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OPINION

of the French Food Safety Agency on the zoonotic risk of the various known strains of TSE in small ruminants

LE DIRECTEUR GÉNÉRAL

1. REVIEW OF THE REQUEST

The French Food Safety Agency (AFSSA) received a request on 25 June 2009 from the Directorate General for Food (DGAL) for an evaluation of the zoonotic risk of different known strains of TSE in small ruminants.

2. CONTEXT

In its opinion issued on 15 May 2006[1], AFSSA declared that it would be premature to conclude that strains of TSE (other than BSE) did not involve any risk to human health. It remains true that, although no epidemiological data indicates any obvious link between animal forms of TSE (other than classic BSE) and human cases of TSE, the diversity of strains of TSE that can be found in small ruminants means that great care should be taken with regard to risk assessment.

This consideration was taken into account in the later opinions by the Agency concerning health enforcement measures for small ruminants[2;3], particularly as regards its recommendations to withdraw from sale all products from animals having sensitive genotypes in herds infected with classic scrapie.

On 25 June 2009, the DGAL issued a request to AFSSA to supply as much experimental data or demonstrations as possible on this point, to enable it to back up its arguments when explaining to its European partners the reasons for the precautionary measures taken concerning TSE in small ruminants.

In particular AFSSA was asked to:

- Indicate the different criteria according to which a TSE can be described as zoonosis and review the scientific knowledge available for each TSE in small ruminants.

- Review the reasons why the lack of an epidemiological link between any animal TSE and human TSE does not necessarily mean that there is no zoonotic relationship.

- Summarise the different experiments that have already been performed to assess the

27-31, avenue
du Général Leclerc
94701
Maisons-Alfort cedex
Tel 01 49 77 13 50
Fax 01 49 77 26 13
www.Afssa.fr

[1] Opinion by Afssa on changes to European regulations suggested in the roadmap on transmissible spongiform encephalopathy (TSE) dated 15 May 2006.
[2] Opinion by Afssa on changes to health enforcement measures in sheep and goat flocks where a case of classic or atypical scrapie has been detected, dated 15 January 2007.
[3] Opinion by Afssa on the possible consequences, for animal and public health, of newly-available scientific data concerning intra-specific transmission of the agent of classic scrapie by milk dated 8 October 2008.

effectiveness of the human species barrier in blocking the different strains of TSE in small ruminants (a table for each target species, contamination pathway used and any conclusions drawn from all these and other elements by the scientific panel concerning the level of zoonotic risk).

3. EXPERT ASSESSMENT METHOD

The collective expertise assessment was performed by the "Transmissible Subacute Spongiform Encephalopathies" (TSSE) scientific panel (CES) which met on 05 November 2009, 17 December 2009, 14 January 2010 and 16 February 2010.

This opinion is based on an examination of the scientific literature describing on the one hand the epidemiological data and on the other the data used to determine the ability of different TSE agents to propagate from one species to another.

The example of BSE and the process that led to its zoonotic status being confirmed, was also taken into account by the scientific panel to illustrate its analysis. The zoonotic risk related to the presence of classic BSE in small ruminants is considered to be well-founded and will not be dealt with in this opinion.

4. DISCUSSION

AFSSA's position is based on the opinion of the 'ESST' CES, whose main elements are listed below:

Contents of the discussion

4.1. Introduction 3

4.2. The limits of the epidemiological approach 3

4.2.1. The limits of the monitoring mechanisms for TSE in animals and humans 3

4.2.2. Risk factors associated with the development of sporadic CJD 4

4.2.3. Geographic risk associated with the development of sporadic CJD 5

4.2.4. Conclusion 6

4.3. The insufficiencies of the methods based on molecular phylogeny 6

4.4. Transmission of strains of animal and human prions to the same receiver host and a comparison of the biological phenotypes obtained 7

4.5. *In vivo* evaluation of the transmission barrier between human and PrP and agents of TSE in ruminants 8

4.5.1. Inoculation of nonhuman primates 8

4.5.2. Inoculation of transgenic mice expressing human PrP 9

4.6. Transmission of human TSE to small ruminants 10

4.7. *In vitro* evaluation of the transmission barrier for PrP between humans and agents of TSEs in small ruminants 10

4.8. The different forms of BSE and the difficulty of demonstrating the zoonotic character of animal TSEs 11

5. Conclusion 13

4.1. Introduction

Zoonoses are parasite-based or infectious diseases capable of propagating from animals to humans (or inversely). There is no official list of zoonotic diseases and the zoonotic potential of infectious agents varies widely (rabies is a disease with high zoonotic potential whereas contagious ecthyma in small ruminants has low zoonotic potential).

