The Etiology of BSE and Variant Creutzfeldt-Jakob Disease in Context with the new European Legislation for Material of Animal Origin, Commission Regulation (EU) No. 722/2012 – Regulatory and Scientific Experience after the first BSE/vCJD Outbreak

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Zitat von Justus von Liebig (1803 – 1873):

Die Wissenschaft fängt eigentlich erst da an, interessant zu werden, wo sie aufhört.

Für meine beiden Schätzchen

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Summary

During evolution parasites and hosts are always playing the well-known cat and mouse play of measures and countermeasures at attack and defenses. Since time immemorial zoonoses threaten the health of humans (the most dreaded: pest and influenza, which extinct in each case nearly one third of the total human population in Europe) and the immune system of humans is fighting against different pathogens (viruses, bacteria, parasites, fungi, helminthes).

With BSE, its corresponding disease in humans (vCJD) and other TSEs (e.g. Scrapie: sheep), a new form of zoonosis was identified: the infectious agent is a degenerated protein, so called prion, (PrP), which is, in the normal form, also present in healthy humans. Hence the immune systems of animals and humans have no chance to fight effective against the PrP.

The first BSE outbreak in cattle in the eighties was caused by feeding ruminants with Scrapie contaminated meat and bone meal and several countries /regions worldwide were affected by this crisis. As in UK the first cases of a new variant of Creutzfeldt - Jakob disease (vCJD) in humans were published, scientific investigations identified the same prion protein, which causes BSE in cattle, in such patients. Hence BSE was classified as zoonosis and it was clear, that the disease can threaten human health causing vCJD mainly transmitted by PrP-contaminated beef food. Therefore several legislations come into force for minimizing the risk of TSE transmission by food. The most important are Regulation 999/2001 and Regulation 1069/2009.

At this time also specific legislation were introduced for health care products (medical devices and medicinal products) utilizing material from animal origin with the objective to protect public health by minimizing the risk for TSE infections by such products. One of these legislation, the Directive 2003/32/EG is recently replaced by the Regulation (EC) No. 722/2012, coming into force in August 2013. The existing provisions described in Directive 2003/32/EC, which includes manufacturer risk analysis and risk management of the animal material, have been updated in the new revision taken global experience into consideration. The most important changes are described in detail in this master thesis in conjunction with the consequences of all involved parties (manufacturers, NB and Member States). First of all, the changed legislation form, regulation instead of directive, can be noted. Both are legally binding acts, but regulations are directly binding to all EU Member States and must not be translated into national law. Furthermore a greater percentage of medical devices (AIMDs,

custom-made devices and clinical trial materials) are now controlled by precautionary measures set in the Regulation (EC) No. 722/2012.

Why is there a revision of the legislation at this time point when BSE recently is no more a dominant public threat?

There is still a great concern worldwide that this fatal disease can re-circulate and scientific / regulatory experts recommend that the taken measures should not be decreased for several reasons, which were discussed in this master thesis. One of the reasons is the difficulty in calculation of risk for vCJD transmission because several people can carry the infectious agent without showing clinical symptoms. The unknown incidence of such `silent` carrier, the long incubation period and the lack of experiences due to the rarity of vCJD disease make calculation of the vCJD risk nearly impossible. In addition, so far there is no reliable diagnostic assay available, which can be used as screening tool to detect PrP in e.g. blood. Global CJD surveillance program revealed the main transmission pathways for PrP from human to human by blood and plasma products, which are in general applied intravenously and therefore have a great potential of transmission of pathogens, by organ transplantations (Dura Mater and Cornea) and by surgical interventions using contaminated medical devices (neurosurgical instruments). With this knowledge, attention must be paid for the future in further regulatory framework to minimize the risk for PrP infections especially by contaminated instruments during surgeries, which is recently handled different in each Member State.

List of Abbreviations

AIMD	Active Implantable Medical Device
AO	Animal- Origin
ATMP	Advanced Therapeutic Medicinal Products
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (Federal Institute for Drugs and Medical Devices)
BfR	German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)
BSE	Bovine Spongiform Encephalopathy
CDC	Centers for Disease Control and Prevention
CEN	Comité Européen de Normalisation
CJD	Creutzfeldt - Jakob Disease
CSF	Cerebrospinal Fluid
CWD	Chronic Wasting Disease
DIN	Deutsches Institut für Normung
DNA	Deoxyribonucleic Acid
EC	European Commission
ECDC	European Center for Disease prevention and Control
EDQM	European Directorate for the Quality of Medicines & Health Care (responsible for e.g. European Pharmacopoeia)
EEG	Electroencephalography
EFSA	European Food Safety Authority
FAZ	Frankfurter Allgemeine Zeitung (German Newsletter)
FCS	Fetal Calf Serum
fDC	follicular Dendritic Cells
fCJD	familiar Creutzfeldt-Jakob Disease
FFI	Fatal Familial Insomnia
FLI	Friedrich-Löffler-Institute
FSE	Feline Spongiform Encephalopathy
GBR	Geographical BSE Risk
GSS	Gerstmann-Sträußler-Schenker-Syndrom
IU	Infectious Unit
i.v.	intravenous
IVD	*in vitro* Diagnostics
M	Methionine (amino acid)
MD	Medical Device
MRI	Magnetic Resonance Imaging
MS	Member States of the European Union
NB	Notified Body
OIE	World organization of Animal Health (former: Office International des Epizooties)
PEI	Paul-Ehrlich-Institute (Federal Institute for Vaccines and Biomedicines)
PRP	Proteinaceous infectious Particle
(n)vCJD	(new) variant Creutzfeldt-Jakob Disease
sCJD	sporadic Creutzfeldt-Jakob Disease
SER	Summary Evaluation Report
sFI	sporadic Fatale Insomnia
SSC	Scientific Steering Committee of the European Commission
TME	Transmissible Mink Encephalopathy
TSE	Transmissible Spongiform Encephalopathy
V	Valin (amino acid)
WHO	World Health Organization

1 Introduction

TSEs, especially BSE in cattle, are fatal neurodegenerative disorders caused by the so called prion protein. Shortly after the BSE outbreak in the eighties, it was noted, that the infectious agent crossed the species barrier resulting in a new variant of Creutzfeld-Jakob Disease (vCJD) in humans. BSE is therefore classified as zoonosis and the transmission from BSE to humans by contaminated animal material must be avoided.

Many of medicinal products use material from animal origin, either as active substance or as excipient. In this regard specific legislation come into force to regulate the use of animal tissue in health care products with the goal to protect patients and other persons against the risk of transmitting animal spongiform encephalopathies, especially BSE. One of these regulations is the new Commission Regulation (EC) No. 722/2012 coming into force in August this year, replacing the Directive 2003/32/EC. In view of the fact, that currently the BSE crisis is mostly banned worldwide, the need for an updated regulation at this specific time is not that obvious at a first glance.

Additionally, the new variant of Creutzfeldt-Jakob disease (vCJD) caused by prion protein can also be transmitted from human to human. Therefore not only the infection route from animals to humans by health care products or food must be considered, but also the infection from human to human by e.g. blood products, contaminated medical devices in surgical/medical procedures and other human material (transplantation of organs donated from humans at risk) must be regulated.

Objective of this master thesis is to have a closer look to the BSE topic, summarizing the scientific experiences after the first BSE and vCJD outbreak in context with the new Regulation No. 722/2012. In addition the master thesis presents that the disease is and remain a threat for the health of animals and humans and show the limiting factors which make the disease unpredictable.

1.1 Zoonoses

In the course of the evolution, human beings became sedentary with agriculture and stock farming. Due to the close contact with animals, the risk for zoonosis increased. Zoonoses are infectious diseases which can be transmitted from animals to humans and from humans to animals. "*The term derived from Greek zoon (animals) and noses (diseases) […]".*[1] According to the WHO the definition (1958) is as follows: "*zoonoses* [are] *diseases and infections that are naturally transmitted between vertebrate animals and humans. A zoonotic*

[1] G. W. Beran: Handbook of Zoonoses, Second Edition, Section B: viral1994

agent may be a bacterium, a virus, a fungus or other communicable disease agent."[2] Often several experts need to overcome massive challenges regarding diagnostic and therapy of zoonoses. Often, the close cooperation of health care professionals, veterinarians, infectiologists and microbiologists are essential to understand the etiology, epidemiology, and the complex exposure routes of zoonotic agents including diagnostic, therapy and disease symptoms. According to the WHO over 200 zoonoses are described, which can be transmitted by viruses, bacteria, fungi, protozoa, parasitic helminthes or arthropods. The most widespread form is the so called zooanthroponoses, infectious diseases which are transmitted from animals to humans. On the other hand, anthropozoonoses, infectious diseases which are transmitted from humans to animals are rare. Certain professionals with close contact to potentially infected animal material (veterinarians, livestock farmers, employees of slaughterhouses/animal laboratories/zoos and hunters) have a higher risk for the infection with zooanthroponoses [13].

Historically the most famous and disastrous zoonosis was pest in Europe between 1347 and 1353, which was caused by the bacterium *Yersinia pestis.* The pest-bacteria were principally transmitted to humans by fleabites from the rat flea *Xenopsylla cheopis.* Nearly one third of the European population was wiped out during this epidemic disease. Not only fleabites can transmit zoonotic agents, infection can also cause by penetration and/or inhalation of infectious agents by the mucosa of gastrointestinal tract or lungs.

Some of the zoonotic agents (e.g. *Bacillus anthracis*, hemorrhagic fever viruses) have the potential to serve as biological weapons [2].

Few months ago in China a new influenza virus (H7N9) originated from avian influenza killed several people and there is a great concern worldwide that the influenza virus will mutate to a highly virulent virus strain which results in an effective human- to human transmission. Such an influenza mutant strain can have the potency to lead to pandemic disease greater than the outbreak of the Spanish Influenza pandemic between 1918 and 1920.

1.2 International Cooperation - OIE

Worldwide tourism, transport of food producing animals across borders, expanded use of materials from animal origin (xenotransplantation, cultures isolated from animal cells) and state-of-the-art medical technologies, such as transplant and transfusion medicine, opened

[2] http://www.who.int/neglected_diseases/diseases/zoonoses/en/index.html

new possibilities for the risk of transmission of infectious diseases from animals to humans and from humans to humans [2].

Strategies for the prevention and combat of infectious diseases in humans are of international interest as well as "*timely dissemination of information is crucial to containing outbreaks*" and need international cooperating network. "*The World Organization for Animal Health is the intergovernmental organization responsible for improving animal health worldwide. It was created by an international agreement as the international Office of Epizootics (still known by its French acronym Office International des epizooties – OIE) on 25 January 1924."* The mission of this organization is available on the webpage and includes the following tasks:

- "TRANSPARENCY: *ensure transparency in the global animal disease situation*
- *SCIENTIFIC INFORMATION: collect, analyse and disseminate veterinary scientific information*
- *INTERNATIONAL SOLIDARITY: encourage international solidarity in the control of animal disease*
- *SANITARY SAFETY: safeguard world trade by publishing health standards for international trade in animals and animal products*
- *PROMOTION OF VETERINARY SERVICES: improve the legal framework and resources of national veterinary services*
- *FOOD SAFETY AND ANIMAL WELFARE: To provide a better guarantee of food of animal origin and to promote animal welfare through a science-based approach"* [3]

The OIE published in weekly reports all immediate notifications of zoonoses worldwide including the follow-up reports every week. BSE is a zoonosis which is under the observation of OIE.

1.3 TSE/BSE

In the United Kingdom 1985 the first cases of an unknown disease by cattle were detected and the disease was widely spread in the United Kingdom. One year later, in 1986, it was diagnosed as an independent disease and referred to as BSE "Bovine Spongiform Encephalopathy". BSE belongs to the "transmissible spongiform encephalopathy" (TSE), which summarizes a group of encephalopathy's with an extremely long incubation time (for

[3] http://en.wikipedia.org/wiki/World_Organisation_for_Animal_Health

example in cattle: 3-6 years). TSE occurs in both humans and in several mammalian species and the following table[4] gives a summary of TSE examples [2].

