although reliance upon the results requires an assumption that experimental rodent data can be extrapolated to the conditions of natural disease in humans and other animal species, the data from both experimentally-induced and natural TSE suggest that blood has at least the potential to transmit disease. The reasons for the discrepancy between this laboratory evidence and the epidemiological evidence that has so far failed to identify any blood-related cases of TSE, are probably multiple: the absence of significant blood infectivity until the onset of symptomatic disease, and comparatively low levels of infectivity during the symptomatic stage of disease: the need for 5 to 10 times more infectious agent to transmit disease by the intravenous than intracerebral route. For some blood products, there is a further reduction of infectivity during the course of blood processing (for example an estimated reduction of 3 logs for the "133°C/20'/3bars" treatment).

REGARDING SOURCING

Slaughtered cattle are used to source blood for food, feed and a variety of other purposes including the manufacture of medicinal products and biologicals. Farmed sheep, goats and deer could supply blood for these purposes. All these species are susceptible to TSE both naturally and experimentally. BSE as a natural disease has only been reported in cattle. The possibility of BSE being in sheep and goats cannot be excluded. Furthermore, one hypothesis for the origin of BSE is from a scrapie-like agent from sheep. It is possible that such a source still exists or that the BSE agent from cattle has returned to sheep and goats. If it exists it may no longer be recognisable if natural sub-passage has occurred.

No validated tests exist to detect TSE in live cattle, sheep, goats or deer. Clinical TSE has never been reported in deer in Europe. A post mortem inspection will not enable detection of any TSE in any species and will not improve upon the ante mortem inspection. Close

surveillance for the disease and effective ante mortem clinical inspection of all slaughter animals therefore remain essential.

Exogenous contamination of blood with CNS material in the form of emboli (and hence infectivity) is most likely in TSE-infected animals stunned with a stunning pistol that injects gas into the cranial cavity under pressure. This can also occur if a conventional cartridge –fired captive bolt is used in combination with pithing. Exogenous contamination of blood could occur post mortem if SRM are not kept separated from collected blood.

REGARDING RISK ASSESSMENT

The SSC considers that the most important aspect of risk assessment relates to brain tissue contamination. The SSC proposes a general approach for the risk assessment for blood within a given area, which basically involves 3 aspects:

- **Slaughterhouse**

Basically at the level of each individual slaughterhouse, the following risk factors should be recorded:

1) number, species and age of slaughtered animals ;
2) number of potentially infected cows being killed and their brain material entering the bloodstream related to the stunning method used (pneumatic devices, pithing) ;
3) the average amount of blood collected per animal;
4) the dilution by pooling blood from several animals;
5) the amount of such collected blood going to the industry to be processed for human or animal consumption.

- **Geographical BSE risk and surveillance**

For the geographical BSE risk and surveillance reference is made to the SSC opinions adopted sofar by the SSC.

- **The use of blood**

At present, blood collected hygienically in licensed EU abattoirs can be used for food, feed and a variety of other purposes without any form of processing. For example, it is permissible to incorporate fresh untreated plasma into the materials used for the

production of sausages, and can be spread on land as a fertiliser. Elsewhere, in this opinion, it is concluded that there could be a risk of the occasional presence of low levels of TSE infectivity in blood collected in abattoirs. Levels of infectivity which might represent a risk to animal or human health are not known. Control measures and/or decontamination standards might need to be developed to potentially TSE-infected blood collected in abattoirs.

There is insufficient information on the nature of usage of blood and the resulting blood-products obtained from abattoirs within the EU. The situation is further complicated by the fact that the names used to describe identical or similar products in different countries can be quite different. From the limited information available on the manufacturing processes for blood-products, the most rigorous methods applied would appear to involve coagulation of the blood at 95-100^{o}C, followed by spray-drying during which the end-product reaches a temperature of 110^{o}C. Several sets of data indicate that this process is unlikely to result in any significant reduction in the titre of TSE infectivity. The alternative is to either apply risk-assessment techniques or, where practicable, adopt the rule that products should be subjected to the "133^{o}C/20'/3bars" standard. The latter approach is applicable only to some products.

At the level of processing of blood for human and/or animal consumption, risk can be further evaluated in respect to

(i) the amount of brain material actually entering the bloodstream following the use of invasive stunning devices. Neither its volume range nor the range of particle size is known. Likewise, no quantitative estimates are available on contamination of blood with SRM materials during the slaughtering process other than by stunning the animals.

(ii) dilution of CNS material resulting from the emboli and

(iii) the efficacy of the various processing steps in respect to inactivating the BSE agent.

There is little doubt that under certain circumstances, humans or animals could be exposed to the BSE agent by consuming blood products.

CONCLUSIONS

The collective data currently available from experimental transmission studies show that there is uncertainty on the presence of infectivity in the blood of TSE-infected ruminants. If PrP^{Sc} has been detected in the blood of clinically normal sheep from scrapie-susceptible flocks using a newly-developed and highly sensitive assay system, infectivity of femtomole amounts remain to be demonstrated.

The relationship between PrP^{Sc} and infectivity is not understood. The two do not always correlate; the presence of PrP^{Sc} does not necessarily imply presence of infectivity. Moreover, the methods for detecting PrP^{Sc} need to be validated for the pre-clinical stage. As far as ruminant blood is concerned, it is considered that the best approach to protect public health at present is to assume that it could contain low levels of infectivity. However, even if this is true, it becomes almost irrelevant compared with the level of contamination that could occur as a result of the methods of stunning used in abattoirs. These procedures are now recognised to release particles of brain-tissue (potentially containing high titres of TSE infectivity) into the bloodstream. The frequency at which this occurs appears to increase with the severity of the stunning process, and this report recognises that this is an area requiring further research. There are also opportunities for the contamination of pooled blood as a consequence of the release of brain-tissue from the hole left by stunning, or with spinal cord during its removal (if a production-line process is not used). Nevertheless, given the low frequency at which apparently healthy animals would have TSE infectivity in the CNS at the time of slaughter, it is considered that the overall potential level of infectivity in pooled blood will be low.

Further specific conclusions are:

- The highest risk of producing CNS emboli follows captive bolt stunning with compressed air into the cranial cavity.
- Cartridge operated captive bolt stunning-followed-by-pithing presents the next highest risk.
- There is insufficient knowledge to advise on the degree of risk from the use of penetrative cartridge-operated stuns without pithing, free bullets or non-penetrative guns.
- There are no published papers on the effect of various stunning methods on sheep and goats and in regard to the generation of CNS emboli.

- More information is required on the possible dissemination of CNS emboli into the systemic circulation.
- TSE risks may exist as a result of the source of animals for slaughter.
- TSE risks may occur independently of the stunning procedure as a result of TSE-infected material from SRM for example entering the blood after exit from the body.
- Improved processing procedures could reduce residual TSE risk in the collected material.
- An accurate *ante mortem* examination performed at slaughterhouse is helpful to increase prevention, whereas this is not the case for *post-mortem* inspection.

1. RECOMMENDATIONS

The Scientific Steering Committee recommends that the present opinion on the safety of ruminant blood is considered in conjunction with its opinions on "Fallen stock" (June 1999)[1] and "Intra-species Recycling"[2].

The SSC also recommends that intraspecies recycling of ruminant blood and blood products should be avoided in situations when a TSE risk exists.

Consideration should further be given to avoiding methods of captive bolt stunning with compressed air or followed by pithing ruminant food animals that increase the risk of CNS material entering the blood stream at slaughter wherever there is a significant risk from TSE[3]. In addition, sourcing from young[4] animals would further reduce the risk.

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1 Scientific Opinion of 24-25 June 1999 of the SSC on The risks of non conventional transmissible agents, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials. Adopted By the Scientific Steering Committee

2 Scientific Opinion of 24-25 June 1999 of the SSC on the risk born by recycling animal by-products as feed with regard to propagating TSE in non-ruminant farmed animals.

3 Changing from pneumatic stunning or pithing, to stunning methods that avoid severe brain damage could go along with an increased risk of physical injury to slaughtermen (particularly during shackling and bleeding out) if the new methods or building facilities are not properly designed.

4 First infectivity in CNS of cattle is detected in most cases in the last quarter of the incubation period. Defining young animals could be done on the basis of the probability of occurrence of BSE according to the age. (See for example the annexes 3 and 4 of the Opinion of 28-29 October 1999 of the Scientific Steering Committee on the *Scientific Grounds of the Advice of 30 September 1999 of the French Food Safety Agency (the Agence Française de Sécurité Sanitaire des Aliments, AFSSA), to the French Government on the Draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom*.

(Improved) methods for reducing the risk of cross contaminating blood with CNS or other SRM post-collection need to be develop or put in place where necessary. Brain spilling from the bullet hole into the blood tank should be prevented; surveys should check the absence of brain material in the blood tanks.

Where an element of risk is perceived, this may be reduced or eliminated by (a combination of) various strategies, as follows:

- Source bovine blood from BSE-free areas or closed herds or other schemes that reduce to a minimum the probability of an animal being infected;
- Subject the product to a 133^{o}C/3 bar/ 20 minute autoclaving process or equivalent validated process.
- Pharmaceuticals: including vaccines, are regulated products, and the use of bovine derived blood products in their manufacture is controlled on a case by case basis. The basic principles included in the present opinion should of course be respected.

2. NEEDS FOR RESEARCH

Further research is needed:

- to determine the comparative (quantitative) TSE risks from various penetrative and non-penetrative methods of stunning and pithing in food animal species
- to quantify the possible presence of CNS material in blood following various stunning methods
- to determine the effect of the "133°C/20'/3bar" treatment on the nutritional value of blood for animal nutrition and the reduction/elimination of possible infectivity in blood.
- on possible TSE infectivity of blood cells and other blood components. Furthermore highly sensitive tests that are presently under development, need to be further validated.

REPORT ON

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THE SCIENTIFIC STEERING COMMITTEE

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DEFINITIONS:

The following definitions are used in the present report:

Blood: fresh whole blood collected from any species of food animal that has passed an official *ante-mortem* inspection at a licensed slaughterhouse supervised by the competent veterinary authority.

Blood products: blood that is treated by physical methods (*e.g.* separation into components of blood, and/or by heat) or chemical methods such as addition of anticoagulant.

Blood products for pharmaceutical and cosmetic use: Blood products intended for use in biological, medicinal or pharmaceutical products, medical devices or cosmetics.

Blood products for use in food: blood or blood products intended for use in human food; post-mortem inspection is mandatory.

Blood products for use in feed: blood or blood products for use in feed for farm animals (*i.e.* those species consumed by man).

Blood products for use in petfood: blood or blood products for use in food for companion animals.

Blood products for technical use: blood or blood products for uses other than those described above, *e.g.* for fertiliser.

Bloodmeal: a blood product intended for use in animal feed or for technical uses that has been coagulated by treatment with steam and dried.

Feedingstuffs/Feed material (of animal origin): processed animal protein, rendered fats, gelatine and hydrolysed proteins, milk and milk products, intended to be used as feed for farmed animals, but excluding petfood.

Laboratory reagents: a packaged product, ready for use by the end user, containing a blood product, and intended for laboratory use as reagent or reagent product, whether used alone or in combination.

Processed animal protein (PAP): animal proteins derived entirely from animal by-products, which have been treated so as to render it suitable for direct use as feedingstuff or as feed material in a feedingstuff for animals or in petfood or as fertilizers. It includes fishmeal, meatmeal, bonemeal, meat-and-bone meal, bloodmeal, dry greaves, feathermeal, [hoofmeal, hornmeal] and other similar products including mixture containing these products.

Products used for *in vitro* diagnosis: a packaged product, ready for use by the end user, containing a blood product, and used as reagent, reagent product, calibrator, kit or any other system, whether used alone or in combination, intended to be used *in vitro* for the examination of samples of human or animal origin, with the exception of donated organs or blood, solely or principally with a view to the diagnosis of a physiological state, state of health, disease or genetic abnormality or to determine safety and compatibility with reagents.

Technical products: animal by-products intended for purpose other than human or animal consumption, (such as tanned and treated hides and skins, game trophies, processed wool, hair, bristles, feathers and part of feathers, apicultural products, serum of equidae, blood products other than blood meal, colouring substances for food, pharmaceutical, bone products for china, gelatine and glue, processed manure, rendered fats derivatives, organic fertilizers, industrial oil, etc.).

1. MANDATE AND CONTEXT OF THE QUESTION

Animal (incl. ruminant) blood is currently legally fed to animals (including ruminants) after very gentle processes, spread on pasture as fertiliser, or used for other products that may reach man or animals (incl. ruminants). There is some concern that animal TSEs might be spread via blood or specific blood components or blood based products that are still permitted to enter the market. It is therefore necessary to establish if animal TSEs can be transferred via blood and the SSC was requested to:

A. Assess for ruminant blood in general and if possible for each component the risk that it could harbour the BSE (TSE) agent and hence transfer the disease.

B. Identify the main ruminant blood-based products that are currently on the market, including products containing ruminant blood or those in which ruminant blood is used in their manufacture.

C. Assess for each product, the function of the blood component included and the risk that that product could transfer BSE (TSE) to ruminants.

D. Outline possible measures that could mitigate any identified risk, as far as possible together with a (qualitative) assessment of the potential impact of the measure on the risk

2. MATRIX OF PRODUCTS AND USES

Table 2.1. hereafter provides a non exhaustive draft matrix of products and uses of ruminant blood. Table 2.2. is a non-exhaustive draft list of the ruminant blood derived materials used for human or veterinary medicinal products or for medical devices.