Two complementary and nonexclusive approaches are generally used to identify the zoonotic nature of a conventional infectious agent (bacteria and virus):

i. epidemiology, to establish any spatio-temporal association between the onset of human diseases and (i) activities involving animals or (ii) the circulation of an infectious agent in animals,
ii. the phylogeny of the infectious agents (generally based on the characterisation of the sequences of nucleic acids) by which the identity of the infectious agents found in the animals and the patients affected can be established unequivocally.

It should be noted however that the zoonotic origin of certain pathologies, even when they are strongly suspected, can be extremely difficult to demonstrate formally (as in the case of certain haemorrhagic fevers, such as Ebola).
Finally, even when it is shown that the same infectious agent is responsible for the disease in both humans and animals this does not necessarily demonstrate that there is a risk of transmission to humans from the animal in question, as some pathogenic agents can propagate themselves to both humans and animals from an external source (e.g. tetanus).

For prion diseases, it is impossible to compare the characteristics of the infectious agents observed in humans and animals, because the infectious agent has not been completely identified and because in the current state of knowledge it does not include specific nucleic acids. At the present, the biological characterisation of infectious agents depends almost exclusively on the characteristics of experimental transmission (rate of transmission and incubation period) to a host receiver (most frequently a laboratory rodent) and on the detailed characterisation of the phenotype obtained (molecular profile of the pathological prion protein in the brains of the affected animals, neuropathological lesions etc.).

4.2. The limits of the epidemiological approach

4.2.1. The limits of the monitoring mechanisms for TSE in animals and humans

Concerning animal TSEs, only bovine species are thoroughly monitored over a large area, and only since 2001. The monitoring procedure is based on the detection of the pathological prion protein (PrP^{Sc}) in the central nervous tissue of animals at slaughterhouses and rendering plants.

This procedure has revealed the existence of previously unknown forms of animal TSE, atypical BSE of types H and L [2, 3].

Nonetheless, there is much less epidemiological data concerning small ruminants on farms. The active monitoring procedure applied to these species has generally been limited to surveys over a relatively short period.

However, this active monitoring procedure for small ruminants has:

i. shown that the estimated prevalence of TSEs in small ruminants over the preceding decades was inaccurate for these species (underestimation, with no cases detected in certain geographical areas),

ii. also contributed to the discovery of another previously unknown form of scrapie: atypical scrapie. It now appears that atypical scrapie is at least as prevalent in Europe as classic scrapie or even more so [4]. The recent discovery of an indigenous case of atypical scrapie in a sheep in New Zealand, an area previously thought to be unaffected by animal TSEs, has underlined the limitations of our past and present estimates of the distribution of these diseases.

The classic forms (meaning non-atypical or having no similarities with BSE) of TSE in sheep and goats are grouped together for operational purposes under the generic term of 'classic scrapie'. This term in fact covers a disease which can be caused by a variety of agents with different biological properties (including the potential for being transmitted experimentally from one species to another). We still have only a limited knowledge of the diversity of strains of prion circulating among small ruminants and more generally in animals and man.

Apart from exceptional cases, past and current tools for epidemiological monitoring are incapable of distinguishing among the different agents responsible for scrapie. At an epidemiological level, this can lead to the wrong conclusion that all cases of 'classic' scrapie involve the same risk of interspecific transmission.

In the form found most frequently, human TSEs (what is known as sporadic Creutzfeldt-Jakob Disease (CJD)) mainly occur in older individuals (between 50 and 60 years old) with an incidence in the region of 1.5 cases per million and per year [5]. Identification of these cases depends on the level of medical care in the country and on the possibility of confirming the disease after death. Despite improved information for health professionals and national and transnational initiatives designed to improve the identification of cases suspected of being CJD, it remains difficult to evaluate the sensitivity of the epidemio-surveillance system for human TSEs.

4.2.2. Risk factors associated with the development of sporadic CJD

Different studies have been made to determine whether there is an epidemiological link between sporadic CJD and exposure to animal tissues and products, in particular those from animals affected by scrapie.

The earliest works on the subject did not really follow an epidemiological approach based on a scientifically defined methodology but on the discussion of old observations and data, many of which were of questionable quality [6,7]. Although these studies have been and continue to be widely repeated in the literature, considerable reservations should be applied concerning the relevance of this work and the conclusions to be drawn from it. The only relationship observed in the study by Chatelain et al. [7] was a link between frequency of CJD and the level of urbanisation of the geographical areas, which was very likely the result of a monitoring bias by man, as the authors themselves recognised that detection is easier in urban areas. Masters et al. [6] gave a descriptive analysis of the characteristics of 1435 patients suffering from CJD throughout the world. They mentioned that patients had often either worked in the medical professions or in activities bringing them into contact with animals or animal products, but it is not possible to deduce any epidemiological link since the frequency of these professional activities among the patients was not compared in this study with any case-controls.