Name	Host	Other species	Transmission in experiments
Scrapie	sheep	Goat, cattle	hamster, mouse and other
TME	mink		
CWD	elch, deer, mules		
CJD	human	Unknown	gorilla, chimps
GSS	human	Unknown	
Kuru	human	Unknown	Chimps
BSE	cattle	Cats, human, zoo animals	hamster, mouse and other

Table 1: Prion diseases in humans and animals

BSE is a progressive transmissible neurodegenerative disorder of cattle caused by an unusual agent, the so called proteinaceous infectious particle (prion, PrP) and is always fatal. The major transmission pathway for BSE is possibly feeding cattle with BSE- or scrapie-infected meat-and-bone meal. Investigations show later, that technical modifications during the manufacturing of meat-and-bone meal and other products (reduction of temperature and reduction of pressure which both in the past reduce the infectious amount of PrP) were responsible for the outbreak of BSE. The reduced conditions during the production of meat- and bone meal were insufficient to inactivate present BSE agents. Due to import of infected meat-and-bone meal and/or infected cattle from UK into other countries, the BSE was distributed nearly worldwide into other European Member States, Canada, Japan and Israel [3].

1.4 Health care products with potential for transmission of infectious diseases

The major part of medicinal products and medical devices are produced with animal origin as starting material or as excipient. For example collagen or gelatin was one of the first biomaterials used in health care products and has been used for many years when a material was needed that combined the properties of high tensile strength, biocompatibility and absorbability in living tissue. At current stage there are numerous collagen products on the market in form of sutures, ligatures, hemostatic agents, wound dressings, artificial skin, nerve guides, dural grafts and other prosthetic devices such as tubes, films and sponges. They can be used topical for wound care management or as implants. For years, the hemostatic effect of collagen, isolated from skin of swine, tendons from cattle or horses, has been well known.

The following list contains examples of detailed gelatin/collagen products:

[4] H. Kraus, A. Weber, M. Appel et al. „Zoonosen – Von Tier zu Mensch übertragbare Infektionskrankheiten", Deutscher Ärzte-Verlag, 3. Auflage, 2004

- Gelatin tampon to stop venous-capillary or parenchymatous bleeding or used for filling dead space in dental coagulum
- Vascular prosthesis impregnated with absorbable bovine gelatin for reconstructive procedure on the aorta and in peripheral area
- Collagen sponges with or without antibiotics as implant for local hemostasis of capillary bleeding
- Collagen Implant for treatment of bone defects in orthopedics surgeries
- Collagen membrane as matrix for cultured autologous cells as part of an ATMP

The application as implant might have a higher risk for transmitting zoonotic agents, because of its invasive character and its direct contact to human blood during and after surgery.

But it is not necessary for the potential of transmitting the BSE-causing agent that the substances of animal origin are directly part of the health care products, often material of animal origin is only used as supplement for cell culture media or is used during the manufacturing process and afterwards removed, but despite that the substances are no more present in the final product, the risk of transmitting prions might remain due to the great resistance of the prion. An example for supplements in cell culture media is the well-known and often used “Fetal Calf Serum (FCS)” and amino acids. All available substituents are not as good as FCS. In the new medicinal product group, the Biologicals (such as therapeutic antibodies), which are often expressed by cell cultures, these supplements are often part of the cell culture media for the establishment of the Master Cell Bank. The Master Cell Bank serves as “archive” for specifically engineered cells and is defined as a collection of cells of uniform composition derived from a single tissue or cell. From aliquots of the Master Cell Bank, the manufacturer’s Working Cell Bank is generated, which is used for routine manufacturing of e.g. antibodies. It is strongly recommended that in the Working Cell Bank, such critical supplements (FCS) should be removed from cell culture media.

Additionally a lot of enzymes (e.g. Pepsin) are also extracted from animal material (in the case of Pepsin from swine mucosa) and are components of manufacturing steps. Despite these components being removed from the final products, the risk of transmitting zoonotic agents may remain.

For these reasons given as examples above many regulations and legislation must be obeyed by manufacturers of medicinal products or medical devices using animal material to ensure safety for humans by minimizing the risk for transmission of potential zoonoses, especially prions.

2 Overview of regulations in regard to minimize the risk for transmission of animal spongiform encephalopathies for health care products utilizing animal material

2.1 In general

Generally European pharmaceutical legislation is divided into legally binding acts (Regulations and Directives) and in legally not binding soft law (Resolutions, Communications, Guidelines, MEDDEV = medical device guidance documents, Standards) [13].

Both, regulations and directives are legally binding acts within the European Union. "A *Regulation is an act of general application, binding in its entirety and directly applicable in all Member States. It does not require any transportation by the national authorities"* whereas *" a Directive is a legal act binding upon the Member States to which it is addressed, as for as the results to be achieved are concerned; leaving the national authorities the choice of form and methods. A directive always leads to complementary national measures. In order to take effect a directive must be transposed into the legal order of the Member States."* [5] Directives are "*a bit weaker than regulation"*[6] and that the directive needs "*transfer into national legislation"*[6] by definite intention, so the text can be modified by the member state which results in a wiggle room for interpretation.

Technical specifications for prevention of risks during manufacturing and packaging of medical devices can in addition be defined in standards. When a standard is drawn up by an internationally organization then it is called ISO standard. DIN EN ISO standards are drawn up at first by an internationally organization and are then transmitted into European (EN standards) and finally into national standard (e.g. in Germany: DIN standard). Conformance with harmonized European standards guarantees compliance with the essential requirements defined in the European Union. Harmonized standard are compiled by the European Committee for Standardization (e.g. CEN) and can be initiated on request from the European Commission.

In the European Union, three directives exist for the regulation of medical devices reflecting that three main groups of medical devices exist:

[5] B. Friese, B. Jentges, U. Muazzam "Guide to Drug Regulatory Affairs; Editio Cantor Verlag Aulendorf, 2007
[6] Presentation of the BSE-symposium "European legislation for material of animal origin is changing – what manufacturers need to consider", organized by TÜV Süd: "Revision Commission Directive 2003/32/EC – Consequences and Requirements for Medical Device Industry", Dr. Scholl, Aesculap AG, 23 Oct 2012

- active implantable medical devices (AIMD) covered by **Directive 90/385/EEC (amended by Directive 2007/47/EC)**
- Active, non-implantable and inactive medical devices covered by **Directive 93/42/EEC (amended by Directive 2007/47/EC)**

  and
- In-vitro Diagnostics covered by **Directive 98/79/EC (amended by Directive 2007/47/EC)**

To get legally binding directives must be translated into national law. In Germany, the three directives are transformed into one law, the Medical Device Act.

In principle, medical devices can only be placed on the market within all EU Member States, (analogous to the centralized procedure by Medicinal products) if following criteria are fulfilled:

1) Medical devices must fulfill "Essential Requirements" regarding their quality, safety and efficacy (analog to medicinal products)
2) Compliance with "Essential Requirements" is confirmed by a conformity assessment of a Notified Body (for some Medical Devices)
3) the issued CE-certificate allows affixing the CE mark and with this mark, the medical device can be marketed within the EU (free movement of goods)

According to Directive 93/42/EEC, the nature and the extent of conformity assessment by Notified Bodies vary and depend on the risk of the medical devices. In principle four risk classes are defined based on their hazard potential for humans:

- Medical Devices with low risk: class I
- Medical devices with middle risk: class IIa
- Medical devices with high risk: class IIb

  and
- Medical devices with very high risk: class III

The main criteria for the risk classification are application time, invasive capacity and activity/non-activity of the medical device. The rules for classification of medical devices are listed in Annex IX of Directive 93/42/EEC. According to rule 17 of this Annex, medical devices using animal origin are mainly classified as class III products "*all devices manufactured utilizing animal tissue or derivatives rendered non-viable are Class III except where such devices are intended to come into contact with intact skin only*".

Therefore in the following section only the conformity assessment of class III medical devices according to Directive 92/42/EEC are described:

Manufacturer of class III products can select between two different conformity assessment procedures, according to article 11 of Directive 93/42/EEC: the EC declaration of conformity of the full quality assurance (Annex II) or the EC type examination (Annex III).

If the manufacturer select procedure one, then Notified Body inspect the full Quality Assurance System in regularly intervals and approve significant changes of the Quality Assurance System before implementation. If manufacturer select the alternative procedure, then Notified Body approve significant changes in the manufacturing process and perform regularly inspections of either the Quality Assurance System_Production (Annex V) or the product (EC verification, Annex IV).

As material from animal origin, non-viable tissues or tissue derivatives originated from bovine, caprine species, deer, elk, mink and cats, is used in manufacturing of health care products, a series of additional requirements is defined to minimizing the risk for TSE transmissions:
The "Essential Requirements" for products underlying the Medical Device Directive 93/42/EEC, 8.2, Item 3 requires the following "*Processing, preservation, testing and handling of tissues, cells and substances of animal origin must be carried out so as to provide optimal security. In particular safety with regard to viruses and other transferable agents must be addressed by implementation of validated methods of elimination or viral inactivation in the course of the manufacturing process."*

In contrast, the section for "Essential Requirements" as set in the Directive 90/385/EEC for active implantable medical devices is currently: "*The devices must be designed and manufactured in such a way that, when implanted under the conditions and for the purposes laid down, their use does not comprise the clinical condition or the safety of patients. They must not present any risk to the persons implanting them or, where applicable, to other persons."* and does not include viruses and transferable agents, which is a regulatory gap. This gap is closed, because AIMDs are now included in the Regulation No. 722/2012 (see later).

Analog to medical devices, medicinal products containing or using animal material must in compliance to Annex I of the consolidated Directive 2001/83/EC, Part I, Module 2, paragraph 3.2 (9):

"*Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies (materials from ruminant origin): at each step of the manufacturing process, the applicant must demonstrate the compliance of the materials used with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products and its updates, published by the Commission in the Official Journal of the European Union. Demonstration of compliance with the said Note for Guidance can be done by submitting either, preferably a certificate of suitability to the relevant monograph of the European Pharmacopoeia that has been granted by the European Directorate for the Quality of Medicines or by the supply of scientific data to substantiate this compliance.*

Resolution AP-CSP (99) / Ph. Eur. 5.2.8 / EMA/410/01 rev 3

Since 2000, manufacturer using material of animal origin have the possibility to submit a dossier which is in compliance with current legal requirements, especially with the European Pharmacopoeia general chapter 5.2.8 "Minimizing the risks of transmitting animal spongiform encephalopathy agents via medicinal products" (which is identical to the Note for Guidance EMA/410/01 rev 3) to obtain the certificate of suitability (CEP) from the EDQM. The issued certificate confirms the TSE compliance for substances and material used in the manufacture of health care products. Such certificates can be issued, for example, for bovine collagen or FCS, but not for the final product. The dossier must be updated every five years reflecting the updated technical documentation and the possible influence on the quality, safety and efficacy of the substance / material.

The next two regulations belong to the food safety legislation, but apply also partially to manufacturer of medicinal products and medical devices as far as the source and starting material of animal origin is concerned, e.g. intermediate products ("Category 3 material").
All EU food measures on animal TSEs are summarized in the main

Regulation 999/2001 (as amended)

This regulation gathered together the rules for prevention, control and eradication of certain transmissible spongiform encephalopathies, including among others a method for determination of BSE status of a country, rules for BSE monitoring and trade / importation of certain live animals and animal products. With this regulation the use of rapid diagnostic

assays on high risk material (such as brain, lymphatic tissue) get mandatory for bovine material planned for human consumption. The consequence of this measure was that several countries detected their first BSE cases, mainly between 2008 and 2010. In this regulation a list of the specified risk material for BSE can in addition be found and the high risk material have to be removed from the human food chain to avoid cross-contamination.