2.1. Matrix of products and uses[5] of ruminant blood. *Italic applies to non-food animal non-human uses*

PRODUCTS:	***USES***								
	To animals			***To humans***			***For technical purposes***		
	Feed	*Vaccines Sera/biolog*	*Other*	*Food*	*Vaccines*	*Cosmetics*	*Fertiliser*	*Culture media*	*Pharma/ Biologicals*
Blood	+		**+ *pet food*** Cattle blood for transfusion to cattle	**+**		*Unknown what component of blood is used*	**+ *crops/ horticulture***	+	
Blood meal	+		+ *pet food*				**+*Crops/ horticulture***		
Blood serum		+	+ *pet food* Diagnostic tests		Diagnostic tests			+	Immune sera & diagnostic tests
Foetal calf serum*		+			+				+ vaccines
Immunoglobulin	+	+			+				+ IgG
Serum albumin*		+	+ embryo transfer			+			+
Spray-dried blood	+			+					
Spray-dried plasma	+			+					
Spray-dried serum	+			+					
Spray-dried blood cells	+			+					
Haemoglobin		+ (pending)	Oxygen carrier	+ colorant clearing agent (wine & beer)					Oxygen carrier
Globulin								+	

* see table in section 2.2. hereafter for details

5 Not exhaustive

2.2. Ruminant blood derived materials used for human or veterinary medicinal products or for medical devices[6]

BOVINE BLOOD DERIVED MATERIAL	GENERAL USE	SPECIFIC USE	COMMENTS
FOETAL CALF SERUM	Cultivation of mammalian cells and, therefore, in the production of most cell culture derived medicinal products	Veterinary vaccines	Used for preparation of cells, propagation of vaccine strains usually, but not always only after change to serum free medium
		Human vaccines	Preparation of cells, propagation of vaccine strains only after change to serum free medium
		Monoclonal antibodies for use in humans	Preparation of cells, but increasing use of serum free media
		Monoclonal antibodies, used for purification of pharmaceutical products (e.g. coagulation factors)	Preparation of cells, but increasing use of serum free media
		Recombinant proteins from mammalian cell cultures, used as pharmaceuticals	Preparation of cells, but increasing use of serum free media
		Products for gene therapy (preparation of vectors)	Preparation of cells, but increasing use of serum free media
	Stabiliser in buffers	In vitro diagnostics	Usually, foetal calf serum is used, but the occasional use of calf serum or bovine serum cannot be excluded
CALF SERUM	Cultivation of mammalian cells	Some stocks of seed viruses (for the production of vaccines)	Usually replaced by foetal calf serum
BOVINE SERUM			Most often replaced by foetal calf serum

6 not exhaustive

SERUM ALBUMIN	Constituent of “serum free” cell culture media	Monoclonal antibodies and recombinant proteins used as, or for the production of, pharmaceuticals	
	Constituent of buffers	Recombinant proteins as medicinal products	
		Allergen preparations (in vivo diagnostics)	
		In vitro diagnostics	
THROMBIN		Used in fibrin sealant (pharmaceutical)	
		Constituent of some medical devices	
BOVINE HAEMOGLOBIN	Oxygen carrier	In development as replacement of red blood cell concentrates for transfusion (not yet licensed)	

3. EXPERIMENTAL STUDIES ON TSE INFECTIVITY IN BLOOD AND ITS COMPONENTS.

This chapter is structured and based in part on the *Risk quantification for CJD transmission via substances of human origin* adopted on 21 October 1998 by the Scientific Committee on Medicinal Products and Medical Devices.

3.1. General

The transmissible spongiform encephalopathies (TSEs) or prion diseases present as sporadic, acquired and inherited disorders which are usually transmissible on experimental inoculation. Infectivity can be measured only by bioassay. There are two common methods of bioassay: end point titration or incubation time interval assay (Millson *et al*, 1976, Outram, 1976, Prusiner, 1987, Scott 1993). Both methods are slow and costly, especially the former, which has undoubtedly contributed to the infrequent use of classical titration methods. Tissues or body fluids are collected from naturally or experimentally infected animals, and inoculated into healthy inbred assay animals, usually by the intracerebral route (i.c.), but sometimes by other routes - intraperitoneal (i.p), intravenous (i.v.), or subcutaneous (s.c.), or a combination of i.c. and i.p. It is almost always advised that across species barriers i.c. and i.p. should be used. The assay animals are observed over a period of months or years for clinical signs of transmissible spongiform encephalopathy (TSE), which is confirmed by histopathological examination, and in some studies also by Western blot identification of PrP^{res} in the brain.

End point titrations offer the most precise assay of infectivity and are typically performed by the intracerebral injection of ten fold serial dilutions of a tissue sample into groups of rodents. The concentration of infectious agent in the sample is expressed as the highest dilution capable of transmitting disease to one half of the inoculated animals ($\log_{10} ID_{50}$/g.)

Incubation time assays involve the construction of a dose response curve by end point titrations, to enable a titre to be interpolated from the curve for a given dilution of the test specimen. The incubation period assay uses far fewer animals, but its accuracy is dependent upon the skills of the observer in defining the clinical onset of disease, and on the conditions to which the inocula are subjected (for example, dose-response curves are significantly extended after exposure of infectious samples to partially-inactivating procedures) (Taylor *et al*, 1999).

Of special importance for measuring the very low concentrations of infectivity in blood is the fact that at limiting dilutions of inoculum the incubation period becomes an unreliable measure of the concentration of infectivity, and thus many animals (often 30-40) are needed for inoculation of the undiluted specimen in order to attain a statistically accurate estimate of infectivity.

One factor influencing the sensitivity of TSE bioassay methods concerns the species barrier effect, which may result in an underestimate or even absence of apparent infectivity in the specimen being assayed. Ideally, therefore, infectivity assays should be conducted in the same species as that of the donor animal. Assays are conducted using a single inoculation of the assay animals. The effects of repeated inoculations have not yet been investigated in depth.

The route of exposure is another important variable. For example, inoculation of blood or a blood product by the i.c. route does not answer the question of tranmissibility, for example by the oral route in feed.

Finally, it must always be remembered that unexpected or bizarre experimental results require a very cautious evaluation until they can be shown to be reproducible and independently confirmed. Errors of all sorts are possible, from specimen mislabelling to laboratory cross-contamination. Some reports of infectivity in the blood of humans with CJD must be viewed with circumspection because of aberrant experimental results, or unreproducibility. Tateishi (1980), Manuelidis (1985), Tamai *et al* (1992).

Blood comprises fluid plasma and cellular elements, and is the body's essential transport medium. Many organs are highly vascularised (e.g. liver, spleen, placenta) and blood represents a significant proportion of their volume. The blood-forming organs (haemopoietic system) are not only related functionally to the production but also to the degradation of blood components. The haemopoietic system includes the lymph nodes, tonsil and other submucosal lymphoid nodules, thymus, spleen and bone marrow. The destruction of red blood cells occurs mainly in the spleen. It is noted that ruminant species have haemal nodes which function to filter blood. In the foetus and for a period after birth haemopoiesis has a wider distribution to the liver and meninges in cattle for example.

All experimental transmissions were done by using blood taken from animals without brain trauma, that is, living animals.

3.2. Studies of blood infectivity in naturally infected donor species assayed in the same recipient species

There is no literature about same-species assays of blood from cases of naturally occurring TSE.

A study undertaken at the Institute for Animal Health, UK, is examining whole blood and buffy coat from scrapie susceptible Cheviot sheep (PrP genotype VRQ/VRQ) in the pre-clinical phase of natural scrapie. The samples were subsequently transfused into susceptible but scrapie-free sheep (PrP genotype: VRQ/VRQ) sourced from New Zealand, and these are being observed for signs of TSE. The study has been underway for about one year and no clinical cases have so far developed in the blood or buffy coat recipients (N. Hunter, unpublished data).

3.3. Studies of blood infectivity in naturally infected donor species assayed in different recipient species (Table 1)

No infectivity was found in serum or in clotted blood of scrapie infected goats and sheep when assayed in Swiss mice (Hadlow *et al*, 1980, Hadlow *et al*, 1982). The single report of infectivity in the serum of a naturally infected ram by assay in Swiss mice (Gibbs, 1965) was not confirmed. In natural cases of BSE assayed in RIII mice (*Sinc s7*), infectivity has been found only in the central nervous system (CNS: brain, spinal cord and retina). No infectivity was found in about 50 other tissues including bone marrow, clotted blood, buffy coat, serum or foetal calf serum (Fraser and Foster, 1994; Bradley, 1999).

3.5. Studies of blood infectivity in experimentally infected donor species assayed in different recipient species (Table 2)

No infectivity was found by mouse bioassay in blood-clots from scrapie infected goats during a time course study (Hadlow *et al*, 1974).

Tissue infectivity in BSE has been comprehensively investigated in cattle in an experimental pathogenesis study in which cattle were challenged orally with 100g infected cattle brain from natural cases. Clinical disease was first detected at 35 months p.i. (39 months of age). No infectivity by assay in RIII or C57Bl mice was detected in buffy coat of orally infected cattle up to the termination of the study at 40 months p.i., by which time all remaining animals had become ill. (Wells *et al*, 1996; Wells *et al*, 1998; G.A.H. Wells unpublished data). Frozen sera and blood clots have not yet been assayed (Wells *et al*, 1996).

Studies in progress at the VLA, UK are examining the transmissibility, pathogenesis and phenotype of BSE in Romney and Suffolk sheep after oral exposure to affected cattle brain tissue. Buffy coat, obtained from exposed sheep at 6 month intervals p.i. (ARQ/ARQ, PrP genotype) and at 12 month intervals p.i. (ARR homozygous and heterozygous) will be assayed in RIII mice. Infectivity in liver and spleen of the exposed sheep will also be assayed in RIII mice at sequential time intervals of 6 or 12 months p.i. (S. Bellworthy, personal communication).

3.5 Studies of blood infectivity in experimentally infected donor species assayed in the same recipient species (Table 3)

Two studies of scrapie (Pattison and Millson, 1962; Pattison, *et al* 1964) with goats as donor and indicator/recipient animals did not demonstrate any infectivity in blood (fig. 1).

In two time course studies performed in scrapie infected mice, small amounts of infectivity were detected in the blood during later stages of the incubation period in one study (Dickinson and Stamp, 1969), but not in the other (Eklund *et al*, 1967). This difference may have been due to the use of mouse strains (Dickinson and Stamp, 1969). Clarke and Haig (1967) detected infectivity in the blood of diseased mice and rats infected with the Chandler strain of scrapie, but argued that the amount of infectivity may have resulted from contamination of blood samples with other bodily cells.

Infectivity in the blood of hamsters infected with the hamster adapted 263K strain of scrapie has been investigated by two groups (Diringer, 1984; Casaccia *et al*, 1989) using identical methods to concentrate the scrapie agent from whole blood. Both groups found infectivity in the preparations, but one study was terminated 6 weeks after infection, and the other study reported declining levels of infectivity during the later stages of disease. Using the same 263K hamster scrapie agent, Brown *et al* (1998) inoculated hamsters with normal human blood that had been spiked with the agent. Infectivity titres were highest in buffy coat, lower in plasma, and very low to absent in Cohn plasma fractions (see Table 4).

A study undertaken at the Institute for Animal Health, UK, is examining sheep of the ARQ/ARQ PrP genotype orally infected with BSE. Whole blood and buffy coat were taken from the animals at various time points during the incubation period. The samples were subsequently transfused into susceptible but TSE-free sheep sourced from New Zealand and these are being observed for signs of TSE. The study has been underway for about one year and no clinical cases have so far developed in the blood or buffy coat recipients. In continuing studies of the pathogenesis of BSE after experimental oral exposure of cattle (Wells *et al*, 1996, 1998, 1999) assays of pooled buffy coats from cattle at each of the original study time points (6, 18, 26 and 32 months p.i.) are in progress at the VLA, UK. There is

no evidence of transmission to date, but some of these experiments have as yet been set up only for one year (G.A.H.Wells, unpublished data)

Two studies in mink (*Mustela vison*) inoculated with the transmissible mink encephalopathy agent, did not reveal any infectivity in serum (Marsh, 1969) or other blood components (Marsh, 1973). In another study, only a single serum specimen, taken 28 weeks after inoculation and four weeks before the onset of clinical signs, showed a small amount of infectivity (Hadlow, 1987). Experimental models of human TSE have also been used in a search for infectivity in blood. The result obtained in guinea pigs inoculated with the CJD agent adapted to this species is puzzling in that infectivity appeared and disappeared irregularly during the incubation period (Manuelidis, 1978a, see fig. 4). A more consistent picture was obtained in mice inoculated with the Fukuoka strain of human GSS (Tateishi *et al*, 1980; Kuroda *et al*, 1983). In this model, low but increasing levels of infectivity were found in buffy coats obtained during the later stages of disease (fig. 5). In a closely related system, Doi (1991) could not demonstrate infectivity in whole (of which leukocytes constitute approximately 1% by volume).

The same system has been studied at the NIH, USA (Brown *et al*, 1998, 1999). Infectivity bioassays were conducted in healthy mice, and the brains of all assay animals dying during the course of the experiments were examined for the presence of PrP^{Sc}. Infectivity in the blood of animals during the pre-clinical phase of disease (Table 4) occurred in the buffy coat at levels of between 6 and 12 infectious units[7] per ml, and was either absent or present in only trace amounts in plasma and plasma fractions. Infectivity rose sharply at the onset of clinical signs to levels of approximately 100 infectious units/ml buffy coat, 20 infectious units/ml plasma, 2 infectious units/ml cryoprecipitate, and less than 1 infectious unit /ml fractions IV and V. Plasma infectivity was not significantly reduced by either leukodepletion filtration (Table 5) or high speed (17,000 x g) centrifugation (Table 6). Approximately 7 times more plasma and 5 times more buffy coat were needed to transmit disease by the intravenous than by the intracerebral route (Table 7). Taylor *et al* (2000) showed small amounts of infectivity can be found in the plasma of mice when they are infected with mouse-passaged BSE agent. The results are rather similar to those obtained by Brown *et al* (see above) when they looked at the blood of mice infected with mouse-passaged GSS agent.

3.6. Evidence of infectivity in other organs, tissues and cells with functional relationship to blood.

Previous work on the tissue distribution of infectivity (detected by mouse bioassay) in sheep and goats with natural scrapie and cattle with BSE has been summarised (OIE, 1996). In scrapie, beginning after 8 months and detected at 10-14 months of age, low levels of infectivity are detectable in a wide range of lymphoreticular rich tissues including the intestine, lymph nodes, spleen and tonsil, and these levels increase through the incubation period to clinical disease onset. In clinical cases of BSE no infectivity has been detected by mouse bioassay in spleen, tonsil, regional lymph nodes, intestine, bone marrow, liver or placenta. Haemal nodes have not been tested in regard to TSEs so far.

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[7] Within the context of Brown et al., (1999) an infectious unit is functionally defined as equivalent to the number of transmissions/ml.

Data for BSE are based on transmissions attempted from a very small number of animals. Nevertheless, the findings are consistent with studies of the pathogenesis of BSE in cattle after oral challenge. In further examinations of the tissues from this pathogenesis experiment additional same-species assays by i.c. challenge with blood- associated tissues pooled from selected time points are being conducted by the VLA, UK. These tissues, with their respective time point derivations, include: spleen (6, 10, 18, 26 months p.i.), liver (6, 18 26, 32 months p.i.) and bone marrow (22, 26, 32, 36 months p.i.). Inoculations of these tissues began in November 1996 and continued through March 1999. Consequently the assays have been in progress for periods of 10-38 months and there is no evidence of transmission to date (January 2000) (G.A.H.Wells, unpublished data).

Experimental parenteral challenge of cattle and RIII mice in three separate experiments is underway at VLA, UK, with (i) a pool of five brains, (ii) a pool of five spleens and (iii) a pool of lymph nodes from five BSE affected cattle. The brain pool has transmitted disease to both species (in cattle, even when diluted 10^{-7}) but neither the spleen pool nor the lymph node pool has transmitted disease to either species (the cattle study is still incomplete at 84 months p.i. (January 2000)). These experiments have shown that with the increased sensitivity of the same-species assay one can detect about 500 times more infectivity/g of infected material in cattle than in RIII mice (Bradley 1999, G.A.H.Wells, unpublished data).