More recently, studies have been undertaken to identify the risk factors associated with the onset of CJD in patients. Most often, these studies are based on case-control mechanisms, with the most powerful being based on data collected in several countries over a long period.

For example, the study published by Van Duijin et al. [8] was undertaken at a European level on 405 patients suffering from sporadic CJD. The authors looked for a link between CJD and some 40 factors relative to genetic characteristics, medical and surgical antecedents in the patients, professional activities, contacts with animals and diet. Only some relatively weak relationships were found, between CJD and the consumption of raw meat (relative risk (RR) of 1.63 (95% confidence interval: 1.18-2.23))[4], brains (RR 1.68 (1.18-2.39)), frequent exposure to leather (other than garments) (RR 1.94 (1.13-3.33)) and exposure to fertilisers based on hoof and horn (RR 2.32 (1.38-2.91)).
A Swiss study was made involving 69 patients suffering from sporadic CJD following an unexplained increase in the incidence of cases in that country in the early 2000s [9]. More than 130 factors were studied, of the same type as those previously mentioned, but more detailed and with the addition of socio-economic aspects and information about the consumption of medical products, tobacco and drugs. Among all the factors concerning exposure to animal products (diet, contact etc.), only the consumption of kidneys was found to be significantly related to the disease (odds ratio (OR) of 1.96 (1.04-3.68)). It should however be noted that some 20 other factors belonging to categories unrelated to exposure to animals or animal products also appeared to be significant.
In 2003, Cocco et al. [10] published the results of a retrospective study on mortality statistics in the United States, including 636 patients who had died from CJD and more than 3000 controls, which also investigated their professional activities. These were divided into 154 categories, 11 of which could be considered as involving possible exposure to agents of TSSE. Among these, the study showed an epidemiological link between CJD and butchery (OR 6.8 (1.5-30.1)) on the one hand, and being employed by a doctor on the other. Significant statistical relationships were also found with about 20 other professions. It should be noted that the very large number of factors tested in this study introduces a higher risk of falsely positive relationships due to the high number of statistical tests used. This phenomenon is also found in the other studies but to a lesser extent.

These different studies of the risk factors of CJD showed that certain types of exposure could be a factor linked to the risk of CJD, related either to the proximity of animal products or the consumption of animal products but without any indication of the animal species in question (and therefore without any indication of the type of animal TSE that could be a risk for humans).

4.2.3. Geographic risk associated with the development of sporadic CJD

An argument frequently used to reject any possible link between the onset of human TSE and exposure to prions from small ruminants is the fact that the apparent prevalence of CJD does not differ between countries where TSE is endemic in small ruminants and those considered by the OIE to be free of TSE. The recent demonstration[5] that New Zealand was affected by atypical scrapie despite its TSE-free status and the increasingly well supported notion that this form of scrapie may be evenly distributed around the entire world ([4]) clearly indicate the limitations of this type of argument.

There have been various studies into the possibility of there being a link, particularly geographical, between cases of sporadic CJD. A study in the United Kingdom of patients suffering from sporadic CJD identified between 1990 and 1998 [11] found that, statistically, these cases were geographically closer to each other than the control cases, suggesting exposure to a common external factor. In the same way, Huillard d'Aignaux et al. [12] showed spatial heterogeneity in France of the risk of sporadic CJD, with certain clusters of cases.

[4] In the weekly epidemiological bulletin No. 51 (1993), Alpérovitch et al. Indicate the existence of 'small' case-control studies (no references mentioned) showing a relationship between CJD and the consumption of meat (pork and mutton) and in particular lightly-cooked meat.

[5] Memorandum from the New Zealand health authorities dated 28 October 2009 http://www.biosecurity.govt.nz/media/28-10-09/atypical-scrapie-detection

A similar approach applied to variant CJD in the United Kingdom [13] did not show any clusters of cases, with the exception of one in Leicestershire, which was attributed to certain practices in retail butchers shops. The authors concluded that the absence of clusters was probably related to the fact that human exposure to the BSE agent was caused by a medium distributed on a wide geographical scale, such as mechanically-separated meat or the preparation of head meat, for example.