Another important regulation in the handling of animal products is the so called "animal-by-Product-Regulation":

Regulation 1069/2009 (former Regulation 1774/2002)

Scope of this regulation is to set up requirements in hygienic measurements in handling of animal by-products and secondary products, including import/export, transit and trade. In this regulation provisions are defined for the nature of starting tissue. Depending to their risk the animal products are classified in category 1 – 3 and the handling of material depending form their category is defined ensuring reduction of their risk and to improve safety of animal and public health in regard of the food chain. Mandatory is an ante- and post-mortem veterinarian control of the slaughtered animals.

In addition to the Essential Requirements defined in Medical Device Directives manufacturer of medical devices manufactured utilizing non-viable animal tissues or derivatives must comply with the detailed specifications defined within **Directive 2003/32/EC**, which will be replaced by **Regulation No.722/2012**.

Objective is to maintain the high level of safety against the risk of transmitting animal spongiform encephalopathies to patients and other persons via medical devices. In the following chapter an overview of the important changes of previous and new law are stated, including the consequences for all involves parties (manufacturers, Notified Bodies and Member States). The roles for the involved parties are:

Manufacturers for health care products utilizing animal tissue have to perform a risk assessment. In Annex I of the regulation is stated what the manufacturer should be addressed in their risk assessment. Practical hints for performance of the risk assessment can be detached from the DIN EN ISO 22442 series in conjunction with DIN EN ISO 14971. The risk assessment must be taken into consideration the intended use of the medical device, the route of administration and the amount of animal material which will come into contact with the patient. This risk assessment included in the technical documentation must be evaluated

by a Notified Body. Main objective of the evaluation process by the Notified Body is to check the justification for the use of TSE-susceptible animal material in medical devices and to confirm the positive benefit to risk balance of the medical device. Member States must ensure that the Notified Bodies are appropriate experienced and qualified for the risk assessment evaluation and that the rules which are set in the appropriate Medical Device Directive (Essential Requirements) and in the Regulation (Annex I) are followed and met.

Helpful guidance for the performance of the risk assessment can be detached from the standard **DIN EN ISO 22442** series, which must be considered in conjunction with DIN EN ISO 14971:
The scope of DIN EN ISO 22442-1 is providing requirements and guidance on risk management scheme related to relevant information of the starting animal material, identifying hazards (such as viruses, bacteria, TSEs) and evaluating risks. Objective of DIN EN ISO 22442-2 are the controls of sourcing, collection and handling of animal tissue and their derivatives when using in medical devices. Scope of DIN EN ISO 22442-3 is validation of elimination / inactivation of TSE agents during manufacturing processes.

MEDDEV 2.11/1 rev. 2

In this guidance document supportive information is given to manufacturer and NB how the legislation about animal material can be interpreted and how manufacturer should perform their risk assessment. Especially the justification must be evaluated by NB that the products showed a positive risk/benefit balance and are safe for patients. But it is also a useful document for the competent authorities in their task of verification the NBs.

2.2 The new Regulation No. 722/2012 and important changes to the Directive 2003/32/EC[7]

One of the TSE legislation, Directive 2003/32/EC, is now replaced by the new Commission Regulation (EU) No. 722/2012, published on 08 August 2012. Before 2003, several different national legislations exist with the goal to protect public health against the risk of transmissible spongiform encephalopathies. With the Directive 2003/32/EC a harmonized

[7] http://medical deviceslegal.com: NEW EU rules for (active) medical devices utilising non-viable tissues of animal origin

standard was set up within the European Union for the regulation of products using animal material with the obligation for manufacturer to perform a risk management. With this directive in addition the evaluation of Notified Body and their qualification is under the control of Member States.

In the following chapter the important changes between the two legislations, directive versus regulation, are summarized:

At first, the changed form of the new law can immediately be noticed. Compared to the previous valid directive, the new law is a regulation. The consequence of that is that the process remains in principle the same, but now all Member States are involved in the process.

Another important change is the inclusion of active implantable medical devices underlying the Directive 90/385/EEC at first. There are only few examples of active implantable medical devices utilizing animal origin and this might be the reason, why this medical device type was forgotten to be included into the previous Directive 2003/32/EC. This failure is now corrected by including the active implantable medical devices using animal origin, which must now comply with the requirements stated in Regulation No. 722/2012. This result in the new obligation for manufacturers of AIMDs to carry out a risk analysis and risk management scheme with the requirement laid down in this regulation showing the safety for their products. In view of the fact, that some of active implantable medical devices using animal material are already legally on the market, Notified Bodies have now during a fixed transitional period according to their role to re-assess this specific product group for the compliance with the requirements laid down in the new regulation. In Article 7 Section 2 the transitional period was set up to 12 months; therefore by 29 August 2014 it must be confirmed by the NB and MSs that such products can still be marketed within the EU. Have NB and MS enough personnel capacity to fulfill this task within the set time period?

The role of the Member States in controlling the NB is extended: additionally Member States have to inform the commission by 28 February 2013 *"regarding the outcome of the verification and in cases where they recognized the need to amend the tasks of a notified body* (Article 4 Section 2 of Reg. (EC) No. 722/2012). In the past the information was only necessary in case, that NB need to amend the task, but not in that regularly intervals. Could Member States fulfill their role according to Article 4 to ensure that NB "*have up-to-date knowledge of* [active implantable] *medical devices, in order to assess the conformity of those devices […]? Member States shall regularly verify that those bodies maintain the required up-to-date knowledge and expertise"*? In Germany, there is apparently a lack of experience,

because "*currently* [the competent authority] *BfArM is not aware of any active implantable medical devices utilizing tissue/derivatives of animal origin,* [so far]."[8] How can they than adequately control the knowledge of the NBs?

Additionally, the requirement for a risk assessment in the new regulation is also extended "*for custom-made devices and devices intended for clinical investigations, which falls under Article 1(1)"* according to Article 3 Section 2.

For manufacturer of medical device underlying Directive 93/42/EC is the performance of risk assessment according to Annex I not new, but the requirements are more detailed and specified. Some of the requirements stated are completely new and some are only updated.

The most important novelty is, that there is now the obligation for a system to collect post-production information according to Article 5 Section 7 *"The manufacturers shall collect, evaluate and submit to the Notified Bodies information regarding changes with regard to the animal tissue or derivatives used for the device or with regard to the TSE risk in relation to the device"*. The consequence of that requirement is that the process of risk assessment is added by a third key step:

1. material selection
2. inactivation / elimination of potential infectious agents during the manufacturing process
3. Collection of post-production information

After identification of significant changes which could modify the previous risk evaluation such as previously unrecognized hazards, an increased estimated risk or that otherwise the original assessment is no more valid (e.g. changes in manufacturing process), the NB must be informed. If NB concludes that there is an increased TSE risk, then a new assessment of the conformity of the device including the involvement of Member States and Commission is needed. Therefore this novelty reinforces the control by the NB.

For responsible manufacturer this is not a novelty, because this is already stated in several guidance documents (DIN EN ISO 22442-1 Section 4.6, in DIN EN ISO 22442-3 Section 8, in DIN EN ISO 14971 and in MEDDEV 2.11/1 rev 02 Section 8). New is, that this requirement becomes now mandatory.

[8] Presentation of the BSE-symposium "European legislation for material of animal origin is changing – what manufacturers need to consider", organized by TÜV Süd; Commission Regulation No. 722/2012- Implications for BfArM, Dr. Schwertfeger

In addition manufacturer get more detailed instructions for performing audits of tissue suppliers including risk minimization of cross-contamination during all processes, from slaughtering over handling /storage among to the transport of the tissue. Contractual agreement between supplier of animal material and manufacture must clearly define the set requirements of all tissue handling processes which may introduce forwarding information regarding relevant changes.

Other important changes are regarding the geographical sourcing and the nature of the starting tissues, as follows:

Geographical Sourcing

NB shall retain information on the geographical origin of animals.

At first, in July 2000 published a group of experts (SSC) the final opinion of their risk assessment of countries resulting in the Geographical Risk of BSE (GBR). The countries were according to this system classified in four groups, which are listed in the Directive 2003/32/EC. This GBR-system has now been replaced by the Commission Decision No. 2007/453/EC amended in Commission Decision No. 2008/829/EC defining the BSE status of Member States or third countries or regions thereof according to their BSE risk with the aim to establish trade rules for each BSE-risk category. The new three classification groups are:

A. Countries/regions with negligible BSE risk such as:
    - Third countries: Argentina, Australia, New Zealand, Paraguay, Singapore, and Uruguay
    - EFTA countries: Iceland and Norway
    - EU Member States: Finland and Sweden

According to Resolution No. 16 from May 2012 the current OIE list A (countries/regions with negligible BSE risk) amended the following countries/regions:

Austria, Belgium, Brazil, Chile, Colombia, Denmark, India, Panama and Peru

B. Countries/regions with controlled BSE-risk
    - Third countries: Brazil, Canada, Chile, Taiwan, Mexico and United States
    - EFTA countries: Switzerland and Liechtenstein
    - EU Member States: Belgium, Bulgaria, the Czech Republic, Denmark, Germany, Estonia, Ireland, Greece, Spain, France, Italy, Cyprus, Latvia,

Lithuania, Luxembourg, Hungary, Malta, the Netherlands, Austria, Poland, Portugal, Romania, Slovenia, Slovakia, the United Kingdom

C. Countries with undetermined BSE-risk; all countries/regions not listed in either Category A or B

This classification applies particularly to BSE but can also be used to determine risk from other TSE relevant species, such as goat, sheep. In this regard the TSE definition in Article 2 of the Regulation No. 722/2012 is extended according to Regulation (EC) No. 999/2001 which is therefore emphasized particularly and includes now all other TSEs which might occur in the future (e.g. CWD in deer and elks, TME in minks, FSE in cats): "*TSEs: all transmissible spongiform encephalopathies with the exception of those occurring in humans."* In contrast to this, the previous definition in Directive No. 2003/32/EC `*transmissible agents*` *means unclassified pathogenic entities, prions and such entities as bovine spongiform encephalophaties agents and scrapie agents"* addressed specially BSE and scrapie.

In general manufacturer should prefer starting material with minimal risk for BSE contamination, e.g. originated from young healthy animals (< 6 months), from closed herds without any hint for BSE infection and from a country of Category A, subject to veterinarian ante and post-mortem investigations. Minimizing BSE-infection from scratch is preferred in contrast to minimizing risk be removal of pathogens by inactivation or elimination.

Nature of Starting Tissue

Rules for the use of animal tissue for manufacturing medical devices are now updated by referencing the new "Animal By Products Regulation" in Article 1(3) of Regulation No. 722/2012: "*Collagen, gelatin and tallow used for the manufacturing of medical devices shall meet at least the requirements as fit for human consumption laid down in Regulation (EC) No. 1069/2009"* and this regulation is therefore emphasized particularly. In contrast to this in the Directive 2003/32/EC the invalid previous Regulation No. 1774/2002 is cited. For tallow derivatives special precautionary measures are stated.

The WHO tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies, updated in 2010 according to new scientific experiences and therefore the categorization of tissue in regard to their risk is changed and must be adapted. In the past there were four classification groups available, which were recently reduced to three groups.

Following tissues and tissue products were classified in an increased risk category: skin, heart/pericardium, milk and urine. Now there exist three categories instead of four categories before:

1. Category A: High infectivity
2. Category B: Tissue with lower infectivity
3. Category C: Tissue with no detected infectivity

All these measures result in the conclusion, that the justification for animal material in manufacturing of medical devices is strengthened compared to the Directive 2003/32/EC and the replacement of TSE-susceptible animal material with non-TSE or synthetic alternative material should be taken into consideration. The benefits of the medical device must outweigh the residual risks.