Another study conducted by the VLA, UK, to detect possible infectivity in the foetal membranes and placenta of cattle with clinical BSE, recipient cattle were dosed oronasally with a pooled tissue homogenate from BSE cattle. The recipients were killed at 24 and 84 months p.i. with no evidence of disease (G.A.H. Wells and S.A.C. Hawkins, unpublished data). Buffy coat, serum and spleen from each of the recipient cattle also gave negative results when assayed in RIII mice (Wells *et al* 1996,1998).

Further studies at VLA, UK, have examined infectivity in pigs exposed experimentally to the BSE agent. Pigs were inoculated i.c.,i.v., and i.p. with BSE brain tissue. Mouse bioassays of CNS pools from animals that were clinically and pathologically affected (17-37 months p.i.), or clinically normal but pathologically affected (killed at 24 months p.i.) were positive, but liver and spleen pools were negative. In another experiment, a group of ten pigs were fed a total of 12 kg BSE brain divided into three equal amounts and given at 1-2 weekly intervals. None developed disease after an 84 month observation period. Spleen, liver, distal ileum, and various lymph nodes from animals killed 24 months and 84 months p.i. were examined for infectivity with negative results, although not all assays on tissues from the 84 months p.i. group are completed (G.A.H. Wells and S.A.C. Hawkins, unpublished data).

3.7. Indirect evidence of infectivity in blood from TSE pathogenesis studies

Many studies have been reported in rodent models of scrapie which have been directed toward an understanding of the pathogenesis of the TSEs after infection by peripheral (non-neural) routes. After peripheral infection a transient viraemia probably accounts for the spread of infectivity (or PrP^{Sc} as a proxy for infectivity) and resulting in the establishment of infection throughout the lymphoreticular system early in the incubation period. The major, if not sole, means by which the agent gains access to the CNS from the periphery appears to be via nerves; however, an accessory hematogenous neuroinvasion cannot be discounted.

The role of leukocytes in pathogenesis and neuroinvasion is debated. In experiments using mice with genetic defects affecting different functions of the immune system, an important role was claimed for B lymphyocytes in the pathogenesis of neuroinvasion (Klein *et al*, 1997). It has also been argued that peripheral lymphocytes may carry infectivity, and thus account for the presence of infectivity in lymphatic organs like peripheral lymph nodes and spleen. However, in animal experiments in which TSE infectivity in blood is demonstrable, this infectivity never parallels the infectivity in spleen (Eklund, 1967, Kuroda, 1983, Casaccia, 1989). This lack of correlation between infectivity in spleen and peripheral blood is also supported by the recent observation that lymphocytes isolated from spleens of infected mice carry infectivity, while lymphocytes isolated from the peripheral blood of the same mice do not (Raeber *et al*, 1999). Identical results had previously been obtained in studies of TME infected minks (Marsh, 1973).

Other work has implicated follicular dendritic cells and not B lymphocytes as the critical element for neuroinvasion by at least one strain of scrapie (K. Brown *et al*, 1999). The conflict between such findings may be the result of differences in scrapie strains, and raises the possibility that a viraemic phase of disease may be a function of agent strain and not obligatory in the pathogenesis of all TSEs.

3.8 Evidence of infectivity in blood and blood associated tissues implied by the presence of detectable PrP^{Sc}

With increasingly sensitive methods for the detection of the disease specific form of PrP in tissues of infected animals has come an increasing use of the PrP^{Sc} as a proxy for measuring infectivity to establish distributions of agent after experimental inoculation. However, the precise relationship between infectivity and PrP^{Sc} concentrations is not fully understood.

Most of the available data suggest that PrP^{Sc} is associated with infectivity. However, immunoassay of disease associated PrP does not always correlate with the level of infectivity. Also, the absence of PrP^{Sc} at present levels of detectability does not necessarily imply absence of infection. (See also: the pre-opinion of 2-3 March 2000 of the Scientific Steering Committee on Oral exposure of humans to the BSE-agent: infective dose and species barrier.)

The application of capillary electrophoresis and similar technologies to measure PrP^{Sc} in tissues and blood for which there is accurate infectivity titration data should help to clarify these discrepancies. Experiments are in the planning stages at the National Animal Disease Center (NADC, U.S.A.) to infect sheep with blood from scrapie infected sheep and to measure the amount of PrP^{Sc} in the blood of the recipients. Similar experiments are underway for hamster blood. As additional assays are developed with sensitivities in the zeptomole range [10^{-21}], the number of molecules of PrP^{Sc} required to transmit disease may be determined (Schmerr, personal communication).

3.9. Conclusion

The majority of assays of infectivity in blood have been carried out in animals or humans with clinically overt TSE and in consequence there remains substantial ignorance about the early pathogenetic involvement of the blood, especially in the naturally occurring diseases. In BSE, transmission has not been achieved either in

natural and experimental disease. However, not all such experiments are complete. In natural scrapie, attempts to transmit disease from blood have been unsuccessful. Whereas in experimental scrapie, blood components obtained during both the pre-clinical and clinical stages of disease from rodents (but not goats), have revealed the presence of the infectious agent (Clarke and Haig, 1967; Dickinson and Stamp, 1969; Tateishi *et al*, 1980; Kuroda *et al*, 1983). These results are consistent with recent data from time course studies in mice infected with mouse-adapted human TSE (Brown *et al*, 1998, 1999), and in hamsters infected with hamster-adapted scrapie under limiting dilution conditions (Rohwer, personal communication June 1998).

Although reliance upon the results requires an assumption that experimental rodent data can be extrapolated to the conditions of natural disease in humans and other animal species, the data from both experimentally-induced and natural TSE suggest that blood has at least the potential to transmit disease. The reasons for the discrepancy between this laboratory evidence and the epidemiological evidence that has so far failed to identify any blood-related cases of TSE, are probably multiple: the absence of significant plasma infectivity until the onset of symptomatic disease, and comparatively low levels of infectivity during the symptomatic stage of disease: the need for 5 to 10 times more infectious agent to transmit disease by the intravenous than intracerebral route; and, for plasma products, the further large reductions of infectivity during the course of plasma processing (average reduction of 3 logs for the "133°C/20'/3bars" or equivalent treatment).

4. SOURCING

4.1. SOURCE ANIMALS

4.1.1. Species supplying blood and risks from non ruminant species

In theory any food animal species could be a source of blood for human or animal consumption, for use in medicines, cosmetics, fertilisers or other purposes. In practice blood is formally collected, with a view to a specific use as a separate item, from cattle, pigs and poultry. This includes use for human consumption. There is no reason why in principle blood could not be collected from sheep, goats, deer for purposes other than use as a fertiliser or for rendering. It is noted that if blood is not specifically collected at slaughter it is likely to be disposed of with abattoir waste. Thus in assessing risks the questions to be asked:

- Where does blood from slaughtered animals go?
- What processes is it subjected to? and
- What is it used for?

In the context of the question (namely risks from TSE) only ruminant species need be considered as pigs and poultry are not known to be naturally susceptible to TSE. In this context uncontaminated blood from these two species can be assumed to have a negligible risk. If this was infected with a TSE agent there is a possibility of cross contamination of the blood *post mortem* from food in the stomach. Any risk would depend upon the measures in place and their effective enforcement. If this was done risks could be contained to a negligible level. The risk could be avoided altogether if feed containing MBM was not fed in the immediate pre-slaughter period. Poultry for human consumption may not be fed immediately prior to

transport and slaughter therefore any contamination from stomach contents would likely be minimal.

4.1.2. Risks from ruminant species

4.1.2.1. Deer

American deer

Chronic wasting disease (CWD) occurs in several species of *Cervidae* in North America but is for the most part, believed to be geographically localised both in wild and farmed *Cervidae* (Williams and Young, 1992). The clinical signs are severe and unlikely to be missed at an *ante-mortem* inspection. The tissue distribution of infectivity has not been reported in deer with CWD so there is no knowledge about infectivity in the blood. If the disease followed the pattern of scrapie, which it does in some respects, any inherent infectivity in blood would be expected to be at a very low or negligible level.

European deer, as well as reindeer

European deer have never been reported to develop TSE. It is noted that the surveillance for TSE in deer in Europe is probably sub-optimal for detecting TSE but if regarded adequate then risks from TSE in European deer would be probably be very low. If there is a risk of TSE in European deer, then at best guess the risk from blood would be expected to be similar to that in sheep and goats with scrapie. The Working Group strongly recommends that research on the possible prevalence of TSEs in European deer populations should be undertaken.[8]

4.1.2.2. Sheep and goats

Both species are naturally susceptible to scrapie. Scrapie is commoner in sheep than in goats. BSE is not known to occur in sheep or goats as a field disease. However, BSE can be transmitted experimentally to sheep and goats, including by the oral route (Foster, Hope and Fraser, 1993). The tissue distribution of infectivity in sheep and goats with experimental BSE is not known but may resemble that in sheep and goats with scrapie. This judgement is based on the fact that the spleen and brain of clinically affected sheep after experimental oral challenge with the BSE agent harbours infectivity (Foster *et al*, 1996). By contrast there is no detectable infectivity or PrP^{Sc} in the spleen of cattle with natural or experimental BSE. Thus the pathogenesis of BSE in sheep and goats, after oral challenge**,** if it were to occur, would perhaps be more likely to follow that of scrapie in sheep than BSE in cattle. This might include a substantial component of maternal and horizontal transmission. [Maternal transmission of BSE in goats has so far been negative (N. Hunter, personal communication]. For further information on the risks of BSE in sheep and goats see the Opinion of 24-25 September 1998 of the SSC on The risk of infection of sheep and goats with Bovine Spongiform Encephalopathy agent.

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8 See Commission Decision 98/272/EC

Geographical sourcing

To eliminate any risk that there may be from sheep and goat blood, a solution might be to source animals from countries or regions where the disease is absent or from flocks certified to be free from scrapie.

Selection of scrapie 'resistant' sheep by *PrP* genotyping.

This issue is dealt with in detail in the SSC opinion of 22-23 July 1999 on *The policy of breeding and genotyping of sheep, i.e. the issue of whether sheep should be bred to be resistant to scrapie.*

One hypothesis for the origin of BSE in cattle (Wilesmith *et al,* 1991) suggests a scrapie-like agent from sheep may be responsible. Thus in countries with scrapie but without reported BSE, where sheep or goat blood is intended to be collected for a high risk activity without effective post-collection treatment, it may be sensible to operate to the restriction of use / precautionary measures (as listed in this opinion) as in countries where BSE is known to occur. An alternative might be to only permit blood collections for such activities from sheep from scrapie-free countries or regions or flocks.

4.1.2.3. Cattle

Of all the ruminant species cattle are the most likely to have been exposed to BSE infectivity but only in certain countries or regions. Even within such at-risk areas there are herds that are certified as BSE free by a method such as, for example, described in the SSC opinion of 22-23 July 1999 on *The conditions related to "BSE Negligible risk (closed) bovine herds*" or akin to that operated in the UK beef assurance scheme (MAFF, 1996).

Although most cases of BSE occur in dairy animals and in cows rather than bulls there are sound epidemiological reasons for this. They relate to the different feeding methods in dairy herds compared with beef suckler herds and to the small size of the bull population to the cow population (Bradley and Wilesmith, 1993).

There appears to be no genetic predisposition to BSE in cattle and thus all cattle should be assumed to be susceptible. Cattle from 20 months to over 18 years have succumbed to BSE. However, as most cattle have been infected as calves (Wilesmith *et al.,* 1988) and in view of the small risk of maternal transmission of BSE it would be wise to consider that all ages of cattle could be infected with the BSE agent.

The distribution of BSE-infectivity in field and experimental cases of BSE is dealt with in another section of this report as will the possibilities for *post mortem* cross contamination of blood collected from cattle.

4.2. TESTING FOR PrP^{Sc}

There are currently no practical, validated tests available for testing live cattle for BSE infectivity or PrP^{Sc}.

There are validated tests for detecting PrP^{Sc} in the brain or spinal cord of cattle with clinical disease *post mortem* (Moynagh and Schimmel, 1999). Work is continuing on the evaluation of test capability using tissues taken from experimentally infected animals before they exhibited clinical signs of BSE. A successful test that can be used on animals in the incubation period depends on its ability to detect low

amounts of PrP*, but also on the presence of this marker in the tissue examined. Thus, negative test results could possibly give a false assurance of non-infection in early cases of the disease.

There are new tests being developed to detect PrP^{Sc} in the blood of living animals. In due course they become validated and if they are it may be possible to differentiate infected from non-infected animals, assuming that positive detection of PrP^{Sc} is a proxy for infectivity. If the non-infected animals are segregated from infected animals any risks there may be in the blood would be reduced. It will be necessary to be confident that PrP^{Sc} negative animals were not infected and remain negative at repeated subsequent testings.

4.3. EPIDEMIOLOGY / SCREENING AND INCIDENCE

Assessment of Risk for TSE transmission by ruminant blood must obviously include data on the source of the population used for consumption.

Because of the long incubation period, clinically healthy but infected animals enter the food chain in areas where TSE occurs. Therefore, it is necessary to determine the number of such animals.

4.3.1. Diagnostic Assays

4.3.1.1. In vivo.

Clinical signs alone are insufficient to confirm TSE. In regard to BSE, there are no reliable *in vivo* tests available yet; diagnosis can only be confirmed by *post mortem* techniques. In scrapie, biopsies from lymphatic tissues can be used to determine infection in live animals in the early stages of incubation (Schreuder *et al*., 1998 ; O'Rourke *et al*., 1998).

Other and perhaps more sensitive assays have been developed based on antibody detection of PrP^{Sc} *e.g.* in the blood of sheep with pre-clinical scrapie (Schmerr, 1999) such as capillary electrophoresis. However, the suitability of such assays for fast routine diagnosis is not known at present.

<u>The new capillary electrophoresis test on blood</u>

A new method has been developed for extracting PrP^{Sc} using an organic solvent followed by a chromatography step. This extraction method concentrates PrP^{Sc}. A method using capillary electrophoresis using fluorescent peptides and a specific antibody to prion peptides is used in a competitive immunoassay format to detect the abnormal prion. Although the assay was originally developed using brain material, it has been adapted for blood buffy coats. The sensitivity or specificity of the test is not yet known.

The results from the blood assays in sheep show a good correlation with that of lymphoid tissue results, particularly tonsil. When sheep from high risk scrapie flocks were tested, it was found that of the 50 sheep that tested lymphoid tissue positive, 47 were blood assay positive. It has been observed that when some of the sheep are in the later stages of clinical disease, the blood assay becomes negative. These animals were positive on earlier tests. The three animals that the blood assay did not correlate were also brain positive. For sheep with negative lymphoid tissues that came from the same high risk flocks, there is approximately 5% of these animals that were positive on the blood assay including some animals with the

polymorphism of QR at codon 171. These sheep will be held and monitored by blood assay.