4.2.4.Conclusion

In conclusion, the available epidemiological studies reveal a weak but recurrent relationship between human TSEs and exposure to animals and/or animal products. It nonetheless remains impossible to identify with any precision the exact risk factor or factors behind this relationship. Considering the conditions under which these studies were performed (location/time), there is only a slight probability that the links observed can be attributed to BSE. Studies of the potential links between cases of sporadic CJD have shown the probable existence of 'clusters of cases' suggesting the possibility of exposure of some of the patients suffering from sporadic CJD to one or more common external factors.

To interpret these data correctly, it should be remembered that epidemiological studies investigating a possible link between animal and human TSEs suffer from significant limitations, particularly:

i) the low number of cases of CJD included, which restricts the power of analysis;

ii) the incubation time of CJD which restricts the relevance of retrospective studies, in particular any reliable evaluation of the exposure of individuals during their lives to risk factors of interest. Indeed, the study of Kuru, a human TSE that is transmitted subsequent to cannibalistic funeral rites, has shown that incubation times for the disease in man can be several decades when contamination is by peripheral infection;

iv) imperfect knowledge of the genetic parameters of susceptibility that can affect the onset of cases;

v) phenomena such as the exchange of goods and merchandise and also travel, which complicate the analysis of geographical correspondence between human and animal cases;

vi) insufficient knowledge or failure to take account of the differences in exposure to the risk for different subpopulations (for example, exposure related to professional or leisure activities), which can contribute to a failure to reveal any relationship due to dilution of the effect;

vii) the wide range of agents responsible for TSE in small ruminants. Indeed, under the hypothesis that only certain forms (strains) of scrapie may be potentially zoonotic, there would have to be a way of evaluating the exposure to these particular strains before any epidemiological link could be established between this animal TSE and CJD (i.e. studying the specific risk created by a specific strain of classic scrapie for a given type of CJD). It does not seem possible in practice to attain such precision when characterising strains identified through the monitoring of cases of TSE because of the extremely cumbersome methods used for identifying strains of the TSE agent.

4.3. Methods based on molecular phylogeny are not adequate to the task

The exact nature of the infectious agent responsible for TSEs remains uncertain. Its essential constituent is a protein of the host (PrP^{C}) which during the pathological process is converted into an abnormal conformer (PrP^{Sc}) and accumulates in the form of aggregates in the nervous and sometimes lymphoid tissue of affected individuals. The absence of specific nucleic acids means that methods based on phylogeny, currently used to compare classic infectious agents, are inappropriate.

PrP^{Sc} can have specific biochemical characteristics (electrophoretic profile in SDS-PAGE gel, resistance to proteases or denaturing agents, etc.) allowing several strains to be differentiated within a given host species. However, the PrP sequence of the host directly influences the biochemical profile of the PrP^{Sc} of a strain [14]. Consequently, a direct comparison of the biochemical profiles of human and animal isolates of TSE is not sufficient to demonstrate the existence or absence of zoonotic risk associated with agents of TSEs. A comparative study might however provide results to encourage a more thorough investigation for a more precise characterisation of the infectious agent.

In the absence of any molecular approach for the direct and reliable identification of the agents responsible for TSE, the experimental transmission of animal and human TSEs to a common host and the comparison of the biological phenotype obtained is the most promising approach for identifying the implication of a common infectious agent in these two diseases.

4.4. Transmission of strains of animal and human prions to the same receiver host and a comparison of the biological phenotypes obtained

Traditionally, the comparison between two agents of TSE coming from different species depends on the transmission to the same host of a third species (laboratory rodents). This approach is the basis for the strain-typing tools developed by the Neuropathogenesis Unit (NPU) in Scotland at the beginning of the 1960s. According to its general principles, the isolates of TSE to be typed are transmitted sequentially to different strains of consanguineous mice and several parameters are measured in the animals at the terminal stage of the disease or at the end of life: length of survival time, nature of the clinical signs, detection and electrophoretic profile of the PrP^{Sc} in the brain (quantity, size and proportion of the different glycoforms of PrP observed using the Western Blot technique) and, at a neuropathological level, the distribution and nature of the deposits of PrP^{Sc} together with the distribution and intensity of vacuolisation (profile of the lesions) in the cerebral tissue. Crosschecking all this information makes it possible to establish any similarity in the characteristics of the different isolates initially transmitted.

This approach nonetheless remains limited because of the lack of intrinsic susceptibility of certain hosts to certain agents of TSEs originating in different species. Thus, although congenic strains of mice with polymorphic PrP genes (C57BL/6 mice, RIII mice, etc.) [15] have historically been very useful in establishing a link between BSE and vCJD [16], they have highly variable susceptibility to the isolates of classic scrapie. Furthermore, they seem refractory to atypical scrapie [17]. Finally they are only slightly permissive[6] to cases of sporadic CJD [18,19,20].