Notified Bodies have now to summarize their result in form of the Summary Evaluation Report, according to Annex II where a template is shown. This additional documentation and the fact that changes increased the TSE risk initiate the whole process, might lead that the NB need more experiences and qualified personnel, which must be educated. The SER is prior to CE marketing forwarded to all Member States, not only the concerned MS as stated in the past according to Directive 2003/32/EC, allowing them to comment within defined timeframes according to Article 4 Section 5: "*The competent authorities of the Member States may submit comments on the summary evaluation report"*. The defined timeframes to comment on are for MD with an EDQM-Certificate within four weeks, without the EDQM-Certificate within 12 weeks from the submission of SER to coordinating authority on. In the past"[…] *Raw material or Medical Devices for which a TSE certificate has been issued, a consultation with all European competent authorities is not required.*", which mean that consultation with competent authority "[…] *was not necessary for medical devices with EDQM-Certification. This additional assessment by Member States is a clear downgrade of the importance of the TSE-Certification."*[9]

The procedure including all Member States raises a number of questions:

- NB must file a SER, which can raise more additional question/requirements from NB to manufacturer. Does this mean that NB need more time for their document review?
- In the updated procedure all Member States are involved. Must manufacturer therefore calculate a longer time before they can bring their products on the market compared to the previous process?
- An issued EC certificate for medical devices allows manufacturer marketing the product within the whole EU, but what happens, if one of the Member States does not

[9] www.tuev-sued.com/medinfo; Medical Devices utilizing material of animal origin

agree? Does this result in non-issuing the EC-Certificate although most of Member States agree?

- Will the cost increase for document review? Will NB intensify their control during inspections resulting in increased costs for inspections?
- How is confidentiality protected when e.g. the full name of manufacturer is added in the SER? Usually manufacturers have confidentiality agreement with its Notified Body, but not with the Member States. Is a release control by the manufacturer possible that the manufacturer can blind confidential information before sending to Member States and Commission?

Most of this open question will be answered after experience with the new updated procedure.

In appendix 1 of the master thesis all changes of Regulation No. 722/2012 compared to Directive 2003/32/EG are summarized.

"I*n 2010 exactly 1 195 903 BSE-tests were carried out in Germany, without positive result"* [10] and in this context one question arises: is BSE still a relevant disease and why a new regulation at this time?

3 BSE and the experience approximately 30 years after the epidemic outbreak

In the following chapter the experiences approximately 30 years after the with BSE outbreak are summarizes.

3.1 Measures in EU against BSE

In response to the outbreak of epidemic BSE in 1985, European and global scientific expertise are coordinated to initiate regulatory framework to combat the epidemic BSE. The main source of transmission pathway of TSEs from animal (e.g. sheep) to animal (e.g. cattle) and from animals to humans was with the likelihood of 99% by food. The BSE crisis was caused by feeding caws with prion-contaminated meat and bone meal. Apparently minor changes in the production of meat and bone meal did no more inactivate sufficiently the infectious agents. So within the EU effective consumer health measures were taken to "*improve EU food*

[10] Presentation of the BSE-symposium "European legislation for material of animal origin is changing – what manufacturers need to consider", organized by TÜV Süd: "Revision Commission Directive 2003/32/EC – Consequences and Requirements for Medical Device Industry", Dr. Scholl, Aesculap AG, 23 Oct 2012

safety, ensure high level of consumer protection and restore/maintain confidence in the EU food supply."[11] Few of the important food safety regulations include:

- In 1994: General feed ban of meat and bone meal to ruminants, in 2001 extended to all animal species (reinforced feed ban); additionally in most EU Member States and in the US: ban of imported live ruminants and most of ruminant products from UK, the country with the most notified BSE cases within Europa (please refer to chapter 3.2)
- In 1996: Definition of the minimum of parameters for treatment of animal waste, adapted in 1998 by following: for effective inactivation of the BSE-infectious agent, temperatures of 140°C at 3.6 bar and for at least 30 minutes is recommended (instead of 133°C, 3 bar and 20 minutes).
- BSE Monitoring rapid testing program: Screening of all dead, slaughtered and/or emergency slaughtered cattle aged 72 months or older:
    - Since 1999: Passive EU Surveillance system for BSE by examination of diseased adult cattle showing clinical signs confirmed by a veterinarian.
    - The development of new rapid post mortem BSE tests e.g. Prionics-Check test based on Western Blot test for detecting the protease-resistant prion fragment (normal PrP = protease K-sensitive ⇔ infectious PrP protease K resistant) allowed an active EU surveillance system (Regulation 999/2001). The diagnostic accuracy and analytical sensitivity of these tests were assessed on brain tissue from clinically affected bovines and approved. Until then, brains of conspicuous cattle were examined by histopathology and immunohistochemistry for PrP.
- Since 2000: Removal of high risk material (please refer to the list included in Regulation 999/2001) from the food-supply-chain by modification of procedures during slaughtering: separation and incineration of high risk cattle products. High risk organs are defined where the agent could be detected such as brain, spinal cord, thymus, spleen, intestines, lymph and nervous tissue listed in Regulation No. 999/2001.
- 2002: Occurrence of several atypical BSE-forms => test systems have to be checked if they are suitable to detect all forms of BSE (EFSA requirements)

[11] http://www.efsa.europa.eu/de/press/news/120130f.htm EFSA Features: Successful EU response to BSE

3.2 BSE cases worldwide?

The OIE data of BSE cases worldwide[12] (see Figure 1) show, that all these measures taken after the BSE outbreak were successful in reducing BSE infections worldwide from thousands of BSE cases in 2001 to 27 in 2011.

BSE wordwide 2001-2011 *Quelle: OIE*

	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Austria	1	0	0	0	2	2	1	0	0	2	0
Belgium	46	38	15	11	2	2	0	0	0	0	0
Canada	0	0	2	1	1	5	3	4	1	1	1
Czech Republic	2	2	4	7	8	3	2	0	2	0	0
Denmark	6	3	2	1	1	0	0	0	1	0	0
Finland	1	0	0	0	0	0	0	0	0	0	0
France	274	239	137	54	31	8	9	8	10	5	3
Germany	125	106	54	65	32	16	4	2	2	0	0
Greece	1	0	0	0	0	0	0	0	0	0	0
Ireland	246	333	183	126	69	41	25	23	9	2	1
Israel	0	1	0	0	0	0	0	0	0	0	0
Italy	48	38	29	7	8	7	2	1	2	0	0
Japan	3	2	4	5	7	10	3	1	1	0	0
Luxembourg	0	1	0	0	1	0	0	0	0	0	0
Netherlands	20	24	19	6	3	2	2	1	0	2	1
Poland	0	4	5	11	19	10	9	5	4	2	1
Portugal	110	86	133	92	46	33	14	18	8	6	5
Slovakia	5	6	2	7	3	0	1	0	0	1	0
Slovenia Rep.	1	1	1	2	1	1	1	0	0	0	0
Spain	82	127	167	137	98	68	36	25	18	13	6
Sweden	0	0	0	0	0	1	0	0	0	0	0
Switzerland	42	24	21	3	3	5	0	0	0	0	2
United Kingdom	1202	1144	611	343	225	114	67	37	12	11	7
U.S.A.	0	0	0	0	1	1	0	0	0	0	0
Total	*2215*	*2179*	*1389*	*878*	*561*	*329*	*179*	*125*	*70*	*45*	*27*

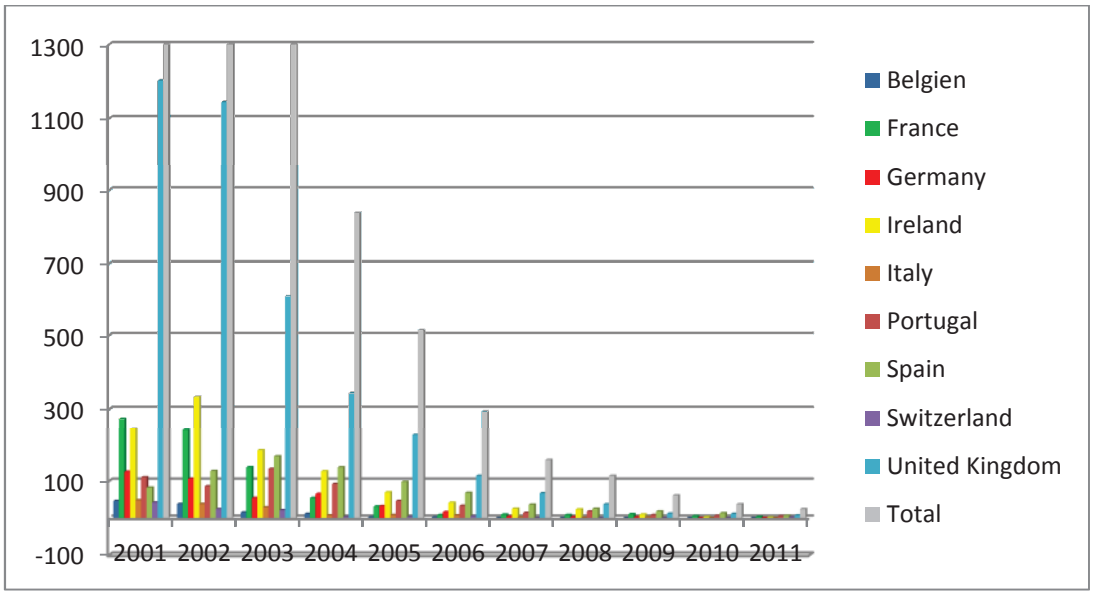

Figure 1: Number of BSE cases (above figure case numbers and below figure geographically presentation).

[12] Presentation of the BSE-symposium "European legislation for material of animal origin is changing – what manufacturers need to consider", organized by TÜV Süd; BSE in Europe – problem fixed, A. Balkema-Buschmann and M. Groschup

UK was by far the country with most detected and notified BSE cases worldwide, compared with Germany 10 times more. Other countries with a higher BSE frequency were Ireland, France, Germany and Portugal. Countries with negligible BSE cases are USA, Austria, Finland and Greece. Since 2001 the BSE cases decreased constantly, which is the consequence of all the taken measures notably the measures, especially Regulation 999/2001.

Is BSE still a relevant disease?

3.3 BSE the unpredictable risk – current level of scientific knowledge

For several reasons BSE remains an unpredictable risk and several aspects of the disease are so far still unclear despite intense scientific research worldwide:

1) the infectious agent (prion) is a protein and therefore
2) the body cannot activate an immune reaction against the prion
3) prion can easily cross species barriers (e.g. Scrapie from sheep => BSE in cattle; BSE in cattle => vCJD in humans)
4) disease and infectious pathway still not fully understood
5) long incubation period with the possibility of transmission of the infectious agent in that time without visible clinical symptoms (silent carriers)
6) no appropriate diagnostic method available before the disease shows clinical symptoms, reliable diagnostic only available on alive patients or on patient which already show clinical symptoms
7) no therapy available; disease is always fatal
8) Prion is resistant against common inactivation treatments
9) existence of atypical BSE forms with unpredictable potential and risk
10) agent become host-adapted: agent inoculated in another species => usually longer incubation period, but subsequent passage within this new species => decreased incubation period

3.3.1 The infectious agent

The infectious agent is a protein and therefore the immune system of the body can not react with an appropriate immune reaction, because the prion is not recognized as foreign. The procedure when the protein degenerate to the infectious protein is still unclear. The prion protein was identified in 1985 at first. It "*is expressed both in normal and infected cells in all mammalians. […] PrP molecules have been found on the outer surface of plasma membranes*

of nerve cells […]"[13]. The normal cellular form of the prion protein (PrP^{C}) is protease-sensitive. Since both, the normal cellular form and the pathological infectious form ($PrP^{TSE\ or\ Sc}$) is expressed, it is essential to have a method to distinguish between both forms. It is still unknown why and when the normal prion protein gets dangerous and infectious causing the disease. "*The prion hypothesis states that once produced, the abnormal* isoform PrP^{Sc} *acts as a template for conversion of more* PrP^{C} *to* PrP^{Sc}*. Thus, a chain reaction is set in motion with more and more* PrP^{C} *being transformed into the pathological* PrP^{Sc} *isoform […]. It has been demonstrated, that mice devoid of* PrP^{C} *(PrP `knockout` mice) do not develop prion diseases when inoculated with mouse* PrP^{Sc}*, demonstrating that […] infection and prion propagation requires the expression of* PrP^{C}*.*[13] It is discussed whether the physical-chemical characteristics of PrP^{TSE} causes its infectivity. The infectious agent forms high molecular aggregates, which can be detected in infectious tissue as so called amyloid plaques [16].