Sheep were infected orally with scrapie infected brain. Some of the infected sheep became positive for PrP^{Sc} by blood assay. This experiment is still in progress and the sheep will be followed for approximately 2 years following the infection.

In order to determine when PrP^{Sc} appears in the blood of animals, lambs born to scrapie infected ewes are being followed by blood assay. The lambs were blood positive at 4-6 weeks after birth. At 6 months the blood assay was negative for some of the lambs but became positive again at 8 months of age. The lambs will be kept under observation to verify whether clinical scrapie develops.

In an elk herd that was implicated for infection with chronic wasting disease by a "trace back", 5 animals were blood positive. One of these animals died in October 1999 and was confirmed to have chronic wasting disease. When the herd of 80 animals was depopulated, two of the other blood positives were positive in the brain by immunohistochemistry. Two others are being investigated. Studies are underway to determine the correlation of the blood assay with Western blot of the lymphoid tissues.

4.3.1.2 Post mortem tests

- Conventional diagnosis of BSE and scrapie is based on histopathological demonstration of spongiform change and neuronal vacuolation in anatomical predilection areas (Wells and Whilesmith, 1995). Spongiform change occurs more or less at about the same time or shortly before clinical signs (Wells *et al*., 1996, 1998).

- Immunohistochemical (IHC) demonstration of PrP accumulation is a very reliable technique to diagnose TSE (Miller *et al*., 1994 ; Haritani *et al*., 1994 ; Graber *et al*., 1995) even in badly preserved and autolysed tissues (Doherr *et al*., 2000). The typical appearance and anatomical distribution of the reaction product are important criteria. With respect to BSE, and on the basis of the limited available data, it appears that PrP accumulation is demonstrable for some months prior to onset of clinical signs and in advance of microscopic lesions in the brain (Wells *et al*., 1996, 1998). IHC is therefore more sensitive than conventional histology. IHC has also been used to detect PrP^{Sc} accumulation in lymphatic tissues derived from sheep infected with scrapie (Schreuder *et al*., 1998 ; O'Rourke *et al*., 1998).

- The Western blot (WB)[9] (Mohri *et al*., 1992) also very reliably demonstrates PrP^{Sc} accumulation even in severely autolysed tissues. In Western blots, digestion of the PrP^{C} is a critical step. In addition to the positive signal, a shift in position of the PrP band helps to confirm diagnosis. WB may be able to detect PrP^{Sc} in cattle some months prior to clinical disease (Schaller *et al*, 1999).

- For cattle, ELISA systems have also been developed and have been shown to be reliable for confirmation of a clinical diagnosis of BSE (Moynagh and Schimmel, 1999). Digestion of PrP^{C} can also be a critical step in ELISA procedures.

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9 In some laboratories, scrapie-associated fibril (SAF) detection methods are still applied.

- Several more tests are under development (Meyer *et al.*, 1999; (Meloen, personal communication, March 2000).

4.3.1.3. Suitability of diagnostic assays for mass screening

Some of the diagnostic methods described above can be used and have been used for mass screening.

- Histological and immunohistochemical procedures are very reliable but costly and time consuming and therefore not suitable for very large numbers of samples.
- Bulk immunoassays with homogenized fresh brain tissues are necessary to process large numbers. A commercial Western blot system (Schaller *et al*, 1999) is capable to examine large numbers of samples within a relatively short time. The technique has been validated with large numbers of samples that were also screened by microscopic examination of the brain and ICH. Sensitivity (of course in respect to detecting clinical cases) and specificity with respect to histology are near 100%. Positives are confirmed by microscopic examination of the brain and immunohistochemistry (Schaller *et al*, 1999 ; Moynagh and Schimmel, 1999).
- Three microplate immunoassay systems and a Western blotting procedure based on the detection of the protease-resistant fragment of PrP^{Sc} have been evaluated for the diagnosis of BSE in cattle for the European Commission. Two of the immunoassays and the WB procedure showed excellent potential for detecting or confirming clinical BSE for diagnostic purposes or for screening dead or slaughtered animals for such cases. One test (D, CEA) could detect a positive signal in BSE-affected cattle brain homogenate ($10^{3.1}$ mouse i.c/i.p LD50/g) diluted 130 times. If infectivity parallels PrP^{Sc} presence in the bovine brain, though it might not, this would be equivalent to approximately 4 mouse i.c/i.p LD50/g. However the absence of data on the timing of accumulation of PrP^{Sc} and its relationship with infectivity during the development of BSE in bovines means that the sensitivity of these biochemical methods for pre-clinical detection of infection remains unknown. Recently a BSE ELISA was reported using heat and chemical denaturation to discriminate between abnormal and normal PrP (Meyer *et al*, 1999). Current initiatives by WHO and OIE to provide standard preparations of human and animal TSE tissues should facilitate the comparison and validation of future diagnostic assays.

Remark: The European Commission's test evaluation programme is continuing in 2000 with emphasis on early detection and the living animal.

4.3.2. Surveillance[10] and modelling

A reliable surveillance system to detect TSE cases is essential. A combination of passive and active targeted surveillance is probably optimal to determine the estimated or probable incidence of clinical BSE. Based on such data, mathematical models can be applied to determine the number of BSE-infected animals coming to slaughter, from which blood may be sourced, if the model accepts the taking into

[10] Surveillance is being dealt with into more detail by a working group of the Scientific Steering Committee.

account of age. These calculations can then be used to assess the risk of blood in regard to TSE in the area under study.

4.4. The Slaughterhouse: possible exogenous contamination of blood.

4.4.1. General considerations.

Apart from the potential risk that blood from cattle with BSE might contain very low levels of endogenous infectivity, the question of contamination of blood from external sources must also be addressed. The possible sources are most logically divided between the central nervous system (CNS), in which the level of infectivity is high, and all other body organs and tissues, in which the levels of infectivity are either very low or non-existent. A further reason for considering the CNS separately is that because of the procedures used at slaughter, the CNS has a greater likelihood of contaminating blood than any other tissue or organ.

Most large slaughterhouses are dedicated to a single species, e.g., cattle, sheep, or poultry; however, smaller facilities may process one predominant species, and accept other species when they are offered, or may even process all species for which they have capability. These non-dedicated plants could provide opportunities for cross-contamination from cattle with unsuspected BSE to blood obtained from other species. In practice, such risk would be limited to slaughterhouses that processed both cattle and pigs, which are the two preferred species for blood processing (poultry blood, when obtained, is being processed only for non-human uses).

4.4.2. Slaughterhouse procedures (detailed in annex 3)

Cattle. In the typical situation, arriving cattle are held in lairage until a clinical antemortem inspection. Only animals passing this inspection are permitted to enter the slaughterhouse, where they are put into a restraining "knocking" box and stunned into unconsciousness by the slaughterman, using a captive bolt pistol, or less commonly, any one of a number of different methods (vide infra). Immediately afterwards, a hind leg is shackled to a chain and the animal is mechanically hoisted to a height convenient for exsanguination by severance of the carotid arteries and jugular veins. Blood gushes into a tank or trough used for the collection of pooled blood from multiple animals. Blood for human consumption is usually collected hygienically into individual containers to await the result of the post mortem examination. This blood is not always collected into individual containers, but the blood of 8-10 animals is collected and mixed. Only after passing the post-mortem examination, the blood is destined to human consumption. In some countries/abattoirs blood from 8-10 animals may be pooled. Blood, whether from a single animal or a batch of animals, is diverted for animal consumption if the carcass of any of these animals fails to pass post mortem examination. The last part of the blood that leaves the carcass is normally only used for animal feed. The suspended carcass is skinned and eviscerated and then split in half. After post mortem examination the carcass usually goes to a chiller and is subsequently cut up for sale in a separate cutting room or at a cutting plant. Edible offals and other usable products are despatched for sale or to processors. Condemned material and specified risk materials are despatched separately for appropriate destruction under the control of the veterinary authority.

The details of this procedure can vary from country to country, and in different slaughterhouses within a single country, depending on national regulations and local preferences. They may also vary depending on the size of the slaughterhouse, with smaller facilities often executing the entire procedure in a single room, whereas larger facilities may render animals unconscious and bleed them in one area, before transferring them to another area for further processing.

Sheep : Sheep are handled in much the same way as cattle, whereas the physical plants for smaller animals are correspondingly smaller and less complicated. One major variable is the method used to achieve unconsciousness of the animals. Whereas some form of captive bolt stunning is the overwhelming method of choice for cattle, sheep are usually stunned by electrocution (but may also be stunned by captive bolt), and some are pithed. Game and fur-bearing animals are usually anaesthetized with carbon dioxide or anaesthetic drugs.

4.4.3. Stunning devices

Stun guns can be either penetrating or non-penetrating. The commonest form of penetrating guns fire a captive bolt through the cranium into the brain. Guns firing a free bullet are sometimes used. Captive bolt pistols are of three general types: cartridge-fired stunners, pneumatic-powered stunners, and pneumatic-powered air injection stunners. All three types induce gross cerebral trauma when fired into the skull, and the air-injection variety produces a particularly profound injury. In addition, the practice of 'pithing' often accompanies the use of penetrating non-air injection devices, which involves inserting a rod into the hole made by the bolt and agitating the underlying deep brain structures and cervical spinal cord. Non-penetrating guns stun by concussion from a heavy mushroom-shaped head, potentially minimising but not altogether eliminating the potential for embolisation of cerebral tissue. (see annex 3).

4.4.4. Dissemination of brain tissue emboli during stunning.

It has been known for some time that, in humans, severe brain trauma can be followed by disseminated intravascular thrombosis due to the coagulating effects of microscopic brain emboli (Ogilvy *et al.*, 1988). In cattle, it has also been shown that 'marker' bacteria applied to a captive bolt gun can be recovered from spleens, and from spleens and muscle tissue when applied to a pithing rod (Mackey & Derrick, 1979).

Since 1996 there have been several studies on the dissemination of CNS material into the bloodstream by different methods of penetrative stunning used to stun cattle slaughtered for human consumption. Garland, Bauer and Bailey (1996) and Bauer, Garland and Edwards (1996 Vet Pathol 33,600 – Brain emboli in slaughtered cattle) in the USA reported finding macroscopically visible brain tissue in the left and right branches of the pulmonary artery of 2.5-5.0% of cattle stunned with ‘The Knocker’ (by Hantover). This is a pneumatically operated captive bolt gun that also injects compressed air into the cranial cavity, thus ‘scrambling’ the CNS tissue. A more recent study (Schmidt *et al.*, 1999) correlated the occurrence of large blood clots in 33% of the right ventricle of hearts with the use of this type of stunner. Some of these clots contained CNS tissue. A modified form of this stunner that did not inject air had a lower percentage of clots (12%) compared with 1% where cartridge-fired stunners were used. It was not mentioned if pithing was used.

In the UK, Anil *et al* (1999) confirmed that pneumatic stunning resulted in CNS material being found in the jugular vein and identification of this was facilitated by immunochemical detection of S100β protein. These authors also compared the results using a conventional captive bolt pistol (with and without pithing) and a non-penetrative captive bolt gun. Of these three methods only the penetrating captive bolt pistol with pithing resulted in the detection of brain material in the jugular vein. Finally, Munro (1997) in the UK, reported a histological study of the pulmonary arteries of 200 slaughter cattle. All but one (stunned with a free bullet) were stunned using a conventional captive bolt pistol. 70% of the latter were pithed as well. No evidence of CNS embolism was found. Some of these studies employed the use of marker proteins or enzymes to support the morphological findings. None of the studies examined other organs or tissues for the presence of brain fragment emboli.

None of these studies examined other organs or tissues for the presence of brain fragment emboli. Thus, a recent study by Schmidt *et al* (1999), in which several different peripheral organs and tissues were examined for the presence of glial firbrillar acidic protein (GFAP) following stunning is particularly important. No GFAP was found in muscle from any of the stunned animals. In contrast, as yet unpublished results from a group at Texas A&M have documented microscopically visible brain fragments in liver after stunning - presumably, under the pressure of injected air, the fragments are forced into the paravertebral venous plexus, which has direct connections to the portal vein system.

Collectively, these studies show that severe brain trauma resulting from the use of stunners that inject air under pressure in the cranial cavity will produce CNS emboli in a significant number of instances. The practice of pithing, no matter what method of stunning is used, appears less traumatic but cannot be relied upon to not produce emboli. Penetrative stunning without pithing appears to be safest of the three methods of stunning in regard to the production of CNS emboli. More research is needed to determine the effects of penetrative versus non-penetrative stunning in sheep, goats and cattle and to what extent CNS tissue can enter into the peripheral blood circulation and consequently cause potential contamination of peripheral organs.

4.4.5. The potential for environmental contamination of blood.

The slaughterhouse environment is far removed from the sanitised conditions of the laboratory, and offers continuous opportunities for cross-contamination of tissues and fluids by common use tools that may be cleaned but not sterilised, or by tissue debris accumulating in the workplace. If brain emboli were to gain access to peripheral (non-CNS) tissues by virtue of head trauma suffered in the course of captive bolt stunning, then any organ or tissue of the carcass could in principle become vulnerable to contamination. Therefore, it is possible that under circumstances where different steps in the slaughtering process are not conducted in different rooms, blood that is siphoned through troughs into collection vessels of pooled blood could be contaminated by particles or aerosols from other bovine specified offals being handled by the slaughterhouse personnel (see annex 3). Moreover, contamination might occur from brain tissue escaping from the stunning bolt wound.

4.5. CONCLUSIONS

1. Slaughtered cattle are used to source blood for food, feed and a variety of other purposes including the manufacture of medicinal products and biologicals. Farmed sheep, goats and deer could theoretically supply blood for these purposes. All these species are susceptible to TSE both naturally and experimentally. BSE as a natural disease has only been reported in cattle. The possibility of BSE being in sheep and goats cannot be excluded. Furthermore, one hypothesis for the origin of BSE is from a scrapie-like agent from sheep. It is possible that such a source still exists or that the BSE agent from cattle has returned to sheep and goats. If it exists it may no longer be recognisable if natural sub-passage has occurred.
2. TSE has never been reported in European deer. Surveillance for the disease is recommended.
3. No validated tests exist to detect TSE in live cattle, sheep, goats or deer.
4. Effective ante mortem clinical inspection of all slaughter animals is essential.
5. Post mortem inspection as currently practised will not enable detection of any TSE in any species and will not improve upon the ante mortem inspection.
6. Exogenous contamination of blood with CNS material in the form of emboli (and hence infectivity) is most likely in TSE-infected animals stunned with a stunning pistol that injects gas into the cranial cavity under pressure. This can also occur if a conventional cartridge–fired captive bolt is used in combination with pithing.
7. Exogenous contamination of blood could theoretically occur post mortem if SRM are not kept separated from collected blood.