When TSE isolate is transmitted from one species to another (in this case to conventional rodent models), what is known as primary (or first passage) transmission results in phenotype characteristics (incubation time, profile of lesions, etc.) that may vary from one animal to the next and which are in many cases unstable. Several successive passages in the same host are generally necessary to obtain stable and reproducible characteristics: this is known as adapting the agent to the new host (which may have a totally different phenotype to the agent found in the original isolate). Consequently, for a comparison of two TSE isolates to be complete using these models, several sub-passages are required. Such work takes a very long time and is both fastidious and costly.

All these factors place considerable limitations on the possibility of comparing TSE isolates taken from different species by the use of conventional rodent models and very little validated data has resulted from these models. One study has shown however that C57BL/6 mice inoculated with an isolate from classic scrapie after a first passage had a lesion profile similar to that observed after transmission of cases of sporadic and iatrogenic CJD [21]. Because of the limitations of the model described above, the significance of this result nonetheless remains relative.

[6] Capacity of permitting an infectious agent to develop

More recently, transmission without any apparent species barrier [7] of cases of sporadic CJD to the Bank Vole (*Myodes glareolus*) has been used to explore these similarities/differences between human and animal isolates of TSE [18]. At present, the experiments published do not suggest any phenotype similarity between the panel of isolates of scrapie and the panel of cases of sporadic CJD transmitted. However, the limited diversity of the isolates from inoculated small ruminants does not allow the conclusion that scrapie in general does not have a zoonotic character.

4.5. *In vivo* evaluation of the transmission barrier between human PrP and agents of TSE in ruminants

The degree to which agents of TSE can be transmitted between hosts of difference species varies widely: while certain agents propagate without difficulty, it can be very difficult or even impossible to transmit others. This phenomenon, called the 'transmission barrier', or 'species barrier' is currently considered to be totally unpredictable.

The evaluation of the zoonotic potential of agents of TSEs depends mainly on the use of animal models (all of which are imperfect) which reproduce some of the constituent elements of the transmission barrier. It is now agreed that the two essential elements determining the capacity of an agent to propagate in a new host are the sequences of amino acids of the host's PrP protein and the strain of the infecting prion. The development of strains of transgenic mice expressing a PrP sequence identical to that of the donor species usually makes it possible to reduce or eliminate the transmission barrier considered.
Different animal models which might reproduce this transmission barrier for the human species have been suggested:

i) primates in which the PrP protein has a very high degree of homology (>96%) with the human protein,
ii) transgenic mice expressing the different alleles of the human PrP gene, in particular the Met/Val polymorphism at codon 129 which is known to modulate the natural susceptibility to different forms of CJD [22].

4.5.1. Inoculation of nonhuman primates

Historically, primates have provided the proof of the transmissible nature of human TSEs. After many fruitless attempts to transmit the disease to various animal models, the transmissibility of Kuru was demonstrated by Gajdusek's team after inoculation of different species of primates with brain homogenates taken from diseased patients [23].

The earliest elements supporting the zoonotic nature of classic BSE come from experiments involving the transmission of BSE to the *Cynomolgus* macaque [24]. The resulting disease is similar in many ways to human vCJD (florid plaques, electrophoretic profile of the PrP^{Sc}, etc.). Since then, the primate model has been used as a model for vCJD in studies designed to evaluate the human transmission barrier against BSE [21,25,26].

The susceptibility of primates to a limited number of scrapie isolates has been tested, either (i) by inoculation of brain matter taken directly from small ruminants, or (ii) by inoculation of scrapie isolates previously propagated iteratively to one or more intermediary hosts.

Thus the *Compton* isolate (a scrapie isolate passed iteratively nine times to goats and then eight times to mice) induces a clinical disease in the *Cynomolgus* macaque after five years of incubation [27]. Other species of monkey included in the same study (rhesus macaques, green monkeys, chimpanzees) seem to be less permissive. It is nonetheless difficult to interpret these experiments considering that propagating the infectious agent to mice could have altered its properties. In this same publication, the authors announced that two macaques (*Cynomolgus* and rhesus) and one chimpanzee infected by the *Compton* isolate that had passed through goats or by an ovine scrapie

[7] Apparent absence of adaptation phenomena (maximum efficacy of transmission at the first passage, slight or non-existent reduction in the period of incubation during successive passages in the host under consideration)

isolate originating in the USA had not developed a clinical disease four years after intracerebral injection (the results of this experiment have not yet been published).
Studies of the transmissibility of atypical scrapie to the *Cynomolgus* macaque are currently under way. However, they are not yet sufficiently advanced to allow conclusions to be drawn (E. Comoy, personal communication).