The infectious agent, the PrP^{TSE} is identified in several species and furthermore even animal models reveal that several species could be infected in parallel.

An alternative hypothesis to the prion theory is *the so called "virino-hypothesis". "Some scientists still believe the transmissible agent is virus-like, and that it contains DNA.[…] The strongest argumentation in support of the viral hypothesis is the presence of different strains of agent found in hosts […].* [13]

3.3.2 BSE infection and pathogenesis

Several transmission pathways (intragastric = via oral exposure and intracerebral inoculation) have been studied to investigate factors such as efficiency of TSE transmission, route of entry, the incubation period, species barriers….

Despite the efficiency of orally exposed TSE transmission being low, as shown in results from animal studies, this was the main pathway, which occurs in reality and is best-studied: BSE infection was caused by feeding cattle with PrP^{Sc}-infected meat and bone meal. Recent scientific investigations suggest that B-lymphocytes play an important role during the pathogenesis:

After oral uptake of the PrP^{Sc} by beef food, the lymphatic tissue of the pharynx is colonized by the infectious agent. From there the agent was spread along the sympathic, parasympathic and spinal cord routes into lymph nodes, spleen and lymphatic tissue of the intestine. The role

[13] WHO manual for surveillance of human transmissible spongiform encepahlopathies including vCJD; Chapter 2

of B lymphocytes is to support the maturation of follicular dendritic cells (fDC) by distribution of lymphotoxine ß. It seems that matured fDCs, where BSE replication occurs, serves as long-term reservoir for circulating prions. "*The agent probably reaches the brain from the spleen via sympathic fibres of the splanchnic nerves, which connect to the mid-thorical spinal cord.*[13]

3.3.3 New atypical BSE cases: H-Type and L-Type

In the past only one uniform BSE-strain was identified, but since 2002, new "atypical" BSE cases (in total 62 cases, please refer to Table 2) are described in France, Italy, Japan, Canada and USA [3].

Country	**H-Type**	**L-Type**
Austria	1	2
Canada	1	1
Denmark	0	1
France	14	13
Germany	1	1
Ireland	1	0
Italy	0	4
Japan	0	1
Poland	2	8
Sweden	1	0
Switzerland	1	0
The Netherlands	1	3
United Kingdom	3	0
USA	2	0
In total	28	34

Table 2: Number of atypical BSE cases worldwide (as of February 2011)[12]

The two BSE types, which were named as H and L depending on the molecular mass of protease resistant prion protein compared to the classical BSE (H = higher molecular mass; L = Lower molecular mass), occur spontaneously in animals aged eight years or older [43]. The infected animals showed no clinical symptoms, but biological and biochemical investigations detect the infectious agent. Recently these atypical forms are identified as sporadic BSE forms, but this is still not confirmed. It must be concluded, that in future single cases of BSE can occur without being associated by an infective source. The question: is atypical BSE infectious? can so far not be answered. In context to the appearance of new atypical BSE cases additional questions raises regarding the origin of BSE, but seem to be emphasize the virino-hypothesis.

Studies revealed that the L-Type has a much higher zoonotic potential than the other two BSE forms (H-Type and the classical BSE form). In France and in Poland most of the atypical BSE-Types are notified.

In contrast to the atypical BSE-Types, where the risk for humans is unknown, the risk of the classical BSE for humans is confirmed.

4 BSE and the risk for humans: variant Creutzfeldt-Jakob Disease

The first case of a new variant of Creutzfeldt-Jakob-Disease (vCJD) in humans was described 1996 in UK by H.G. Creutzfeldt and A. Jakob. This variant of the disease was different from the former well known classical Creutzfeldt-Jakob-Disease (CJD). Appendix 2 summarizes in brief the differences between the two variants of CJD, the classical form versus the new variant CJD.

4.1 Correlation between BSE and vCJD and the transmission pathway from animals (food) to humans

Histological investigations showed that the infectious agent causing vCJD is identical to the infectious agent causing BSE in cattle. Therefore it was clear that BSE must be classified as zoonosis and the correlation between the epidemic vCJD with the epidemic BSE (see figure 2) confirms the transmission of BSE to humans.

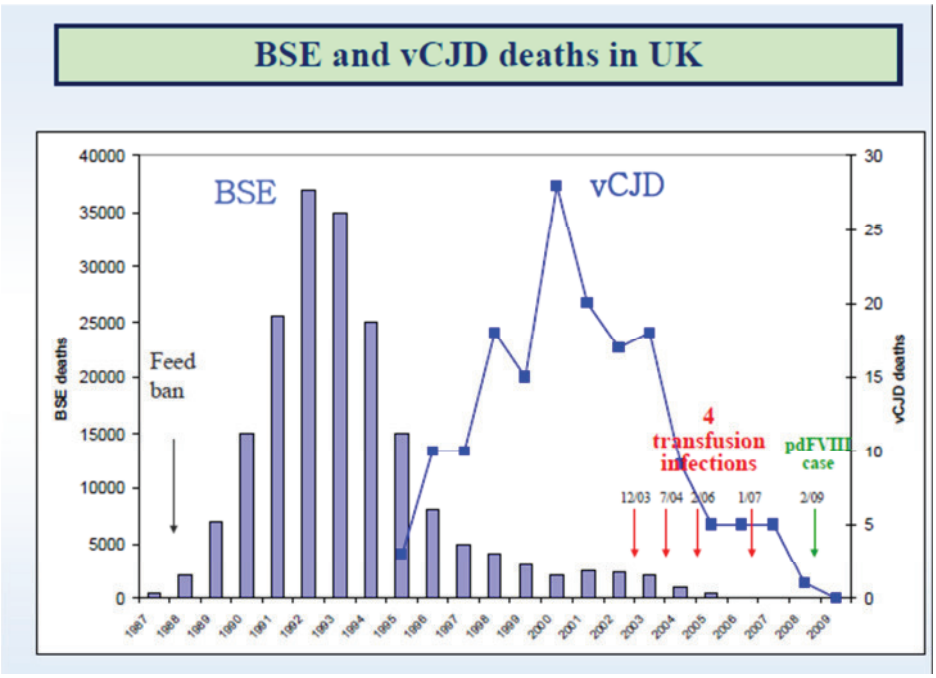

Figure 2: Correlation between epidemic BSE and occurrence of vCJD in humans[14]

The peak of BSE cases in cattle was in 1992 and approximately eight years later, the identified and confirmed cases for vCJD in humans reached the maximum in 2000. This results in the conclusion that the two diseases must stay in close correlation together. The delayed peak for vCJD might be caused by the human specific incubation period. Although the efficiency of the oral exposure in studies is classified as low, it has been shown, that the main transmission pathway of BSE from animal to humans is by PrP^{TSE}-contaminated beef food.

In contrast to beef food, there is (to date) no reported cases worldwide that a person was infected by a health care product manufactured with TSE-susceptible animal material. Nevertheless, immediately after the first BSE outbreak in 1985, the first regulatory rules (see chapter 2.1) are published for health care products utilizing animal tissue.

4.2 Global CJD surveillance program

The detection of the first case of vCJD patient is the result of a successful national CJD surveillance program in UK which was initiated in 1990. The premise therefore is, that human spongiform encephalopathy (except of the familiar forms) requires notification. In Germany, the notification of several diseases including human TSEs is defined in paragraph 6d of the German Infection Protection Act (Infektionsschutzgesetz) from 2001.

[14] Presentation of D. Asher during the BSE-symposium "European legislation for material of animal origin is changing – what manufacturers need to consider", organized by TÜV Süd;

The main key elements for initiating of surveillance systems are mainly:

- Collection of diagnosed case reports/referrals by experienced health care professionals, normally neurologists and neuropathologists, during conferences and academic rounds for adequate monitoring of trends
- Sharing state-of-the art diagnostic capacities
- Close cooperation of surveillance with scientific research with the ability to recognize early the future risk of infectious disease in humans and for a better understanding of this rare disease

  So called cohort studies, for example in families were a familiar CJD already was detected or in countries were the exposition of humans with BSE contaminated material (food or blood products) is very likely (UK, France…), identifying cases were "*the risk of TSE would be higher than among the general population"* with the goal to avoid further spread
- Review of death certificates

In general two surveillance systems can be performed, the active and the passive. Advantages of the active surveillance, e.g. periodic analyses of national multiple cause-of death data, are that clinicians can encourage to report cases and to affirm, that absence of reports correctly implies the absence of cases and a reminder program. The disadvantage is that this form of a surveillance system is expensive and most of the countries, especially emerging countries, can't perform this surveillance form. Therefore the second, the passive surveillance, is often put into practice and this form "*depends on the interest and ability of clinicians to report cases and may result in receipt of death reports (autopsy findings) only."*[15]

Especially for the surveillance system of human TSEs two obstacles exist: the rarity of the disease and the missing preclinical markers for diagnostic purposes. The first obstacle can be undergoing by a centralized national case collection and a close cooperation between "*neurologists, neuropathologists, laboratories conducting diagnostic test* (so called reference centres), *public health departments and [the] international network [is necessary]"*[15]. Nationally collected and evaluated data (e.g. in Germany, this is among others the responsibility of the Robert-Koch-Institute) are forwarded to the WHO, the European Commission and to other national authorities of the European Member States.

[15] WHO manual for surveillance of human transmissible spongiform encephalopathies including variant Creutzfeldt-Jakob disease, Chapter 8

Since 1993 epidemiological surveillance of CJD has been undertaken in a small numbers of EU Member States (EuroCJD including 11 countries), which was extended internationally by another surveillance network, NeuroCJD in 1998: the initial EuroCJD was extended with other ten Member States. Other projects also initiated by the European Commission are Prion Net for studying the neuropathology of human TSEs and SEEC-CJD to observe CJD in countries of Central and Eastern Europe and China, which started in 2001. In US "*in 1996-1997, the National Prion Disease Pathology Surveillance Center was established by the Centers for Disease Control (CDC) in collaboration with the American Association of Neuropathologists to facilitate prion disease surveillance."*[16]

Several human neurodegenerative diseases (classical CJD form, Alzheimer disease, brain tumours and cerebral vasculitis,…) show similar clinical symptoms as vCJD. Therefore common case definitions are essential to get proper interpretation of the reported cases and effective differential diagnostic. All the listed diseases must be clearly distinguished from the vCJD cases that the collection of data is exclusive and usable for specific calculation of risk for vCJD. According to the WHO recommendations following methods should be performed on living patients to make a suitable clinical differential diagnosis:

<u>*Cerebrospinal fluid (CSF):*</u>

"14-3-3 has been examined as a laboratory marker of CJD. 14.3.3 is a neuronal protein involved in cell signaling and is present in high concentrations within the central nervous system.[17]

> The use of this novel and simple diagnostic test for 14-3-3 positivity alone is not at diagnostic weight, but in conjunction with the other methods is the test suitable. The advantage of this method is, that the protein is stable at room temperature and can be easily shipped to the available centers were the CSF assay can be performed (Australia, Canada, some European Member States and USA). Although this test could not distinguish between vCJD and sCJD, this test method could be clearly distinguished between CJD forms from other dementing illnesses.

[16] Belay, E., Holman R. and Schonberger L (2005): Creutzfeldt-Jakob Disease Surveillance and Diagnosis, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
[17] WHO manual for surveillance of human transmissible spongiform encephalopathies including variant Creutzfeldt-Jakob disease, Chapter 9

Electroencephalography (EEG)

"The EEG was first recognized as an important aid to the diagnosis of CJD in 1954 and was included as a component of the first published diagnostic criteria in 1970."[17]

The typical EEG appearance with characteristic periodic changes which can be detected in sCJD cases, is not a feature of vCJD.

Magnetic Resonance Imaging (MRI)

"In over 90% of patients […] a characteristic distribution of symmetrical hyperintensity of the pulvinar nucleus […] of the thalamus […]"[17] the so called pulvinar sign was observed and seems to be highly sensitive of the vCJD disease.