5. INACTIVATION OF THE TSE AGENT IN BLOOD-DERIVED PRODUCTS, RECYCLING AND DISPOSAL.

5.1. Introduction

At present, blood collected hygienically in licenced EU abattoirs can be used for food, feed and a variety of other purposes without any form of processing. For example, it is permissible to incorporate fresh untreated plasma into the materials used for the production of sausages, and can be spread on land as a fertiliser. Elsewhere, in this document, it is concluded that there could be a risk of the occasional presence of low levels of TSE infectivity in blood collected in abattoirs. Because it is not known as to what levels of infectivity might represent a risk to animal or human health, there is a need to establish an EU-wide policy as to what control measures and/or decontamination standards might need to be applied to potentially TSE-infected blood collected in abattoirs, regardless of whether it is discarded or used for other purposes. The shaping of such a policy requires the availability of a considerable amount of information to answer a number of key questions that are identified below. The answers to a number of these questions are provided elsewhere in this document, to which reference will be made. Additional commentary has been added as appropriate.

5.2 The questions

5.2.1. *For what purposes is blood that is collected in abattoirs used, and how is it processed?*

It is apparent from the tables in section 2.1 and 2.2 of this document, that the nature of usage of blood and the resulting blood-products obtained from abattoirs within the EU is wide and incompletely understood. This is compounded by the fact that the information that has been made available to the Working Group has been provided by a relatively small number of member states. The situation is further complicated by the fact that the names used to describe products in one country can be quite different from the descriptions, and methods of manufacture, in other countries. For example, a product known as "cooked blood" in one country is described in another as "blood meal," and is likely to be produced in other member states under other names. It is known that the heating procedures used to produce "cooked blood" are somewhat different from those used to produce "blood meal," and there are likely to be further variations in the methods used to produce comparable products (but with different names) in other member states. From the limited information available on the manufacturing processes for blood-products, the most rigorous methods applied would appear to involve coagulation of the blood at 95-100^{o}C, followed by spray-drying[11] during which the end-product reaches a temperature of 110^{o}C. Several sets of data indicate that even this process is unlikely to result in any significant reduction in the titre of TSE infectivity (Brown *et al*., in press; Brown *et al*, 1990; Taylor & Diprose, 1996 ; Taylor, 1996; Steele *et al*, 1999a & 1999b). Given this, and the other imponderables, there can be no prescriptive process that sets separate standards for the effective TSE decontamination of individual products. The alternative is to either apply risk-assessment techniques or introduce a generic rule that all such products should be subjected to the 133^{o}C/20'/3 bars autoclaving procedure[12] now used to render mammalian protein within the EU when it is to be fed to non-ruminant species. The latter approach would be likely to result in a number of products being unsustainable. Although individual manufacturers could opt to apply the 133^{o}C autoclaving process, risk-assessment would appear to be the more general way forward. Nevertheless, when this autoclaving procedure is applied to blood products or raw blood, these should be regarded as containing a negligible risk level regardless of the region from which the donor animals were sourced and passed fit for slaughter.

5.2.2 *Is there evidence that the blood of apparently healthy ruminants might contain infectivity at the time of slaughter if they are incubating TSEs?*

Earlier discussions have indicated that there is no proof of this at present, but suggest that this might prove to be the case when applying the more sensitive methods of detection that are becoming available. It is considered that the best

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[11] One type of blood meal production is the spray system. During this process, blood undergoes different phases: homogenisation and filtration; concentration under vacuum at T=30-40°C for 2' ; spray drying with air injection at 450-500°C for 30'. At the end of this phase, the meal that exits of the spray tower has a temperature of about 110°C. During the whole process, the system is hermetically closed. There is also the roller system, which comprises different phases: 90° C with steam, centrifugation, drying at 100° C during 90'. One company prepares bovine and porcine blood for animal feed uses by spray drying of plasma at 230°C for >20 secs input and 90°C output.

[12] 1999/534/EC

course at present is to assume that such blood could be infected at very low levels. Experimental studies have shown that the detection of infectivity in the blood of TSE-infected rodents becomes increasingly more successful as one reaches the stage of overt clinical neurological disease (Brown *et* al, 1999). This suggests that the age at which ruminants are slaughtered could have a significant effect on whether or not infectivity might be present in the bloodstream. However, there is no uniform policy within the EU as to the age at which different ruminant species should be sent to abattoirs. For its own reasons, relating to BSE, the UK currently requires that products for human and animal consumption must be obtained from cattle that are slaughtered before the age of thirty months (with the exception of cattle in the beef assurance scheme that can be killed up to 40 months of age). Given that the average incubation period for BSE is around five years, this measure aims at eliminating as far as possible the likelihood that the organs (and blood) of potentially BSE-infected cattle will have no detectable infectivity at the time of slaughter. However, this policy is confined at present to the UK, and other countries slaughter their bovines at whatever age seems appropriate.

As far as scrapie-infected sheep (and probably goats) are concerned, PrP^{Sc} can be detected in the lymphoreticular tissues of natural cases as early as four months of age, suggesting that infectivity may also be present in the blood of very young animals (Schmerr, personal communication, 2000). This indicates that the risk of scrapie infectivity being present in the blood of slaughtered sheep and goats has to be considered, regardless of their age at slaughter.

5.2.3 *Are the TSE-related risks associated with the collection of blood in abattoirs confined to the blood of TSE-susceptible ruminant species?*

The initial remit was to consider TSE-related risks associated with the usage or disposal of ruminant-derived blood. However, it would appear that the problem is not confined to ruminant blood. Although the TSEs that currently exist within the EU are confined to cattle, sheep and goats, it is recognised that the blood of currently-unaffected ruminant or monogastric species such as deer and pigs could become contaminated in pooled blood collected in abattoirs that slaughter a variety of species.

5.2.4 *Even if the blood of TSE-affected ruminants is infected only at a low level, are there abattoir practices that could enhance the titre of infectivity in the collected blood?*

As discussed, it is now evident that the various combinations of stunning and pithing that are commonly used during the slaughter of cattle can result in particles of brain-tissue being released into the bloodstream, and this has been highlighted as an area that needs further research. Although this problem has only been studied so far in cattle, there is no reason to suppose that it does not apply also to sheep or goats. Thus, in all of these species, there is the possibility of TSE infectivity invading the bloodstream as a result of the slaughtering procedures if there is infectivity in the brain.

As has already been discussed, there are further risks of blood becoming contaminated if the complete slaughtering process, including bleeding, is carried out within a single area in the abattoir, rather than in a progressive production-line fashion where bleeding is carried out in one area but other parts of the meat-production process are carried out downstream in physically separate areas of the abattoir. Under such confined conditions there could be a somewhat greater degree

of contamination of blood. This could occur through the potential release of central nervous system tissue as a result of skull-splitting [prohibited in the UK as the head is SRM] and spinal cord trauma, in addition to removing lymphoreticular tissues of sheep and goats such as spleen (the lymphoreticular tissues of cattle with BSE – with the possible exception of that in the distant ileum- appear to be uninfected). As a result of blood collection procedures, the contamination of collected blood, with brain tissue that leaks out of the captive bolt wound in the skull, is possible. In contrast, there is evidence that the lymphoreticular tissues of sheep and goats infected with scrapie are likely to be infected at a relatively early stage in the scrapie disease process. This means that significant numbers of young, and apparently healthy, sheep and goats could be slaughtered at a time when the lymphoreticular system (but not the brain or spinal cord) is infected. However, the size and discrete nature of the spleen (the largest lymphoreticular organ) is such that it is likely to be removed intact after slaughter. What is unknown is the degree of trauma (leading to contamination) that might be sustained by infected lymph-nodes during the process of carcass preparation but this is thought to be minimal. Consequently, even under the worst conditions, the titre of infectivity in pooled blood will be modest compared with that in the brain-tissue of clinically affected animals.

5.2.5 *What is the current status of scrapie in sheep and goats with regard to human health considerations?*

The risk of BSE being present in small ruminants has been addressed in the SSC opinion of 24-25.09.98 on *The risk of infection of sheep and goats with BSE agent*.

There is long history of dietary and occupational exposure of humans to the scrapie agent, and no evidence that this has ever resulted in human disease. However, there is experimental evidence that the BSE agent can be transmitted to sheep and goats by the oral route (Foster *et al*, 1996). The resulting disease is indistinguishable from scrapie, and this has posed the question as to whether sheep and goats that were fed meat and bone meal obtained from BSE-affected countries (before exportation was prohibited) could have become infected with the BSE agent. At present, this is an open question, and studies are in progress to determine whether or not this has actually occurred. Thus, until the results of ongoing studies become available, it would be sensible to assume that the BSE agent could be present in the sheep and goat populations of countries where scrapie occurs if sheep and goats have been fed imported meat and bone meal (or home-produced meal in the case of countries with indigenous BSE) since 1980.

5.2.6 *Should there be restrictions on the source of blood used to prepare commercial products supplied to laboratories?*

Companies that supply animal serum for incorporation into viral culture media are already well aware of the problems associated with the TSEs; this is also true for companies that manufacture bacterial culture media that contain animal blood or its components. Scientific advice and provision of guidelines for sourcing materials has already meant that such companies have probably already taken or considered to take measures to ensure that their products are (as far as posible) free from TSE-associated risks. With regard to serological products, the small amounts of TSE infectivity that might be present in any of these products has to be viewed as representing a minimal risk to human health. Any such minimal risk would be further reduced by the careful *modus operandi* that should prevail in laboratories

that handle biological agents. There is also an obligation for such laboratories to carry out risk-assessments relating to the procedures that they carry out, and introduce measures for the protection of laboratory personnel as appropriate. It is therefore considered that the commercial (or private) manufacture of laboratory reagents derived from animal blood collected in abattoirs does not need to be the subject of control measures.

6. RISK ASSESSMENT

The Working Group considers that the most important aspect of risk assessment relates to brain tissue contamination and proposes the following general approach for the risk assessment for blood within a given area:

Because of a large number of risk factors involved, risk assessment for ruminant blood needs to be made on a regional base. A distinction may have to be made between BSE and Scrapie.

The assessment involves basically 3 levels:

6.1. Slaughterhouse

On a regional base, basically at the level of each individual slaughterhouse, the following risk factors can be objectively evaluated:

1) species and age of slaughtered animal ;
2) frequency of infected cows being killed and brain material entering the bloodstream related to the stunning method used (pneumatic devices, pithing);
3) the average amount of blood collected per animal;
4) the dilution factor by pooling blood from several animals;
5) the amount of such collected blood going to the industry to be processed for human or animal consumption.

6.2. Geographical BSE risk and surveillance

The geographical BSE risk and surveillance are covered in detail in a number of SSC opinions adopted since early 1998. In addition, a working group of the Scientific Steering Committee is presently addressing ways to further improve TSE surveillance systems.

An essential risk factor which can be quantified on a country by country basis (using appropriate surveillance data and calculation models) is the incidence of BSE in the population, which allows to estimate the number of BSE infected animals of each age category entering the slaughtered population.

6.3. Use of blood

At the level of processing of blood for human and/or animal consumption, risk can be further evaluated in respect to (i) further dilution of CNS material and (ii) the efficacy of the various processing steps in respect to inactivating the BSE agent.

There is little doubt that under certain circumstances, humans or animals could be exposed to the BSE agent by consuming blood products. The frequency of such exposure could be estimated from case to case on a highly regional base. An essential risk factor, which cannot be quantified on the base of present knowledge,

is the amount of brain material actually entering the bloodstream following the use of invasive stunning devices. Neither its volume range nor the range of particle size is known. Likewise, no quantitative estimates are available on contamination of blood with SRM materials during the slaughtering process other than by stunning the animals.

A more general qualitative approach to risk assessment could be proposed as summarised in the following table:

Increased risk	Decreased risk
high incidence of BSE in slaughter animals	low incidence of BSE in slaughter animals
animals > certain age slaughtered	Animals < certain age slaughtered
invasive stunning devices	non-invasive stunning devices
small slaughterhouses	large slaughterhouses
blood frequently collected	blood infrequently collected
amount of blood pooled *	amount of blood pooled *
large amount used for consumption	small amounts used for consumption
mild processing of blood products	severe processing of blood products
Feeding to same species (no species barrier)	Feeding to humans/different species (species barrier)

* A high degrees of dilution would result in a lower residual infectivity per unit of volume, but may also imply that a much higher number of individuals be exposed to (lower) infectivity. In terms of possible infections, the outcome may eventually appear to be the same (see also the Pre-opinion of 2-3 March of the Scientific Steering Committee on *Oral exposure of humans to the BSE agent: infective dose and species barrier.)*

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 General

As has been discussed, the collective data that are currently available from experimental transmission studies suggest that there may be no infectivity in the blood of TSE-infected ruminants. In contrast, low levels of PrP^{Sc} have been detected in the blood of clinically normal sheep from scrapie-susceptible flocks using a newly-developed and highly sensitive assay system. However, the precise relationship between PrP^{Sc} and infectivity is not understood; the two do not always correlate quantitatively, and the absence of PrP^{Sc} does not necessarily mean the absence of infectivity. As far as ruminant blood is concerned, it is considered that the best approach at present is to assume that it could contain low levels of infectivity. However, even if this is true, it becomes almost irrelevant compared with the level of contamination that could occur as a result of the methods of stunning used in abattoirs. These procedures are now recognised to release particles of brain-tissue (potentially containing high titres of TSE infectivity) into the bloodstream; the frequency at which this occurs appears to increase with the severity of the stunning process, and this report recognises that this is an area requiring further research. There are also opportunities for the contamination of pooled blood as a consequence of the release of brain-tissue from the hole left by stunning, or with spinal cord during its removal (if a production-line process is not used). Nevertheless, given the low frequency at which apparently healthy animals

would have TSE infectivity in the CNS at the time of slaughter, it is considered that the overall potential level of infectivity in pooled blood will be low.

Tables 2.1 and 2.2 show that ruminant blood and blood-products are used in a variety of fashions for feed, food, and other purposes. For reasons discussed in the report, it is considered necessary at present to conclude that bovine blood might occasionally facilitate foodborne transmission of BSE to animals or humans in high-risk areas. It was also concluded that the blood of other ruminants and pigs could pose a similar problem if collected in abattoirs that also process cattle. Raw blood is incorporated into feed and food-products, and even the heating procedures used to process some blood-products appear not to have any great capacity to inactivate the BSE agent. It is therefore considered appropriate to attach conditions to the use of blood for various purposes depending on the BSE-risk status of the country of origin. With regard to restrictions on the use of ruminant blood or its derivatives in the manufacture of cosmetics and pharmaceuticals, this is already covered by existing international guidelines. This Working Group recommends adherence to such guidelines.