The Bolivian squirrel monkey is permissive via the intracerebral pathway (transmission in 14 months, [19]), and the oral pathway [28] in 25-32 months, to the *Compton* isolate, when the latter has been subjected to 3 extra intermediary passages via hamsters. In this model, the inoculation of human cases of sporadic CJD induces similar periods of incubation (11-48 months via the intracerebral pathway, 23-27 months via the oral pathway).

More recently, an isolate of ovine scrapie (PG 85/02) was transmitted by the intracerebral pathway to a marmoset, with an incubation period shorter than for BSE [29].

It is important to remember that New World monkeys (Bolivian spider monkeys and marmosets) are phylogenetically more distant from man than Old World monkeys (macaques and chimpanzees). This difference could have consequences for the relative pertinence of the data obtained in the two experimental models concerning the evaluation of the zoonotic risk of agents of TSEs. The Bolivian spider monkey for example is perfectly susceptible to isolates taken from deer suffering from Chronic Wasting Disease (7/8 isolates in 33-53 months), while macaques seem more resistant (no transmission 70 months after inoculation, [30]).

At the time when most of these experiments were performed, there was little documentation about the concept of diversity of TSE agents. These few studies do not allow any overall assessment of the zoonotic potential of the agents of TSEs in ruminants considered in all their diversity.
However, the data obtained show the capacity of certain isolates other than classic BSE to propagate in these models.

4.5.2.Inoculation of transgenic mice expressing human PrP

It is difficult to transmit cases of sporadic or hereditary CJD to conventional strains of mice [18,19,20]. However, transgenic mice expressing human PrP (with either a methionine or a valine at position 129 on the PrP gene) when the PrP's murine gene has been deleted (hereafter referred to as tgHu) enable researchers to propagate the agents responsible for these diseases without any apparent species barrier [20,31,32,33]. These 'humanised' strains provide interesting models for primary evaluation of the relative zoonotic potential of TSE isolates from animals and/or characterisation of possible similarities between these isolates and human cases of TSE. In tgHu mice, classic BSE, whose zoonotic character has been demonstrated, can be used as a benchmark for evaluating the zoonotic potential of other animal TSEs.

The comparatively short life cycle of mice (~2 years) can handicap these transmission studies considering the incubation period observed in humans which can exceed several decades. The over-expression of PrP by certain models of tgHu mice allows incubation periods to be shortened to some extent, though without eliminating this problem entirely.

Isolates of classic BSE can be transmitted to tgHu mice [20,34,35]. However, the integration periods and the attack rates of less than 100% (sometimes despite the presence of PrP^{Sc} in the tissues of the mice) observed after intracerebral inoculation of these isolates suggest that there is a significant transmission barrier. During iterative passages, the biological phenotype observed tends to become superimposed on that obtained after transmission of cases of vCJD [34], suggesting (if this were still necessary) a link between these two agents.

The transmission of atypical bovine isolates of type-L BSE has recently been reported on different models of tgHu mice expressing the Met129 allele [36,37]. The incubation time and attack rates observed with type-L BSE (after intracerebral inoculation) suggest that this type of agent can be transmitted much more easily than classic BSE. The absence of any reduction in incubation times and the stability of the phenotype characteristics observed during the iterative passages suggest that type-L BSE is propagated without any apparent species barrier to the human sequence of PrP.

Tests for the transmission of other cases of atypical type-H bovine BSE to tgHu mice have so far proved negative [34].

These studies indicate that:

i) even if type-L bovine BSE cannot be considered as a proven zoonotic agent in the current state of knowledge, it may still have the potential, and probably to a greater degree than classic BSE, to pass through the human species barrier,

ii) the permeability of the human transmission barrier (modelled with tgHu mice) depends largely on the TSE agent considered (see the three bovine prions considered: classic, type-L and type-H BSE).

iii) extrapolation of this concept to TSEs in small ruminants, whose exact biological diversity remains unknown but which seems greater than that observed in the bovine species, should be treated very cautiously.

To our knowledge, there have been few reports of experiments concerning the transmission of natural TSE isolates from small ruminants to models of tgHu mice. The only experiments published seem to indicate the absence of transmission of some isolates of classic scrapie in chimeric mice jointly expressing mouse and human PrP [38]. Considering the impact of chimerism on the propagation of isolates of TSE [39], it is difficult to interpret these results.