4.3 vCJD cases worldwide

Result of the global surveillance systems is that so far more than 200 vCJD cases could be detected worldwide. Table 3 shows the vCJD cases worldwide as of August 2008[18] and of August 2012[19].

	Number of vCJD cases	
Country	**August 2008**	**August 2012**
UK	166 + (3 +1)***	176*
France	23	24 +1*
Ireland	4	2+2*
Italy	1	2
Portugal	2	2
Spain	4	5
The Netherlands	2	3
China (Hong Kong)	nd	1*
Canada	1	1+1*
USA	3	1**+2*
Taiwan	nd	1*
Japan	1	1(*)
Saudi Arabia	1	1*

*Relation to stay in UK assumed

**The third US patient with vCJD was born and raised in Saudi Arabia and has lived permanently in United Stated since late 2005

*** Secundary transmission via blood (one case likely vCJD-Transmission)

Table 3: vCJD cases worldwide

[18] "Stellungnahme zur Entwicklung und Einführung von Testsystemen zum Screening von Blutspendern auf vCJK

[19] Presentation of the symposium "European legislation for material of animal origin is changing – what manufacturers need to consider", organized by TÜV Süd; Update on variant Creutzfeldt-Jakob disease and risk for medical devices, J. Blümel

The identified vCJD cases in humans between 2008 and 2012 are not significantly increased.

The surveillance data of vCJD specifically from UK are summarized as follows [36]:

- 122 of 176 cases are definite confirmed, 54/176 cases probable infected
- 75/176 cases (= 43%) are women
- Median age at onset of clinical symptoms 26 years, 28 years at death
- Youngest 12 years, the oldest 74years
- Genetically MM at codon 129 of prion protein (except of one case, which have MV at codon 129, published in 2008)
- No case in individuals born after 1989
- Epidemic peak in 2000 with 28 reported deaths
- Current incidence 1-2 deaths / year

Some of this notified vCJD cases were caused by human to human transmission via blood transfusion. In the following chapter the possible transmission pathways for human TSEs (CJD and vCJD) are considered.

4.4 Transmission pathways of CJD/vCJD from human to human

4.4.1 Blood transfusions (full blood or "buffy coat" = leucocytes/thrombocytes)

In the Blood Directive 2002/98/EC and the Directive 2004/33/EC requirements are given to ensure high level of human health protection in connection with the application of blood and its constituents, especially: donor selection and prion reduction during manufacture. Contamination of blood, plasma or blood used as starting material for medicinal products by viruses, bacteria, PrP can have fatal or severe consequences for patients, which are people already weakened. In the Blood Directive standards regarding the quality and safety were set as minimum, requirements for the quality system are set in Directive 2005/62/EC.

The definitions are according to this "Blood Directive" *"blood shall mean whole blood collected from a donor and processed either for transfusion or for further manufacturing"* and "*blood products shall mean any therapeutic product derived from human blood or plasma"*[20]

Transmissibility of vCJD by blood was already described and confirmed in several cases. The difficulty is here, that all blood donors at the time of blood donation showed no clinical

[20] Directive 2002/98/EC of the European Parliament and of the Council (27 Jan 2003) setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC

symptoms. The disease first become visible in the blood donors one to three years later: "*four apparent transmissions of infection by blood transfusion from donors later developing vCJD have been identified (resulting in three clinical and one asymptomatic disease courses in the recipients at the time of death)"*[21]. Due to the traceability, a requirement of article 14 of the Blood Directive, and the surveillance program in total 48 potentially contaminated blood donors are identified and monitored in UK. From the blood donors developed 15 humans later on vCJD and three cases are reported were the transmission of vCJD via blood donation is confirmed between 1996 and 2006. In all three cases the patients were treated with erythrocytes-concentrates which were not leucocytes-depleted. Two of this patients died by vCJD, the third patient died otherwise, but the vCJD-agent could be detected histopathologically in the spleen and in lymph nodes, but not in the central nervous system [1]. Some of the Member States introduce the depletion of leucocytes, although it is doubtful, that the leucocytes-depletion is really effective, because animal model show that the infectivity could not be significantly reduced by this procedure, (according to animal studies only to 42%). Due to scientific data the French authority AFSSAPS assumed an infectivity as 20 IE-i.v./ml blood as worst case, with leucocytes depletion 10 IE-i.v./ml.

The transmission of BSE by blood transfusions is further confirmed in animal models using sheep. The donor animals developed later on clinical symptoms and the transmitted agent could be detected. "*Experiments indicate that approximately half the infectivity is in cellular components* [of the blood]*, mainly the "buffy coat", and the remainder in the plasma."*[22]

To calculate the risk of an infection with vCJD via blood transfusions following aspects are decisive according to Bennett and Daraktchiev (Lit [29]), who published a mathematical model in Feb 2013. With this model a range of possible scenarios can be generated that might be happen in the future.

Their key inputs are:

- How many individuals in one population were potentially carrying the vCJD agent?
- How much of the "vCJD agent- carrier" did not develop any clinical symptoms of the disease?
- Is the blood of these "silent" carriers infectious or not?

[21] MEDDEV 2.14/4: Guidelines relating to the application of: decision 2002/364/EC on common technical specifications for *in vitro* diagnostic medical devices

[22] EMA/CHMP/BWP/303353/2010: CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products

In another case the recipient of blood donation died by vCJD, despite the blood donor did not develop clinical symptoms for vCJD, but the vCJD agent could be detected in the concerned blood donation.

- What is the infectious dose in the silent carrier?
- Is the survival of the recipients long enough to develop symptoms of vCJD?
- Recently all diseased patients were MM in Codon 129 of the PrP gene. What role plays heterozygote in Codon 129 by developing the disease?

Shortly after the publication and confirmation of the first clinical transmission of vCJD the EU-Commission made the following key statements "*There was agreement that optimal use of blood may further reduce the risk of transmission of vCJD by avoiding unnecessary exposure to allogeneic blood transfusion. In addition avoiding unnecessary transfusion may improve the availability of blood for transfusion; this in turn may facilitate the introduction by Member States of additional donor deferrals if required*"[23].

Limiting factor for the development of an enough sensitive test method for PrP^{SC}, directly detecting the infectious agent useful for screening purposes, is the low concentration of pathogenic agents PrP^{SC} in blood or other body fluids (i.e. urine). Concentrations of approximately 1-10 IU per milliliter blood are assumed. The sensitivity of currently approved postmortem diagnostic tests is 10^9 IU per gram of infected tissue.

"*Blood screening assays undergo a more stringent assessment, including involvement of Notified Bodies and fulfillment of Common Technical Specifications (CTS). In principal, vCJD blood screening tests should fulfill minimal requirements equivalent to other IVDs qualified for blood screening. Recognizing that the current CTS cannot be adapted easily to vCJD assays, this guideline* [MEDDEV 2.14/4] *has been generated to identify basic quality requirements for vCJD assays."*[24] [45].

Diagnostic assays should have the following desirable characteristics:

- Possibility to detect abnormal PrP in the preclinical phase to ensure precautionary measures

[23] Transfusion Medicine and Hemotherapy (2006): Possible Measures for Reduction the Potential Risk of vCJD Transmission by Blood and Blood Porducts

[24] MEDDEV 2.14/4: Guideline relating to the application of decision 2002/364/EC on common technical specifications for *in vitro* diagnostic medical devices

- The sensitivity of the assay should be high enough to detect infectious agent in the blood , which is estimated to 1 to 10 infectious doses per ml whole blood based on results of animal models
- All forms of abnormal PrP agents must be detected by the assay

In general, diagnostic medical devices that are used to test diseases in Europe must meet the requirements which are stated in Directive No. 98/79/EC. The IVD tests can be divided into two main groups. The main group includes IVDs without any risk for patients and which can be used by trained personnel. Such IVDs can be marketed generally under the solely responsibility of manufacturers. In contrast to this group needs the second group the involvement of a Notified Body, because false results either positive or negative could have dramatic consequences for the recipient, third parties or the public. This second group includes, for example, blood diagnostic tests for HIV or Hepatitis infections. The situation for diagnostic PrP^{TSE} tests in blood or other human fluids (e.g urine) is analog to them and is therefore also classified in the second group of IVDs. The ability to identify positive samples as positive (= diagnostic sensitivity) and the ability to identify negative samples as negative (= specificity) are of great importance of any diagnostic test. High risk IVDs are included in list A of Annex II of the IVD Directive where currently tests to determine blood type, detect HIV 1 and 2, HTLV I and II, Hepatitis B, C and D are added. The European Commission recently amended this directive, upon request from UK, to include vCJD assays for blood screening and diagnosis [44].

On the one hand blood can be transfused as full blood or enriched cells, but on the other hand isolated blood components can be part of medicinal products. In contrast to blood transfusions, the infectivity of health care products utilizing human blood components is not yet clarified but cannot be entirely ruled out: in UK, no further vCJD case was published associated with blood transfusion in 2010, but one case was reported 2010 carrying vCJD infection in the spleen without clinical symptoms, but probably infected by clotting agents. It was a hemophilia-patient [36].

4.4.2 Blood components (pooled Fresh Frozen Plasma – FFP and from FFP isolated products e.g. clotting agents, such as Factor VIII, IX)

Cryoprecipitate (Fibrinogen and Factor VIII) and from FFP-supernatant isolated products (immunoglobulins, Albumin, Thrombin, Factor XIII / IX) are often used in medical devices.

Such products are industrial manufactured products resulting by pooling of blood from thousands of donors. On the one hand the potential of vCJD-contamination is diluted by such a high number of pooled samples, but on the other hand the risk, that one of the thousand samples is vCJD-infected is increased.

Since 1980 with the outbreak of the HI-Virus pathogen inactivation steps are established in the manufacturing of plasma derivate to ensure safety to the patients treated with plasma products. Plasma products are isolated from plasma pools which are collected from many thousand individual donations. The manufacturing process for plasma derivatives so called fractionating concludes mainly alcoholic precipitations at different alcohol concentrations, temperature and ionic strength. Alcohol precipitation conditions result in an aggregation of the prions and therefore they could be eliminated by appropriate sieves. Currently also specific prion affinity filters (e.g. a chromatographic resin matrix) are under investigation for its prion binding capacity.

Since 2003 manufacturer of blood products must check and validate whether their manufacturing process is suitable for inactivation/elimination of PrP to ensure safety before marketing authorization according to the European guidelines EMA/CHMP/BWP/303353/2010 (CHMP Position statement on Creutzfeldt-Jakob disease and plasma-derived medicinal products with regard to vCJD risk, June 2011) and CPMP/BWP/5136/03 (Guideline on the investigation of the manufacturing processes for plasma-derived medicinal products with regard to vCJD risk). The validation includes tests which showed an appropriate reduction of the model pathogen by each manufacturing step. It could be shown that the usually used manufacturing process effectively eliminates PrPs. But recent scientific data suggests "*that endogenous infectivity might persist through the fractionation process to a greater extent than would be expected from spiking studies."*[22]. For the evaluation of the elimination of prions a defined spiking material is used, which is isolated from brain tissues of infected animals [29].

The guideline includes also urine-derived products, but only low levels of infectivity have been detected in urine of scrapie-infected rodents and in CWD of deer. In vivo studies confirm the infectivity of urine either by intracerebral or intraperitoneal administration. "*The infectivity titre of the urine was calculated to be around 3.8 infectious doses/ml."*[22]. The confirmed TSE infectivity in urine leads to the consequence, that WHO classified urine in the category of lower-infectivity tissue. But recently, *there is no epidemiological evidence of CJD*

and vCJD transmission by urine-derived medicinal products."[22] e.g. the widely used human gonadotropin (hGN).

Despite of all these measures, on 10 June 2005 a case notification was made by the German company Biotest to the PEI that the batch of coagulation factor IX, released by the French authority AFSSAPS, contain plasma of a blood donor who develop the vCJD disease. An immediately performed batch recall of the affected batch could not avoid, that some products are applied on patients. In this context the experts within the authorities begin to calculate the risk of the patients. The result of the final estimation of the infection load by PEI was at least 1.8×10^7 ID_{50}/unit and the risk of patients was therefore low, even if several units are applied in the same patient. The risk analysis of the French authority AFSSAPS result in a residual risk of 3.1×10^6 ID_{50} / annual doses.