A particular problem has been to determine whether BSE might have infected sheep and/or goats as a result of including (potentially BSE-infected) meat and bone meal in their feed. Experimental data have demonstrated that sheep are susceptible to BSE by the oral route, and that the resulting disease is clinically and neurohistopathologically indistinguishable from scrapie. It is difficult to resolve whether transmission of BSE to sheep or goats might have occurred in countries within which scrapie, but not BSE in cattle, is endemic, and where imported meat and bone meal might have been fed to sheep and/or goats. The situation is even more complicated in countries where both endemic BSE and scrapie occur if sheep and goats were fed meat and bone meal. In the UK, where such a situation exists, there are a number of ongoing studies that are designed to determine whether the BSE agent has infected sheep but these may not produce unequivocal data. One further clue might be provided by strain-typing the agent/s that have caused a few single cases of scrapie in sheep-flocks in Switzerland during the 1990s. The relevance of such a study is that Switzerland appeared to be scrapie-free before the emergence of its BSE epidemic. Also, it would be extremely unusual for only single individuals to succumb to scrapie in any given flock of sheep. It can be seen in the report that precautionary measures have been recommended that take account of the possibility that BSE has already infected sheep and goats, and is masquerading as scrapie.

7.2 Specific conclusions and recommendations.

- There is an increased risk of producing emboli of CNS material by use of stunners that inject gas under pressure into the cranial cavity, or by the use of pithing rods following any method of stunning.
- The highest risk of producing CNS emboli follows captive bolt stunning with compressed air into the cranial cavity.
- Cartridge operated captive bolt stunning followed by pithing presents the next highest risk.
- A lower risk occurs following use of other methods of penetrative stunning, including cartridge-operated stun guns without pithing.

- There is insufficient knowledge to advise on the degree of risk from the use of penetrative cartridge-operated stuns without pithing, free bullets or non-penetrative guns.
- More information is required on the possible dissemination of CNS emboli into the systemic circulation.
- TSE risks may exist as a result of the source of animals for slaughter. The lowest risk results from the slaughter of animals from TSE free countries, regions, herds or flocks.
- TSE risks may occur independently of the stunning procedure as a result of TSE infected material from SRM for example entering the blood after exit from the body.
- Processing procedures could reduce residual TSE risk in the collected material.
- The greatest risk of dissemination of infected material, if present in the final product, would be by exposure of the same species as the donor species.
- Risks would be greatest when efficient routes were used and the dose was greater than a minimum infective dose.
- Whereas ante-mortem examination is an essential prelude to slaughter, post-mortem inspection performed in the slaughterhouse as currently conducted is irrelevant to blood safety in regard to TSE, because it will not detect BSE or scrapie that escaped detection during the ante-mortem inspection.

RECOMMENDATIONS

- Consideration should be given to prohibiting methods of stunning ruminant food animals that increase the risk of CNS material entering the bloodstream at slaughter wherever there is a risk from TSE. In addition, sourcing from young[13] animals would further reduce the risk.
- Develop methods for reduding the risk of cross contaminating blood with CNS or other SRM post-collection.
- Brain may often spill from the bullet hole via the gutter into the blood tank. Given the low temperatures at which bloodmeal has been prepared and is prepared, it is not excluded that contaminated blood would have contributed and contributes to the spread of BSE. It is therefore recommended that surveys are done to verify the presence of brain material in the bloodtanks.
- A change from pneumatic stunning or pithing, to stunning methods that avoid severe brain damage could go along with an increased risk of physical injury to slaughtermen (particularly during shackling and bleeding out) if the new methods or building facilities are not properly designed.

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13 First infectivity in CNS of cattle is detected in most cases in the last quarter of the incubation period. Defining young animals could be done on the basis of the probability of occurrence of BSE according to the age. (See for example the annexes 3 and 4 of the Opinion of 28-29 October 1999 of the Scientific Steering Committee on the *Scientific Grounds of the Advice of 30 September 1999 of the French Food Safety Agency (the Agence Française de Sécurité Sanitaire des Aliments, AFSSA), to the French Government on the Draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom*.

FURTHER RISK REDUCTION

Depending upon their geographic source, blood and blood-products obtained from healthy ruminants declared fit for slaughter may, or may not, be considered safe for a variety of purposes depending upon their nature of usage. Where an element of risk is perceived, this may be reduced or eliminated by (a combination of) various strategies, as follows:

1. Source bovine blood from closed herds or equivalent schemes.
3. Subject the product to a 133^{o}C/3 bar/20 minutes autoclaving process or equivalent validated process.
4. Source blood for manufacturing cosmetics and pharmaceuticals only according to corresponding international recognised standards.

These strategies should be implemented by taking also into account also other opinions of the Scientific Steering Committee, e.g., on "Fallen Stock" (June 1999) and "Intra-species recycling" (September 1999).

RESEARCH

Further research is needed to:

- Determine the comparative (quantitative) TSE risks from various penetrative and non-penetrative methods of stunning in food animal species.
- Quantify the possible presence of CNS material in blood following various stunning methods.
- Determine the effects of various stunning procedures used in sheep and goats in regard to the production of CNS emboli.
- Determine the effect of the "133°C/20'/3bar" treatment on the nutritional value of blood for animal nutrition and the reduction/elimination of possible infectivity in blood.
- Investigate the possible TSE infectivity of blood cells and other blood components. Furthermore highly sensitive tests that are presently under development, need to be further validated.

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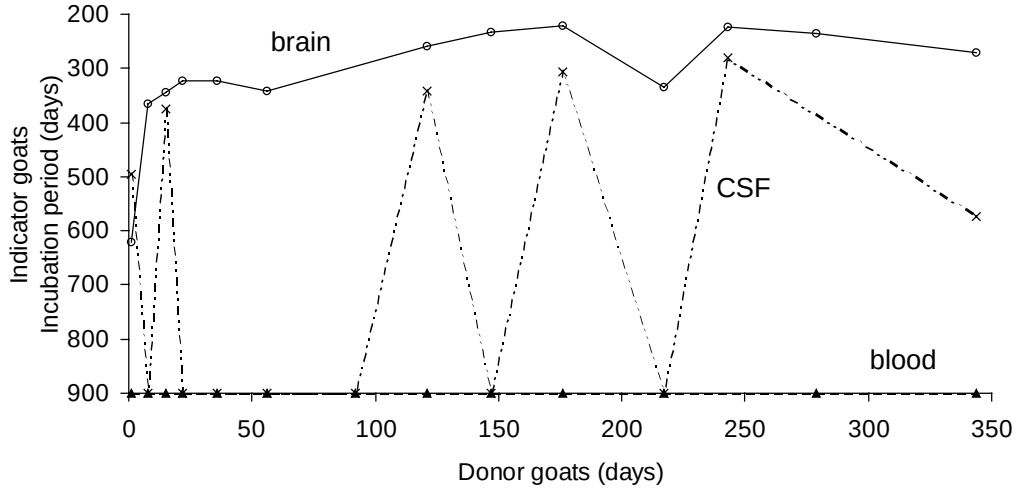
ANNEX 1

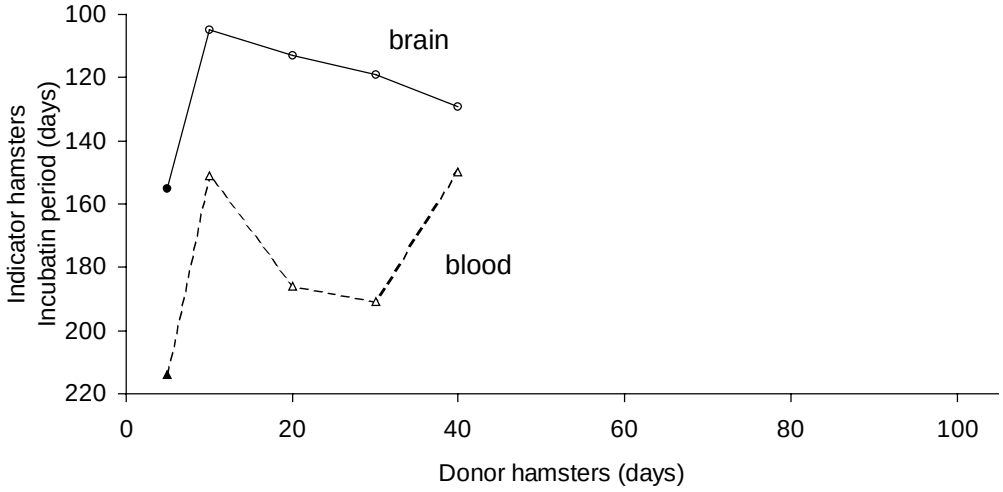
FIGURES

Fig. 1: Tissue infectivity in scrapie goats



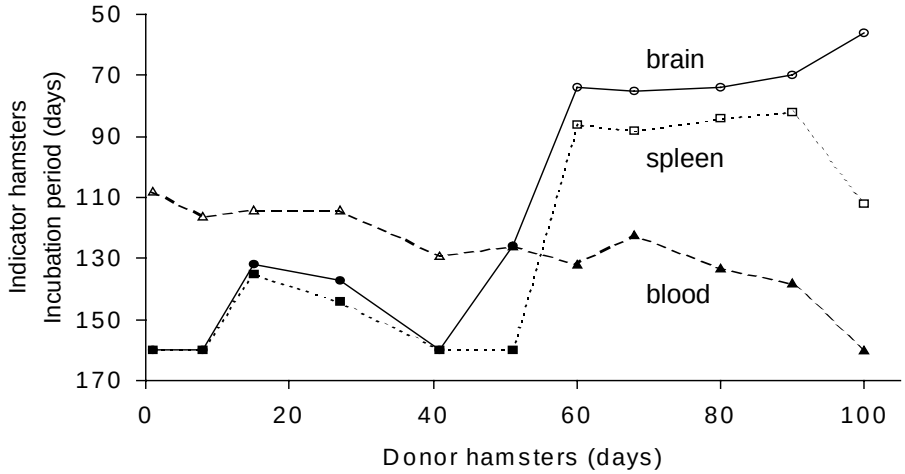
Pattison, I.H., Millson, G.C. Distribution of the scrapie agent in the tissues of experimentally inoculated goats. J.Comp.Path. 72, 233-244, 1962
Donor goats: i.c.
Indicator goats: i.c. 1 ml whole blood

Fig. 2: Infectivity of P_{215S} in scrapie hamsters



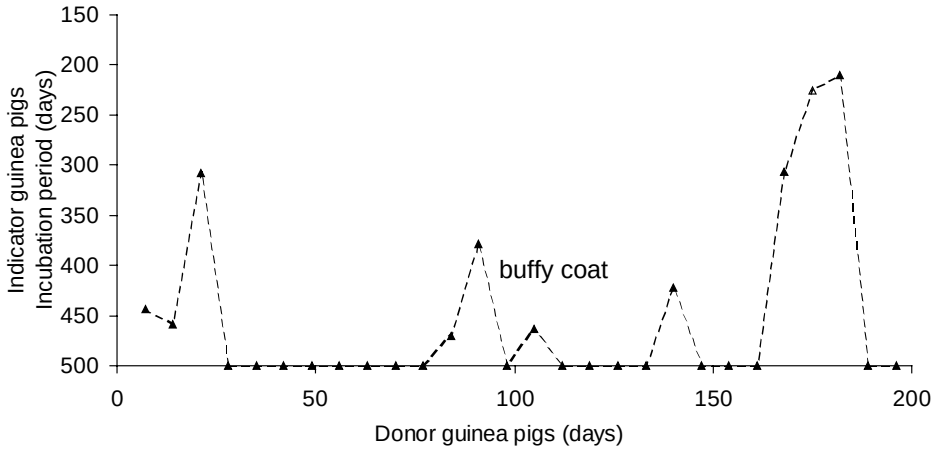
Diringer, H. Sustained viremia in experimental hamster scrapie. Arch.Virol. 82,105-109,1984
Donor hamsters: i.p.
Indicator hamsters: i.c. P_{215S} (equivalent to 2 ml blood)

Fig. 3: Infectivity of P_{215S} in scrapie hamsters



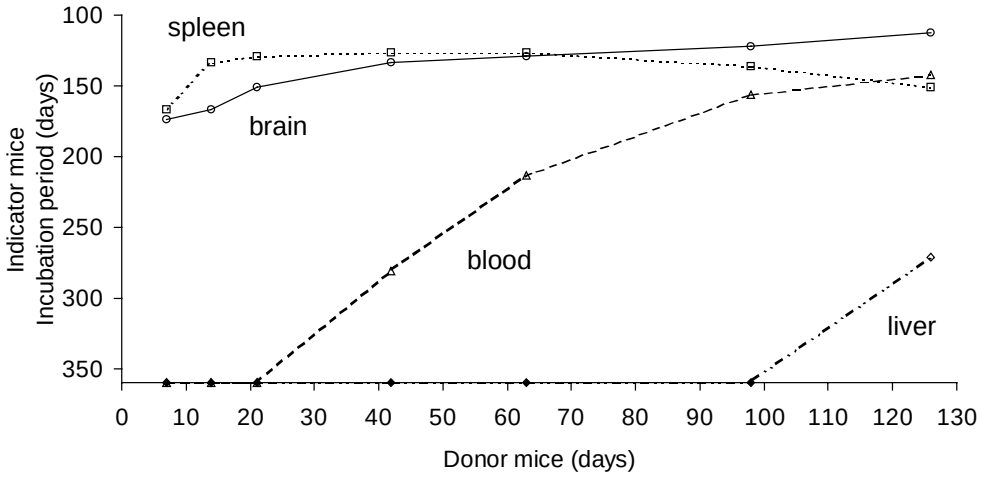
Casaccia, P. et al. Levels of infectivity in the blood throughout the incubation period of hamsters peripherally injected with scrapie. Arch.Virol. 108, 145-149, 1989
Donor hamsters: i.p.
Indicator hamsters: i.c. P_{215S} (equivalent to 0.2 ml blood)

Fig. 4. Infectivity of buffy coat in CJD Guinea pigs



Manuelidis, E.E., Gorgacz, E.J., Manuelidis, L. Viremia in experimental Creutzfeldt-Jakob disease. Science 200, 1069-1071, 1978
Donor guinea pigs: i.c
Indicator guinea pigs: 0.1 ml i.c.+s.c.+i.m.+i.p. buffy coat

Fig. 5: Tissue infectivity in GSS mice



Kuroda, Y., Gibbs. C.J. Jr., Amyx, H.L., Gajdusek, D.C. Creutzfeldt-Jakob disease in mice: persistent viremia and preferential replication of virus in low-density lymphocytes. Infect.Imm. 41, 154-161, 1983
Donor mice: i.c.
Indicator mice: i.c. buffy coat, i.p. serum and erythrocytes

ANNEX 2

TABLES

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: B232 (6/74).
Telephone: direct line (+32-2)29 58554, switchboard 299.11.11. Fax: 29 96301 / 96299.