Studies designed to characterise the capacity of propagation of a panel of TSE isolates from small ruminants to various models of tgHu mice are under way. The results of these studies should be available within two to three years. Initial results from the French National Institute for Agronomic Research (INRA) at Jouy-en-Josas with European isolates of atypical scrapie taken from small ruminants with different genotypes or from transgenic mice expressing ovine PrP (the VRQ allele, [40]) suggest that this agent has a strong transmission barrier to human PrP (Met129 allele; no signs of TSE at first passage nor of PrP^{Sc}, with the second passage currently underway; V. Beringue, H. Laude, personal communication[8]). The possibility of the agent of a TSE propagating in a heterologous species without inducing clinical symptoms [18,41,42] but while remaining dangerous during secondary transmission to another sensitive species, is a supplementary factor illustrating our poor comprehension of these phenomena and the difficulty of modelling them.

4.6. Transmission of human TSE to small ruminants

'Mirror' experiments on the transmission of CJD isolates to small ruminants were performed at the beginning of the 1980s. In 1980, Hadlow and his team gave cerebral inoculations of brain extract from two patients suffering from sporadic CJD to 4 and 3 goats respectively [43]. Forty-three months after inoculation, one animal in each group developed a a pathology undistinguishable from scrapie, suggesting the possibility that the species barrier between man and goat could be penetrated, at least under the experimental conditions used.

4.7. *In vitro* evaluation of the transmission barrier between human PrP and agents of TSEs in small ruminants

At a conceptual level, while some murine cellular models can be used to detect very low levels of infectiousness for the murine prion (no species barrier [44]), they also show strong selectivity concerning the nature of the strains that they can propagate, although the underlying molecular mechanisms remain poorly understood.

There is currently no cellular model expressing human PrP that is sufficiently permissive to envisage the efficient propagation of prions of human origin and *a fortiori* an evaluation of the transmissibility of TSE isolate of animal origin [45,46,47].

It is consequently very difficult to envisage any development in the near future of pertinent cellular models for evaluating the permeability of transmission barriers.

[8] And also the report by the French Human Food and Nutrition Agency (PNRA) Prion Scientific Interest Group: on Atypical Scrapie

The capacity of a prion to propagate on a sequence of heterologous PrP has also been studied using noncellular conversion models. In its simplest version, this method consists in incubating semi-purified pathological PrP with radio-labelled cellular PrP [48]. The conversion efficiency is measured by quantification of the abnormal protease-resistant radio-labelled PrP. Experiments of this type have suggested that it is difficult for human cellular PrP to be converted by pathological PrP extracted from the brains of sheep infected by scrapie [49].

A technique developed recently and known as Protein Misfolding Cyclic Amplification (PMCA [50]) has enabled researchers to overcome this difficulty concerning the very poor conversion efficiency of the earliest non-cellular systems. In the course of PMCA, small quantities of pathological PrP are mixed with a much larger amount of cellular PrP. The mixture is then subjected to cycles of incubation and sonication so that part of the cellular PrP is converted into abnormal PrP. Quantification of the amplified abnormal protease-resistant PrP then provides a measure of the conversion efficiency. The capacity for conversion of polymorphic variants (MM, MV, VV) of normal human PrP by different agents of TSE has been reported very recently [51]. None of the three genotype variants seems to be (easily) transconformable by abnormal PrP extracted from the brains of Suffolk sheep homozygous for the ARQ allele and infected by scrapie (agent or strain not given). Experimentally ovinised BSE (ARQ 2x) on the other hand is capable of converting the MM and MV variants of normal PrP.

Considerable caution is needed when interpreting the results of these methods of *in vitro* conversion. Indeed, it is still not possible today to assess to what extent the data obtained from these models can be transposed to complex biological systems (animals). Many studies, no doubt lasting several years, will be required before these methods of characterising the capacity of prions to penetrate transmission barriers can be validated or invalidated.

4.8. The different forms of BSE and the difficulty of demonstrating the zoonotic character of animal TSEs

The example of BSE in its so-called classic form (BSE-C) fully illustrates the difficulty of formally demonstrating the zoonotic character of a prion disease. BSE-C is currently the only animal TSE whose zoonotic character has been clearly established. Dietary exposure to this TSE agent was responsible from 1995 onwards for the emergence of variant Creutzfeldt-Jakob Disease (vCJD) in man. However, this causal relationship was only demonstrated through a highly particular combination of circumstances:

1 – Despite a massive exposure of the human population, at least in Britain, to tissues infected by the agent of classic BSE, a relatively small number (approximately 200 cases) of vCJD have so far been identified around the world. In quantitative terms, the onset of these cases of vCJD has not had a significant impact on the level of global prevalence of CJD (taking all forms together), including in the United Kingdom. In other words, analysis of the evolution of the prevalence of cases of CJD (taking all forms together) would never have revealed the zoonotic character of the BSE agent.