In general blood and blood products are mainly used regional and therefore such products are regulated nationally. Here are few examples of nationally regulatory differences:

France and Netherlands

- Leucocytes depletion
- Exclusion of persons who stay 1 year in UK between 1980 to 1996 from blood donation
- Exclusion of transfusion-recipients from blood donation

Germany

- from 1994 on: ban of the import of plasma products originated and isolated from blood from UK
- mandatory since 2000: Leucocytes depletion
- Exclusion of people who stay 6 months between 1980 to 1996 in UK from blood donation for the manufacturing of blood products since 2004. This was a consequence for the published first cases of vCJD infections by blood transfusions.
- Exclusion of patients which undergo surgery in UK from blood donation

UK

- the national CJD Surveillance program was immediately extended to blood donation service in 1997 after the detection of the first vCJD cases in humans

- Leucocytes depletion*: from late 1999 on all blood donations have undergone removal of white cells (leucocytes depletion) in order to reduce any vCJD infectivity present*.[24]
- Import of plasma: *from 1999 plasma derivatives have been fractionated from imported plasma (or more recently, manufactured using recombinant methods), rather than being sourced from UK donors* […] *FFP used for children and certain groups of adults needing frequent transfusions is also imported*."[25]
- Exclusion of transfusion recipients from blood donation

4.4.3 Other iatrogenic transmission pathways

The term "iatrogenic" implies transmission of pathogens resulting from activity/treatment by a physician/surgeon e.g. Dura Mater, Cornea transplantation or by using contaminated neurosurgical instruments, such as silver electrodes and by cadaver-derived material such as dural homograft, human growth hormones.

In USA (1974) transmission of the classical CJD by transplantation has been already described between 1950 and 1977 and is therefore also presumed for the new variant of CJD. In UK according to Literature [36] in total 74 cases of iatrogenic CJD are reported. Table 4 summarizes the reported infections in UK and in USA.

treatment	**USA**	**UK**
Cornea transplantation	3	nd
Dura Mater allograft	>168	8
Pituary hormones	>180	65
Neurosurgical instruments	7	nd
In total	>358	74

Table 4: iatrogenic CJD/vCJD infections published in USA and UK

[25] vCJD and transfusion of blood components: an updated Risk Assessment, P. Bennett, M. Daraktchiev, 14 Feb 2013

73 of these monitored individuals in UK have died and one is still alive. Several cases of iatrogenic CJD were reported by using contaminated neurosurgical instruments, such as silver electrodes.

In general, contaminated medical devices can be source of infectivity for humans. To evaluate the risk of transmission of TSE from patients to other individuals (health care professionals, dentists, care workers) by handling surgical intervention more must be learned about the disease and its pathogen agent. It is clear, that surgeries on tissues with well-known high TSE-infectivity such as brain, spinal cord or eye higher precautions must be considered with higher precautionary measures than invasive interventions on tissues with no detectable infectivity. But results of a risk assessment study in the UK performed by an expert group revealed e.g. a the surprising result, that there is a higher potential of prion presence and therefore a higher potential for transmitting CJD/vCJD by flexible endoscopy in the HNO region than in the upper or lower gastro intestinal tract without taking a biopsy. Another example is that in animal studies significant levels of TSE infectivity could be detected in gingival and dental pulp tissues. Experts are unable to come to a consensus to calculate the risk for transmission of vCJD through dental procedures and so far no recommendations were fixed for dental procedures.

In future surgical interventions must be managed with special precautions according to available guidelines. Some of the medical interventions are already classified depending on their infectivity risk for TSE:

a. Neurosurgery with contact with brain, spinal cord, inner ear, hypophysis or area olfactoria
b. Surgical interventions on eye (retina, nervus opticus) e.g. cornea transplantation (risk coming from cross-contamination with tissue of the retina)
c. Miscellaneous surgical interventions with contact to other risk tissue (HNO, Epithelium of olfactoria)
d. Taking a sample of the cerebral liquor (normally not relevant because of using disposable products)
e. especially by vCJD additional surgical intervention on lymphatic tissue (tonsils, Splenectomy, Appendectomy; orthopaedics:…) and furthermore a specific risk material for vCJD is blood

Furthermore, patient should also be involved in the risk assessment depending on their risk for exposure to the TSE agents. The following list is stated in Literature [4]:

Risk Group I:	Persons suffering from or are suspected of suffering vCJD
Risk Group II:	Persons suffering from CJD or are suspected of suffering CJD
Risk Group III:	Persons closely related with a CJD-Patient
Risk Group IV:	Recipients from natural (non-recombinant) human growth hormone and of transplantations of Cornea or Dura Mater
Risk Group V:	Patient with unknown ZNS disease with rapid progress with or without dementia (no concrete suspected CJD)
Risk Group VI:	all other persons

Whenever possible, single-use equipment should be preferred in contrast to re-usable medical devices. In some cases there are no alternatives and medical devices must be re-used in several surgical interventions (e.g. endoscopes, electrodes). In regard of the CJD/vCJD topic, the re-processing of medical devices gets more and more important and there is a need for harmonized regulatory framework within the EU. So far, reprocessing of medical devices was not uniformly regulated within the EU resulting in strong nationally regulatory differences: e.g.

- in Germany and some other Member States: strict regulations
- in France: re-processing is prohibited

The Proposal 2012/0266 of the new Medical Device Regulation (which will replace Directive 90/385/EEC and Directive 93/42/EEC) even includes a definition for re-processing : *"the process carried out on a used device in order to allow its safe reuse including cleaning, disinfection, sterilization and related Procedures, as well as testing and restoration of the technical and functional safety of the used device"*[26]

General steps for re-processing procedure of reusable instruments are recommended:

1) Pre-cleaning and cleaning:

   This step should avoid that tissue or blood will dried and therefore fix on the surface of medical device, followed by the appropriate cleaning procedure. Applicants (external services or in-house instructions) must demonstrate validation of the cleaning and must proof that the material of the instrument is compatible with the

[26] Proposal for Regulation Medical Devices 2012/0266 (COD), Brussel 26.9.2012

performed cleaning methods. According to up-to date knowledge an alkaline environment at pH values > 10 has a better effectivity for inactivation of prions and show best results. Currently WHO recommends treatment with NaOH, NaOCl or Guanidiniumthiozyanat by an incubation time over 10 minutes at 55°C. To remove residuals of the detergents during the cleaning process the medical device must be flushed.

2) Followed by appropriate desinfection e.g. by aldehyde, alkohole, peracetic acid or thermic
3) And Sterilization with approved prion inactivation afterwards
   For thermostable medical devices according current knowledge the best sterilization method is by steam at 134°C for 5 minutes or in cases where the inactivation of prions after step 1 and 2 is not sure, for 18 minutes. For thermolabile medical devices the alternative is e.g. H_2O_2

This procedure must be adapted to each instrument specifically to get optimal result in reducing the TSE-infectivity and to ensure TSE-safety of reusable medical devices.

5 Discussion

After the first outbreak of BSE in the eighties and its corresponding disease vCJD in humans, BSE was scientifically studied to get a better understanding. It is one of the first known diseases which are caused by a protein. Other infectious agents like bacteria or viruses were normally inactivated during manufacturing processes and this can be validated by virus inactivation studies. In contrast to this the elimination/inactivation of the resistant PrP is not yet fully clarified.

The frequency of notified and confirmed BSE cases in cattle varies considerably within the EU Member States. In some Member States the disease occurs more often (e.g. UK, France) but there are also Member States which remain BSE-free (e.g. Austria). The previous Directive 2003/32/EG as a legalization form enables Member States more flexibility in interpretation of the text with the translation into national law as it is the case for the new regulation, which is directly binding to all Member States. The different occurrence of BSE may reflect the different conditions in animal husbandry (e.g. feeding cattle with meat-and-bone meal in contrast to natural feeding with e.g. hay and grass). The protection for any BSE infections, e.g. ban of import of bovine material from BSE-concerned Member States, to remain BSE-free is the major goal for such Member States. For the BSE-concerned Member

States is the goal to reduce the number of new cases by detection and elimination of the source of PrP infections.

With a likelihood of 99% the transmission from prions is caused by the oral route of infection (food), despite this was classified as inefficient in animal studies. Immediately after this finding, appropriate precautionary measures are taken to regulate all ruminant products intended for human food chain. For example, the introduction of rapid BSE tests by material entering the human food chain as laid down in the Regulation 999/2001 revealed, that more cattle are BSE-infected than expected. Therefore the estimated cases of BSE-infected cattle and other animals must be revised to an increased number. The taken food measures were effective and the BSE crisis was banned, e.g. in Germany 2009 the last confirmed BSE case was registered. Despite the successful measures against BSE, the BfR, the German Federal Institute for Risk Assessment published on 14th of January 2013, that all the activities, should remain the same to recognize a potential outbreak of an atypical BSE form and to detect a new epidemic BSE at an early state [19]. Other experts mentioned that the revision of the taken measures is feasible, but need to consider carefully avoiding recirculation of TSE agents. They note that there is not enough knowledge in regard to the variable BSE-strain characteristics after passage through different species. This demonstrates that there is still a great fear worldwide for the BSE disease and authorities would like to avoid recirculation of TSE agents. The high level of uncertainties in science, the uncontrollability, the long incubation period and the involuntary nature of the risk are a few aspects that cause the concern.

In this context, only BSE and the risk of transmitting BSE to humans is of intense scientific evaluation worldwide, but in other countries, other TSEs might be of greater interest e.g. CWD in North America, because people in other countries consume not only beef food, but also other meat such as from elk, deer, and mules. Animal models demonstrate that also pigs have susceptibility for BSE, but failed so far to develop clinical symptoms. Is it possible that in future the infectious agent in swine can infect humans or is the pathogen in swine generally not infectious?

An article in the German newspaper FAZ summarizes the recently published scientific data which conclude that spleen plays a greater role in crossing species barriers then brain tissue did. The author follows, that therefore a new risk evaluation of the transmission pathways of prions from animal to humans should be performed based on the consideration of the difference between spleen and brain tissue. The German prion expert, Martin Groschup from

the German Friedrich-Löffler-Institute negates these warnings and emphasizes, that the currently taken precautionary measures to avoid TSE-infections are sufficient [40].

The vCJD cases worldwide are not significantly increased during August 2008 and August 2012, but this is no reason to be reassured: *"Analysis of vCJD diagnosis and deaths from January 1994 to December 2011 indicates that a peak has passed. While this is an encouraging finding, the incidence of vCJD may increase again, particularly if different genetic subgroups with longer incubation periods exist."*[27] This is a conclusion from the observation that so far all patient developed vCJD were methionine homozygous (MM) at codon 129 of the prion protein (except of one case). It is possible, that this genomic constellation facilitate the protein folding of the prion in its infectious form. Nearly 40 % of the European population has this genetic aptitude [40]. Maybe there exists also other genetically differences enabling the disease, because it was in addition observed, that white people are 2.5 time more accessible to develop CJD than black people, as reported in the surveillance studies from the US [35]. Ethical questions raised in regard of a genetically screening for the codon 129 of the prion protein for diagnostic purposes or before surgical interventions for the possibility to take appropriate precautionary measures. Would people like to know that they have the potential to develop the disease? This question is analogous to other fatal diseases like Korea Huntington, which is transmitted from generation to generation. May a screening test before invasive medical treatments justifiable for adequate protection of other persons like surgeons, hospital nurses and laboratory assistants?

It was estimated that for the future the frequency of vCJD is highly increase by transfusions with contaminated blood and blood products, but fortunately this scenario was not realistic and it looks like that the species barrier from animal-to-humans is higher than from human-to-human. To the most recent scientific evidence, blood transfusions did not play an essential role for epidemic vCJD.