Internet: paul.vossen@dg24.cec.be

Table 1: Studies of blood infectivity in naturally infected donor species assayed by crossing the species barrier

Author	TSE strain, route of administration	Donor species	Tissue	Indicator amount of material, route of administration	Indicator species	Remarks
Gibbs, C.J. Jr (Gajdusek, D.C.) 1965	Scrapie (natural infection)	Sheep (ram)	Serum		Swiss mice (NIH general purpose stock)	From transmission data: infectivity similar to that in brain and not reproducible (Hadlow et al, 1982)
Hadlow, W.J. (1980)	Goats (living with scrapie infected Suffolk sheep) with natural scrapie (clinically affected)	3 goats (Nubian x Toggenburg)	Serum and blood clot	30 µl i.c. (tenfold dilutions, 10 mice per dilution)	Swiss mice	No infectivity in serum and blood clot.
Hadlow, W.J. (1982)	Sheep naturally exposed to scrapie (young sheep, infected, but not clinically ill as well as diseased sheep)	Suffolk sheep	Blood clot, serum	30 µl i.c. (undiluted and tenfold dilutions, 10 mice per dilution)	Swiss mice	No infectivity in serum and blood clot.
Fraser & Foster (1994)	BSE (naturally affected)	Cattle	Buffy coat, blood clot, serum.	0.02ml i.c + o.1ml i.p. (10^{-1} homogenate)	RIII inbred mice	No infectivity
Brown, P. (Gajdusek, C.D.), 1994	Spongiform encephalopathy (natural infection, positive transmission with brain tissues)	Human	1. Whole blood (including units of blood) 2. Leukocytes 3. Serum	Inoculation	Primates (mostly squirrel monkeys)	No transmission 1. 0/7 2. 0/3 3. 0/2

Manuelidis, E.E. et al (1985)	2 CJD patients (second case only with "subtle microscopic changes)		Buffy coat (centrifuged, frozen, cut 0.5cm at each side, 50% crude homogenate)	i.c.	1. 4 guinea pigs 2. 5 hamster, 5 guinea pigs	ad 1: 1 guinea pig positive, brain transmitted to 1 of 6 guinea pigs. ad 2: hamsters positive, guinea pigs negative
Tateishi, J. (1985)	CJD patient	Human	1. Blood clot 2. Cornea (10% homogenate) 3. Urine 4. Brain (10% homogenate)	20µl i.c.	CF1 mice	ad 1: 2/13 ad 2: 1/6 ad 3: 5/10 ad 4: 7/10 (blood of two other CJD patients: negative)
Tamai, Y. (1992)	1 pregnant CJD patient (brain infectious in animals)	Human	~~1. Patient's erythrocytes.~~ ~~2. Patient's leukocytes~~ 3. Patient's plasma (3x concentrated) ~~4. Cord erythrocytes.~~ 5. Umbilical cord leukocytes ~~6. Cord plasma~~	i.c (no volume given)	BALB/c mice	ad 3: 3/8 positive ad 5: 1/10 positive all others negative, also non-concentrated plams
Deslys, J.-P. et al (1994)	Iatrogenic CJD (hGH)	Human	Buffy coat	No information	Hamster	1/5 died with PrP+ brain after an incubation period of 20 months. The other 4, as well as 3/5 uninoculated controls, dies of unknown causes during this period and were cannibalised

Table 2: Studies of blood infectivity in experimentally infected donor species assayed by crossing the species barrier

Author	TSE strain, route of administration	Donor species	Tissue	Indicator amount of material, route of administration	Indicator species	Remarks
Hadlow, W.J. (1974)	Scrapie Chandler strain (derived from Cheviot sheep) i.c.:$10^{7.3}$ mouse i.c. LD_{50} s.c.: $10^{7.7}$ mouse i.c. LD_{50} i.c.:$10^{6.6}$ mouse i.c. LD_{50}	Goats (Saanen breeding)	10% blood clot (whole blood?)	30 µl i.c.	Swiss mice	Exps. include time course studies, no infectivity found in blood clots.
Wells, G.A.H. (1996) Wells, G.A.H. (1998)	BSE (homogenate of brain stems of 74 cases of BSE) 100g single oral dose	Friesian/Holstein male calves	10% buffy coat	20 µl i.c. and 100 µl i.p.	RIII mice or C57Bl-J6 mice	Time course study, no infectivity in buffy coat up to 40 months post inoculation, study completed (Clinical onset approx 35-37 months p.i)

Table 3: Studies with experimentally infected donor species assayed without crossing species barriers

Author	TSE strain, route of administration	Donor species	Tissue	Indicator amount of material, route of administration	Indicator species	Remarks
Pattison, I.H. (1962)	Goat adapted scrapie (passaged 3 x in goats) (1 ml sup. (1500 rpm, 15 min) 10% brain susp.) i.c.	Goat	Whole blood	1 ml i.c.	Goat	Time course study, no transmission with blood. (See fig 1)
Pattison, I.H. (1964)	Scrapie affected foats (no further details, as in Pattison 1962?)	Cross bred goat	1. Whole blood. 2. Blood cells (mainly red blood cells) 3. Serum (each pools of two animals)	1a. 1 ml (0.5ml?) i.c. 1b. 3 x 5 ml s.c. (weekly intervals) 2. 1 ml (0.5 ml?) i.c. 3. 1 ml (0.5ml?) i.c.	Cross bred goat	No transmission
Marsh, R.F. (1969)	TME 1. 1 ml 10% brain of naturally infected mink (Hayward, Wis.) i.m. 2. 0.1 ml 10% brain (2nd passage) i.c.	Mink (Genetics - Dep., Univ. of Wisconsin)	Serum	1 ml s.c. (diluted in ten-fold steps)	Mink	No infectivity in serum, but infectivity in a series of tissues including spleen.
Marsh, R.F. (1973)	TME 1. Late clinical stage. 2. 10^5 LD_{50} i.c.	Mink	1. Whole blood, plasma, 10% red blood cells, 10% platelets, white blood cells ($1.7x10^7$/ml), cultured lymphocytes ($1.5x10^7$/ml), PHA stimulated lymphocytes ($1.5x10^7$/ml). 2. Lymphocytes ($2x10^7$/ml)	0.1 ml i.c.	Mink	1. No infectivity in listed preparations. 2. Time course study, no infectivity in lymphocytes, but in spleen and peripheral lymph nodes.

Hadlow, W.J. (1987)	TME agent Idaho strain, second mink passage, 10^3 LD_{50} s.c.	Royal pastel mink	Undiluted serum	100 µl i.c. (2 animals)	Royal pastel mink	Time course study, serum only at one occasion (28 wks. after inoculation) positive in 1/2 animals (during secondary spread from brain?)
Clarke, M.C. (1967)	Chandler strain (mice) Chandler and Fisher strain (rats)	Weaned white mice (B.S.V.S.) Wistar rats	Serum from blood pools of animals with advanced clinical signs 1. Blood from chest cavity after severing carotid and brachial vessels. 2. Blood from heart puncture.	50 µl i.c. (mice and rats)	Weaned white mice (B.S.V.S.) Wistar rats	More tranmissions in exp. 1 than in exp. 2 (contamination with tissue?)
Eklund, C.M. (Hadlow, J.) 1967	Scrapie Chandler strain (fourth mouse passage), $10^{5.7}$ LD_{50} s.c. (50 µl, 1% mouse brain susp.)	Swiss mice	Blood clot serum (pools of three mice)	30 µl i.c. (10-fold dilutions, six mice per dilution)	Swiss mice	Time course study, no transmission with blood.
Field, E.J. (1968)	Scrapie (Chandler) (50µl 10% brain suspension) 1. i.c. 2. i.p.	Mice	Blood collected from chest cavity. 1. Serum 2. Whole blood	1. 50µl i.c. 2. 50µl i.c.	Mice	Time course for the first 18 hours p.i., procedure 2 more successful (association of the agent with cells?)
Dickinson, A.G. et al (1969)	ME7 scrapie (fourth passage in mice, last two in C57Bl) 20µl s.c. and 10µl i.p. (1% brain susp. After 50g, 5 min.)	C57Bl (s7) or SM (s7) or LM (s7) or VM (p7) (s7, p7: sinc allelles)	Blood (cardiac pncture)	20 µl i.c. (10^{-1} saline susp. Centifuged 300g for 10 min.)	C57Bl weanling mice (11 to 15 mice per sample)	Time course study, blood occasionally positive (3/12 time points, 1 or 2 of 11 to 15 mice)

Diringer, H. (1984)	Scrapie 263K (100µl 1% brain homogenate) i.p.	CLAC hamsters	Blood concentrate P_{215S} (1:1 diluted, sonicated in 1% sarkosyl, centrifuged 20min 22000g, supernatant pelleted 120min 215000g)	50µl (equivalent to 2ml blood) i.c.	Hamsters	Time-course study (see fig. 2)
Casaccia, P. et al (Pocchiari, M.), 1989	Scrapie 263K (50µl 10% brain homogenate) i.p.	Golden Syrian hamster	Blood centrate (1:1 diluted, sonicated in 1% sarkosyl, centrifuged 20min 22000g, supernatant pelleted 120min 215000g)	50µl (equivalent to 0.2ml blood) i.c.	Weanling hamsters	Time course study (infectivity phagocytized by monocytes, cellular turnover) see fig. 3
Manuelidis, E.E. (1978)	CJD (54yr female) 5x serially transmitted in guinea pigs (100µl 1% brain homogenate), i.c.	Guinea pigs (Hartley strain)	Buffy coat from 8ml blood (centrifuged, frozen, cut 0.5cm at each side)	Altogether 0.4ml (i.c. and s.c. and i.m. and ip., 0.1ml each) (equi-valent to 6.4ml blood)	Guinea pigs	Time course study (see fig. 4)
Tateishi, J. et al (1980)	CJD, serially trans-mitted in mice (same strain as Kuroda 1983? No details) (10µl 15% brain homogenate)	Mice (difference strains?)	Whole blood	10µl i.c.	Mice (different strains?)	2/10 indicator mice positive (material from diseased animals?)
Kuroda, Y. eta l. (Gajdusek, D.C.), 1983	Fu strain of "CJD" (atypical case = GSS) 2x serially transmitted in mice (30µl sup. 10% brain homogenate), i.c.	Weanling BALB/c mice	Buffy coat (blood centrifuged, frozen, cut 0.5cm at each side) serum erythrocytes	30µl buffy coat, i.c. 100µl serum, i.p. (?) 100µl erythrocytes, i.p. (?)	Weanling BALB/c mice	Time course study (see fig. 5)
Doi, T. (1991)	"CJD Fukuoka 1" strain (same as Kuroda 1983), 5x serially trans-mitted $10^{5.5}$ LD_{50} I.C. (20µl)	ddY mice	Whole blood (cardiac puncture)	20µl whole blood (undiluted? 20% homogenate?) i.c.	ddY mice (720 days)	No infectivity in blood

Table 4: Infectivity levels in the buffy coat, plasma, and Cohn cryoprecipitate+Fraction I+II+III in groups of mice exsanguinated at intervals of 5, 9, 13, and 18 weeks after inoculation with the Fukuoka-1 strain of TSE. Mice were asymptomatic at 5, 9, and 13 weeks, and showed symptoms at 18 weeks

Time after inoculation	Specimen[1]	Inoculated volume (ml)[2]	Number of inoculated animals	Number of positive animals	Infectious units per ml (CI)[3]	
5 weeks	Buffy coat	0.28	35	3	11.3	(3-29)
	Plasma	0.59	40	1	1.8	(0.1-8)
	Cryo+I+II+III	0.16	29	2	0.5	(0.1-1.5)
9 weeks	Buffy coat	0.15	29	1	6.8	(0.4-30)
	Plasma	0.29	38	0	0.0	(0-11)
	Cryo+I+II+III	0.14	28	3	1.0	(0.3-2.6)
13 weeks	Buffy coat	0.16	28	1	6.4	(0.4-28)
	Plasma	0.29	38	0	0.0	(0-11)
	Cryo+I+II+III	0.15	28	2	0.6	(0.1-1.7)
18 weeks[4]	Buffy coat	0.14	27	11	106.0	(55-184)
	Plasma	0.25	33	5	21.9	(8-47)

1. Cryoprecipitate and fraction I+II+III were prepared from 10 ml plasma.
2. 0.03 ml volumes were inoculated into groups of mice either undiluted, or at dilutions of 1:2, 1:4, or 1:40. These dilution factors were taken into account when calculating the total undiluted inoculated volume of each specimen.
3. CI = 95% confidence interval (based on a likelihood ratio).
4. Cryoprecipitate from this bleeding was not available for inoculation.

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: B232 (6/74).
Telephone: direct line (+32-2)29 58554, switchboard 299.11.11. Fax: 29 96301 / 96299.

Internet: paul.vossen@dg24.cec.be

Table 5: Leukofiltration of plasma from mice inoculated with the Fukuoka-1 strain of TSE

Experimental Conditions	Specimen	Inoculated volume (ml)[1]	Number of inoculated animals	Number of positive animals	Infectious units per ml (CI)[2]
Exp.#1: 16 week plasma pool (symptomatic mice)					
Frozen & thawed (undiluted)	Pre-filtration	0.47	28	12	34.4 (18-58)
	Post- filtration	0.33	35	4	13.0 (4-30)
Exp.#2: 13 week plasma pool (asymptomatic mice)					
Frozen & thawed (diluted 1:4)	Pre-filtration	0.29	38	0	0.0 (0-11)
	Post-filtration	0.26	35	8	34.6 (16-65)
Exp.#3: 18 week plasma pool (symptomatic mice)					
Fresh (undiluted)	Pre-filtration	0.25	33	5	21.9 (8-47)
	Post-filtration	0.23	29	4	19.8 (6-46)

1. 0.03 ml volumes were inoculated intracerebrally at dilutions of 1:2 and 1:4. These dilution factors were taken into account when calculating the total undiluted inoculated volume of each specimen.
2. CI = 95% confidence interval (based on a likelihood ratio).

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Telephone: direct line (+32-2)29 58554, switchboard 299.11.11. Fax: 29 96301 / 96299.

Internet: paul.vossen@dg24.cec.be

Table 6: Centrifugation for 30 minutes at 17,000 x g of plasma from mice inoculated with the Fukuoka-1 strain of TSE

Specimen	Inoculated volume (ml)[1]	Number of inoculated animals	Number of positive animals	Infectious units per ml (CI)[2]
Plasma	0.47	28	12	34.4 (18-58)
Pellet[3]	0.26	34	19	21.8 (13-34)
Supernatant	0.30	40	2	6.8 (1.1-21)

1. 0.03 ml volumes were inoculated intracerebrally at a dilution of 1:4. This dilution factor was taken into account when calculating the total undiluted inoculated volume of each specimen.
2. CI = 95% confidence interval (based on a likelihood ratio).
3. Resuspended in saline.

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: B232 (6/74).
Telephone: direct line (+32-2)29 58554, switchboard 299.11.11. Fax: 29 96301 / 96299.

Internet: paul.vossen@dg24.cec.be

Table 7: Bioassay of infectivity in the plasma and buffy coat of symptomatic mice inoculated 16-18 weeks earlier with the Fukuoka-1 strain of TSE: comparison of levels of infectivity detected by intracerebral or intravenous inoculation of specimens into the assay animals

Specimen	Route of inoculation	Inoculated volume[1]	Number of inoculated animals	Number of positive animals	Infectious units per ml (CI)[2]		Intravenous to intracerebral equivalence
Plasma	intravenous	4.50	30	16	5.1	(3-8)	
	intracerebral	0.47	28	12	34.4	(18-58)	7:1
Buffy coat	intravenous	1.40	28	18	20.6	(12-32)	
	intracerebral	0.14	27	11	106.0	(55-184)	5:1

1. For intracerebral inoculations, 0.03 ml was inoculated at dilutions of 1:2, 1:4, or 1:40. For intravenous inoculations, 0.15 ml was inoculated undiluted (plasma) or 0.2 ml at a dilution of 1:4 (buffy coat). These dilution factors were taken into account when calculating the total undiluted inoculated volume of each specimen.
2. CI = 95% confidence interval (based on a likelihood ratio).

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Internet: paul.vossen@dg24.cec.be

Table 8: Proportions of disease transmissions to hamsters after intracerebral inoculation of 0.03 ml of fresh or formalin-fixed scrapie-infected brain tissue, either unheated or exposed to dry heat at various times and temperatures.

		Log_{10} dilution										
2.1.1.1.Specimen	undiluted	-0.7	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
Fresh tissue												
Unheated control				4/4		7/7		12/12	11/11	10/12	0/12	0/8
Heated 5 minutes												
150°C			8/8	8/8	8/8	8/8						
300°C		34/34	12/12	8/8								
600°C	0/15											
1000°C	0/17											
Heated 15 minutes												
150°C			8/8	8/8	8/8	8/8						
300°C		33/33	12/12	7/8								
600°C	5/18											
Formalin fixed tissue												
Unheated control						7/8		5/12	3/12	1/12	0/12	0/8
Heated 5 minutes												
150°C			8/8	8/8	8/8	7/8						
300°C		34/34	12/12	8/8								
600°C	1/24											

ANNEX 3

STUNNING, KILLING AND COLLECTION OF BLOOD FROM FOOD ANIMALS FOR HUMAN AND ANIMAL CONSUMPTION AND OTHER PURPOSES

INTRODUCTION

Since blood, for human food or animal feed, is only permitted to be collected from healthy animals that have passed an official ante mortem inspection and that are killed in a licensed slaughterhouse, supervised by the Competent Veterinary Authority, only these animals will be considered. This report is concerned with TSE risks only. Therefore, in theory, only ruminant species need to be considered. In practice blood is collected from cattle and pigs and could be collected for various purposes from other ruminant species including sheep, goats and deer, and theoretically also from poultry. Slaughter methods vary between species and between different religious sects so this annex will cover all these aspects.

Killing is accomplished by bleeding. Bleeding out is of considerable importance to the subsequent keeping quality of meat and acceptability by the consumer. Some methods of religious slaughter employ a sharp knife to 'cut the throat' without any prior treatment. Welfare considerations conflict with this aspect of slaughter. In the EU, most animals are stunned before they are bled out. The purpose of stunning is to reduce pre-slaughter stress of the animal, to ensure the slaughter is conducted in a humane manner, to improve operator safety and maintain hygiene to a high standard. In particular, stunning is used to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover whilst being bled out (Blackmore & Delany, 1988). Pigs are particularly prone to pre-slaughter stress. Excessive stress can significantly affect the meat quality of pigs and so produce pale soft and exudative (PSE) meat or dark firm and dry (DFD) meat depending upon the glycogen level in muscles at the time of slaughter.

LEGISLATION

According the Council Directive (1993) on the protection of animals at the time of slaughter or killing, solipeds, ruminants, pigs, rabbits and poultry brought into slaughterhouses for slaughter shall be a) moved and if necessary lairaged, b) restrained and c) stunned before slaughter. Animals must be restrained in an appropriate manner, to spare them any avoidable pain, suffering, agitation, injury or contusions. Animals must not be suspended before stunning or killing; however, poultry and rabbits may be suspended for slaughter provided that appropriate measures are taken to ensure that they are in a sufficiently relaxed state for stunning. Permitted methods for stunning are 1) captive bolt pistol, 2) concussion, 3) electro-narcosis and 4) exposure to carbon dioxide.

According the existing EU legislation, (Directive 93/119/EC), animals shall be spared any avoidable excitement, pain or suffering during movement, lairaging, restraint, stunning, slaughter or killing. The construction, facilities and equipment of slaughterhouses, and

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: B232 (6/74).
Telephone: direct line (+32-2)29 58554, switchboard 299.11.11. Fax: 29 96301 / 96299.

Internet: paul.vossen@dg24.cec.be

their operation, shall be such as to spare animals any avoidable excitement, pain or suffering.

Instruments, restraint and other equipment and installations used for stunning or killing must be designed, constructed, maintained and used in such a way as to achieve rapid and effective stunning or killing in accordance with the provisions of this Directive. The competent authority shall check that the instruments, restraint and other equipment used for stunning or killing comply with the above principles and shall check regularly to ensure that they are in a good state of repair and will allow the aforementioned objective to be attained.

Inspections and controls in slaughterhouses shall be carried out under the responsibility of the competent authority, which shall at all times have free access to all parts of slaughterhouses in order to ascertain compliance with this Directive.

Methods on stunning

Captive bolt stunning is widely used for all farm animals and rabbits. Gunpowder (cartridge), compressed air and spring under tension have been used to drive bolts through the skull of animals. The ideal shooting position is frontally on the head. Pithing is sometimes used to prolong the period of stunning and to improve operator safety. It is not compulsory and many large slaughterhouses do not use it. It is not recommended for hygienic reasons.

A study in the EU-Member states in the seventies showed that the captive bolt was used for stunning of more than 90% of cattle. A few cases were stunned by concussion. Electrical stunning was mainly used for sheep and in a few cases for bobby calves. Gas stunning was not used for cattle and sheep (Mickwitz & Leach, 1977). It can be expected that these figures have not changed dramatically in the last 20 years. Religious slaughtering (bleeding without stunning) is limited in the Member states and shall operate under the responsibility of the official veterinarian.

EFFECT ON BRAIN TISSUE

In general, penetration of a missile into the brain can cause injury in the following three ways, depending on its velocity and shape: a) by laceration and crushing at a low velocity (< 100 m/s), b) by shock waves at a high velocity (about 100 to 300 m/s) and c) by temporary cavitation at a very high velocity (> 300 m/s) (Hopkinson & Marshall, 1967). The velocity of a bolt of a captive bolt pistol is about 100 m/s in the air. This low velocity and shape of the bolt should crush the cortex and deeper parts of the brain either by the bolt itself or by forward shock waves (Lambooij 1982).

When the bolt penetrates the cavity of the skull, the capacity cannot be increased due to the inflexible bony skull. The consequence is that some brain tissue has to leave the cavity via the hole of the penetrated bolt in the skull. When air pressure is used to limit convulsions a lot of brain tissue will leave the cavity. Brain tissue was observed in lungs of cattle slaughtered in the US after being stunned with a pneumatic powered stunner (Garland et al, 1996), however, the validity and relevance was questioned (Taylor, 1996; Munro, 1997). To determine the extent of dissemination of CNS tissue in the heart approximately 2,000 carcasses in commercial beef slaughterhouses in the US were screened. Visible clots (not necessarily containing CNS emboli) in the right ventricle were observed in 33%, 12% and 1% when powered air injection stunners, pneumatic powered stunners and cartridge stunners, respectively, were used (Schmidt et al, 1999). Moreover, multiple fragments of brain tissue were detected in the jugular vein blood of 4

out of 15, 1 out of 16 and 0 out of 15 cattle slaughtered after the use of powered air injection stunners, cartridge penetrating stunner with subsequent pithing and captive bolt stunner, respectively. Brain tissue was not observed in the jugular vein blood after concussion stunning (Anil et al, 1999).

PHASES OF THE SLAUGHTERING PROCESS

The different slaughtering phases can be summarised as follows:

1. **Movement and lairaging of animals in slaughterhouses**
2. **Restraint of animals before stunning, slaughter or killing**
   - Animals must be restrained in an appropriate manner in such a way as to spare them any avoidable pain, suffering, agitation, injury or contusions.
   - Animals which are stunned or killed by mechanical or electrical means applied to the head must be presented in such a position that the equipment can be applied and operated easily, accurately and for the appropriate time. The competent authority may, however, in the case of solipeds and cattle, authorize the use of appropriate means to restrain head movements.
3. **Stunning**

   Note stunning renders the animal unconscious, bleeding out kills. Stunning must not be carried out unless it is possible to bleed the animals immediately afterwards. The person that is responsible for stunning, shackling, hoisting and bleeding of animals must carry out those operations consecutively on one animal before carrying them out on another animal.
4. **Bleeding out**

   For animals which have been stunned, bleeding must be started as soon as possible after stunning and be carried out in such a way as to bring about rapid, profuse and complete bleeding. In any event, the bleeding must be carried out before the animal regains consciousness.

   All animals which have been stunned must be bled by incising at least one of the carotid arteries or the vessels from which they arise.

   Usually, blood destined to human use is collected with a hollow knife. This procedure requires more time than for conventional bleeding. But this procedure is more hygienic. In the case of severing of arteries, blood is collected more rapidly.

   Blood is collected by gravity beneath the hoisted animal into a tank or a canal, to large collection reservoirs. It is absolutely impossible to separe blood from single animals.

   This causes two problems:
   - the post-mortem inspection on carcasses by the veterinarian is carried out downstream in the slaughtering chain. In this manner, if the carcass is not declared fit for human consumption, blood of the same carcass is no longer separable from blood of the other animals;
   - if the captive bolt pistol has not operated correctly and if the carcass of this animal is not seperated, blood with possible cerebral fragments is not separable from the rest of the mass.

   Individual animal blood collections are not usually done from poultry, swine and cattle so cross-contamination is possible.

SLAUGHTER METHODS

1. **Captive bolt pistol**

   Instruments must be positioned so as to ensure that the projectile enters the cerebral cortex. In particular, it is prohibited to shoot cattle in the poll position. When using a captive bolt instrument, the operator must check to ensure that the bolt retracts to its full extent after each shot. If it does not so retract, the instrument must not be used again until it has been repaired. Animals must not be placed in stunning pens unless the operator who is to stun them is ready to do so as soon as the animal is placed in the pen. Animals must not be placed in head restraint until the slaughterman is ready to stun them.

   This method is only permitted using a mechanically-operated instrument which administers a blow to the skull. The operator must ensure that the instrument is applied in the proper position and that the correct strength of cartridge is used to produce an effective stun without fracture of the skull.

2. **Electronarcosis**

   a. Electrodes. Electrodes must be so placed that they span the skull, enabling the current to pass through the brain. Appropriate measures must also be taken to ensure that there is good electrical contact, in particular by removing excess wool/hair or wetting skin.

   b. Waterbath stunners (for poultry)

3. **Exposure to carbon dioxide**

   Only for pigs

The following specific methods are subject to authorisation by the competent authority, which must ensure in particular that they are used by duly qualified staff and respecting the animal.

4. **Free bullet pistol or rifle**

   For various species, in particular large farmed game and deer.

5. **Decapitation and dislocation of the neck: only for poultry**

6. **Electrocution and carbon dioxide (see above)**

7. **Vacuum chamber:**

   This method, which is to be used only for the killing without bleeding of certain animals for consumption belonging to farmed game species (quail, partridge and pheasant).

OTHER POSSIBLE METHODS FOR SLAUGHTERING MEAT ANIMALS

There is a wide variety of captive bolt stunners available. Non-penetrative stunners have a "mushroom-headed" bolt which impacts with the skull but does not enter the brain, causing the stun due to concussive forces alone. Penetrative stunners cause insensibility due to the concussive blow to the skull, and the physical damage resulting from the entry of the bolt into the brain.

There is in addition a particular model of penetrative stunner which provides a blast of air through the centre of the bolt following entry into the brain which is intended to have the same effect as pithing after the stun.

These pneumatic stunners with air injection prevail in USA and North Europe whereas in Ireland, Italy and the UK no slaughterhouse uses this system.

The use of an injection of high pressure air into the brain produces a significant blow back of brain tissue. This produces a smearing of the head of the animal with liquefied brain. The use of this stunner again, following an initial effective stun, leads to a massive quantity of liquefied brain exiting from the original bolt hole (E.C., 1998).

In the case of air introduction in cranial cavity or vertebral canal, there is a possible passage of brain tissue in blood circulation (Schmidt, 1999).

ANIMAL SLAUGHTER AUTHORISATION FOR MUSLIM AND JEWISH RITUALS (Italian Health Ministry Decision of 11/06/80)

All the precautions able to avoid excitement, pain or suffering of the animals must be adopted. Only slaughtering without stunning is authorised and it must be carried out by qualified people. It is to be done by cutting simultaneously, with a sharp knife, the throat, windpipe and the blood vessels in the neck.

According to information available on Internet, the *Muslim ritual consists of* cutting (with a sharp knife) the throat, windpipe and the blood vessels in the neck causing death, but without cutting the spinal cord. The blood has to be drained before the head is removed.

From the same source (internet) appears that, under the *Jewish ritual*, the rabbi, using a razor-sharp knife (about 46 cm long by 3.5 cm wide), made a swift cut from side to side to sever both jugular veins and the two carotid arteries in a single stroke without burrowing, tearing or ripping the animal. The head was then raised further as the blood spurted forth. For beef, the animal is to be cut horizontally across the throat, severing the major blood vessels, trachea and the oesophagus.

BLOOD COLLECTION

1. **For human consumption**

   Blood for human consumption must be collected only from individual animals so that if a carcass later fails post mortem examination the blood from that animal can be discarded. If the carcass passes post mortem inspection the blood can be pooled with blood collected similarly from animals that have also passed post mortem inspection.

2. **For pharmaceutical use**

   Blood such as foetal calf blood used to extract foetal calf serum for vaccine manufacture is collected in a very hygienic and careful manner in order to avoid cross contamination with maternal blood, placenta and environmental hazards (Shailer and Corin, 1999). Cattle from selected donor herds of high health status can be used (Rolleston, 1999).

3. **For animal feed**

   Blood is usually collected into an anticoagulant such as sodium phosphate/sodium citrate.

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