Nevertheless, the threat of vCJD remains, because of its unquantifiability. The frequency of silent infections, without showing clinical symptoms is more often than expected, because investigations of removed human appendices demonstrate prion in lymphatic tissue in three of 12 000 persons and lead to the conclusion that more people carry the prion protein as calculated by mathematically models. *"The greatest risk of vCJD […] will continue to be among persons who as a child or young adult consumed UK beef products during 19080 –*

[27] Twentieth Annual Report 2011: Creutzfeldt-Jakob Disease - Surveillance in the UK, The national CJD & Surveillance Unit, Western General Hospital, Edinburgh

1996, the years when such products were most subject to BSE contamination.“[28] The median incubation time in humans is still unknown, but it is estimated that the incubation time varies depending from transmission pathway and infective doses.

In contrast to beef food, there is no case published where a patient was infected with prions by health care product utilizing bovine material as raw material. However, those products are strictly regulated by specific legislation shortly after the first BSE outbreak. The disease is apparently no more a dominant public threat, but recently the new Regulation (EC) No. 722/2012 comes into force with further control mechanisms for manufacturer of medical devices using animal material. The most important one is the requirement for a system which enables manufacturer to collect and evaluate post-production information of the animal material (including extended control of suppliers and affected processes during slaughtering, handling, transport and storage). When significant changes of animal raw material are observed, than manufacturer have the obligation to report this to their NB. If NB decide that the change increase the TSE risk, they must be initiate a re-assessment process, which may compromise continued use of CE marketing of the concerned medical device. Another fact which increase the uncertainty of manufacturer regarding the CE status of their products, is, that starting material certified by EDQM underlay at first in addition the consultation with all EU Member States (in Directive 2003/32/EC this was not required). Objective of the additional measures is that all involved parties get the chance to detect a re-circulation of the disease as early as possible. Was this the main reason for the new regulation at this time point or exist other reasons? The inclusion of AIMDs, custom-made devices and clinical material close the regulatory gap which was present with the Directive 2003/32/EC where only medical devices falling under Directive 93/42/EEC are covered. Now a greater percentage of medical devices using animal material are under extensive control by NB and competent authorities resulting in safer products for patients. But on the other hand development of devices (clinical testing) and manufacturing of the devices are associated with an increased workflow which is accompanied with additional costs for manufacturer and finally also for patients. The up-to-date scientific knowledge in the BSE topic resulting in new classification systems for geographical sourcing and nature of material, initiates the necessity that manufacturer must update the risk assessments. Most countries improved their BSE/TSE status by effective countermeasures against the disease resulting in safer starting material.

[28] Holman E, et al. (Jan 2010): Human Prion Disease in the United States, PLoS ONE, Volume 5, Issue 1

The involvement of all Member States makes the process more complicated, more personnel-, more time-consuming and more cost intensive. But the high complexity of the new procedure is not associated with an increasing safety of such products. Since 2003, medical devices with animal material underlying Directive 93/42/EEC were under continuous control by NB. This includes the sourcing of animal material, the risk management and the evaluation of risk/benefit by NB. The experience in the past revealed that medical devices utilizing animal material are practically safe regarding PrP infections to patients.

Furthermore due to different background in regard to BSE in each Member State of the European Union, the justification and tolerance for using bovine material in health care product is not uniformly within the EU Member States. This could result in discussions, which might lead to a delay of bringing medical devices on the market: e.g. if one Member State does not agree with the comments, this will induce that NB is not able to issue the EC-Certificate, because all partners within the system must have full compliance.

The work load of NB will also be increased by the facts, that they must document their conclusions in SERs and that changes which increase the previous TSE-risk initiate a re-assessment procedure. This may lead to deficit of experienced and enough qualified personnel.

In contrast to transmission pathway from animal to human (food, health care products), the situation for transmissions by contaminated blood, organ transplantation and surgical medical devices (human to human) differ:

Where ever possible alternative therapies should be preferred. In UK since 1985 there is no more risk for iatrogenic CJD because the human pituary-derived hormones could be replaced by synthetic preparations. But in case of blood transfusions, which are always life-saving therapies, no alternative exists, but wherever possible autologous blood donation should be considered over allogeneic transfusion to minimize the risk of transmitting vCJD. The load of infectious particles by blood transfusions is 6 to 7 times higher compared to natural transmission pathways. In addition recipients for blood transfusion are even weakened because these are sick people more acceptable for possible infections. Especially hemophilia patients need the whole life and often products such as factor VIII and have therefore a higher risk for infections.

Unfortunately after 30 years intense scientific investigations worldwide, there is no reliable test available for the evidence of prion-infections for humans and animal without having clinical symptoms. "*Current diagnostic methods are based mainly on the physicochemical*

differences between PrP^C and PrP^{Sc} which, to date, are the only reliable markers for TSE."[29] and are post-mortem tests. Due to this lack of diagnostics the safety for infection of vCJD/CJD by human products (organs, blood and contaminated equipment) is still obvious.

The risk of transmitting TSE depends on several factors such as used source material, intended use of health care product and the route of administration. "*Finally it is critically important to understand that categories of infectivity are not the same as categories of risk, which require consideration not only of the level of infectivity route by which infection is transmitted. For example, although the level of tissue infectivity is the most important factor in estimating the risk of transmission by instrument cross-contamination during surgical procedures (neurosurgery versus general surgery); it will be only one determinant of the risk of transmission by blood transfusions, in which a large amount of low-infectivity blood is administered intravenously."*[30] Directive 2004/33/EC set-up rules for blood donation taken into account only CJD and not vCJD, which might lead to a regulatory gap, because for vCJD are further measures necessary.

A major step forward would be a diagnostic test with enough sensitivity that also the low titers of infectious agent in blood and blood products can be reliable detected. This will led to a better calculation how many people carry the infectious agent and about the risk of transfections.

The BSE topic remains exciting in all respects and the future will show, if the fear for a re-circulation is justified or not.

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- Prof. Dr. Schwertfeger (BfArM): Commission Regulation (EU) No. 722/2012 – Implications for BfArM
- Dr. Balkema-Buschmann and Groschup (FLI): BSE in Europe – problem fixed?
- Priv. Doz. Dr. Blümel (PEI): Update on variant Creutzfeldt-Jakob disease and risk for medical devices
- Dr. Waesch (Geistlich Pharma): The TSE regulation – Geistlich Pharma perspective
- Prof. Dr. Kloth (TÜV Süd): EN ISO 22442-1: Medical devices utilizing animal tissue and their derivatives
- Dr. Bos (BSI): Medical devices utilizing Materials of Animal Origin (Including those for which a TSE risk is suspected) – Controls on Sourcing, Collection and Handling
- Dr. Rubenwolf (TÜV Süd): The new TSE Commission Regulation and its impact on ISO 22442 part 3
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7 Appendices

Appendix 1: Summary of important changes between Directive 2003/32/EC and new Regulation (EU) No. 722/2012

	Directive 2003/32/EC	**Regulation 722/2012**
general	directive => a bit weaker than regulation; Transfer into national legislation until a date, Intention is definite; modification of text is possible = MS have too much wiggle room for own interpretation	regulation = logical step in the process of the EU regulates more by directly applicable regulation in the life science industry Effective as law within EU. Direct application, no transfer into national legislation, no modification of text
Article 1, Section 1	Concerns Directive 93/42/EEC	Concerns in addition to Directive 92/42/EEC also the Directive for active implantable MD 90/385/EEC => AIMD are included in this regulation
Article 1, Section 2	Remain unchanged	
Article1, Section 3		Modified by adding reference to Regulation (EC) No. 1069/2009 “Fit for human consumption” for collagen, gelatin and tallow regulation is emphasized in particular
Article 1, Section 4		Special requirements for tallow derivatives
Article 2 “definitions”		modified TSE definition according to Regulation (EC) No.999/2001 taken into account other TSE which are detect in future regulation is emphasized in particular
Article 3		added section 2 Inclusion of AIMD, custom-made devices and clinical material
Article 4	MS shall notify the Commission and other MS by amending tasks of NB	Additional requirement for MS by adding Section 2: MS shall regularly verify, that NB maintain up-to-date knowledge MS must inform the Commission and other MS about results of verification NBs by 28 Feb 2013
Article 5, Section 1	Remain the same except of adding AIMDs	
Article 5, Section 2		More detailed specifications AO-material => The justification for the use of animal tissues or derivatives, taking into consideration lower risk tissue or synthetic alternatives More detailed demands for the

		performance of audits => the need to audit matters related to the sourcing and processing of animal tissues and derivatives, processes to eliminate or inactivate pathogens, including those activities carried out by suppliers
Article 3, Section 3		NB may request additional information of starting material
Article 5, Section 4	Information MS and Commission not necessary with EDQM-Certification	Additional rules (Section 5 – 7) Also with EDQM TSE-certification the coordinating CA has to inform MS and the Commission of their assessment carried out by means of SER Additional assessment by MS is a clear downgrade of the importance of EDQM TSE-Certification
Article 6	Remain the same except of adding AIMDs	
Article 7		Transitional period for AIMD by 29 Aug 2014
Annex I	<u>Geographical sourcing</u> four GBR categories for tissue <u>Nature of starting tissue</u> Reference of Regulation No. 1774/2002	<u>Geographical sourcing</u> three categories for OIE categories <u>Nature of starting tissue</u> Updated WHO recommendations Reference to updated Regulation No. 1069/2009 and Regulation No. 999/2001 AND Manufacturer must maintain a system for collection of postproduction information regarding the used tissue (not new, but now mandatory) and must ensure that the risk of cross contamination during slaughtering, collection, processing, handling, storage and transport is minimized In the case, that NB concludes that there is an increased TSE risk, then new assessment of the conformity of the device including the involvement of MS's competent authorities Addition of Section 2.1, 2.2 and 2.3
Annex II	none	SER form
General System set up (allowing MS to comment on the NB's evaluation of high risk devices prior to CE marketing – Dir. 2003/32/EC) stays the same, except for a system for regulatory fast-tracking of SER		

Appendix 2 Differences between classical (sporadic) CJD and the vCJD

	Classical CJD-Form	**(New) variant CJD**
First occurrence	Well known since 1920	First description of patients in 1996 (UK), was develop clinical symptoms in 1994
Infectious agent and infectivity	Different to prion causing BSE in cattle Possible risk by Surgery: transplantation of infected Dura Mater, hypophyse or Cornea	Identical with prion causing BSE in cattle Transfer of BSE to humans by BSE-infected bovine food products is very likely => correlation between epidemic vCJD with BSE outbreak[31] (please refer to figure 2)
Transmission by blood products	unlikely	in UK: Three patients were infected by vCJD-infected blood by blood transfusion
Transmission by medical devices	In single cases described	Unknown but presumed, especially for invasive processes of high risk material (e.g. electrodes implanted in brain)
Frequency	Constant worldwide i.e. Germany: 1 new case/1 million people/year = 80 cases per year = Rare Disease	For the future: increased cases possible (because the possibility of infectivity of "silent" prion carrier, showing no clinical symptoms)
Patients	Older patients (distribution maximum at 65 year old people)	Younger patients under 30 years old
Detection of infectious agent	Only within central nervous system; the abnormal prion protein was detected rarely in spleen and muscle	Abnormal prion protein detected not only in central nervous system, but also in peripheric lymphatic tissue i.e. in spleen, appendix, lymph nodes and palatal tonsils…
Incubation period	Over 10 Years; death 1-2 years after occurrence of clinical symptoms	Estimation about 17 years; median duration of the disease: 13-14 months from clinical symptoms to death
Different forms	- Sporadic (most frequently 85-90% of the cases): sCJD and sFI - Genetic: fCJD, FFI and GSS	Infectious (less than 1%) (n)vCJD
PRNP gene Codon 129	Heterozygous MV or homozygous MM / VV	Homozygous MM (except one case)

[31] Presentation of the symposium "European legislation for material of animal origin is changing – what manufacturers need to consider", organized by TÜV Süd: D. Asher

Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

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Datum , Unterschrift