2 – It can be very difficult to spot fluctuations in the number of real cases of CJD (taking all forms together) from the monitoring data alone. (For example, in the United Kingdom, even before the discovery of vCJD, there were 33 confirmed cases of CJD in 1990 and 62 in 1994, against 53 and 119 suspected cases respectively, with the similarity of the confirmation ratios between these two years clearly indicating the lack of sensitivity of the monitoring system.) In this context, vCJD was only identified because of the sudden appearance over a short period, first in the United Kingdom, of a series of cases with very particular characteristics (10 in 1996) which distinguished them from all other forms of CJD (the age of the subjects, clinical picture, neuropathological lesions such as the presence of florid plaques). It was only because of such particular features as these that experimental investigators focussed on such cases, in order to characterise the infectious agent responsible. It should be noted that the identification was probably facilitated by the context of the very large scale epizootic of classic BSE, which

encouraged much greater vigilance concerning neurodegenerative diseases in man.

3 – The experimental transmission of isolates of bovine BSE-C and cases of vCJD to a panel of conventional mice made it possible to identify similar biological signatures (incubation time for each strain, distribution of spongiform lesions in different parts of the brain), indicating that the agents involved were probably identical [16]. This was an almost 'ideal' situation which does not represent the norm: the transmission of other natural isolates of TSE to these mouse models rarely allows unequivocal discrimination of the agent or agents present. Although some doubts may remain about the validity of this methodology, there is now no doubt about the conclusions concerning vCJD, as a result of the epidemiological data and the arguments supplied by other experimental studies, particularly regarding transmission to macaques [24] and to transgenic mice expressing bovine [52] or human [31] PrP.

These elements explain why it can be difficult if not impossible to establish the zoonotic character of a prion disease.

A practical example of this difficulty is provided by taking the opposite approach with type-L atypical BSE for which there are now convergent experimental findings (see above) arguing that transmissibility to man is considerably higher than for classic BSE (greater virulence compared to BSE after transmission to macaques [53] and 'humanised' transgenic mice [34,37]), but in an epidemiological situation involving a very limited number of cases (28 cases identified by 14 January 2010 in Europe, T. Baron, personal communication and [54]).

Today it would be extremely difficult to demonstrate an epidemiological link between type-L BSE and a prion disease in humans. Indeed, bovine BSE-L is detected (at extremely low frequency) in every country where it is sought and its frequency seems to vary little over time. In this context, it would seem difficult to detect the appearance or increased frequency of a human disease in a determined geographical area, which removes the principal points for analysis of any epidemiological relationship. An alternative approach might consist in a prospective epidemiological study of man, to evaluate the effect of exposure to the risk of BSE-L (which would be extremely difficult to implement, incidentally):

1 – Obviously, no repercussions on the incidence of CJD are expected, especially since the appearance of these rare cases in cattle is probably relatively constant over time, under the probable hypothesis that the disease is 'sporadic'. If such cases were the origin of the disease in man, one would expect that the human cases linked to this exposure would disappear with the withdrawal of Specified Risk Material (SRM) from human consumption, reinforced by screening tests; however, considering the probably very long and heterogeneous incubation period in the case of dietary transmission, it would take several decades before any conclusions could be drawn.

2 – The characteristics of a possible human disease related to these cases of type-L BSE are unknown *a priori*, and might not be revealed by the sudden emergence of a new pathological entity in man. Such a human disease would have to be sought amidst the 'background noise' of human neurodegenerative diseases. We could not be certain of identifying such cases from among the suspected cases of CJD as currently defined. Indeed, it would be unwise to consider the range of human prion diseases as definitive: one example is the recent identification [55], including in Europe [56], of new forms of prion diseases (known as 'protease-sensitive prionopathies') that do not necessarily satisfy all the criteria for suspecting CJD.

3 – Unlike the case of BSE-C, there is currently no body of knowledge from which to establish with any confidence the similarities between the agents responsible for type-L BSE and a form of human prion disease. On the contrary, if we consider the current data obtained by transmission to a very large panel of experimental models (conventional mice, hamster, vole and transgenic mice expressing bovine, ovine or human PrP), the complexity of the infectious agent or agents involved in this bovine disease seems quite disconcerting, making it very difficult to interpret the experimental data for the time being.

5. CONCLUSION

AFSSA considers that it has not so far been possible to establish a formal epidemiological link between animal and human TSEs, with the exception of that between vCJD and classic BSE. It was possible to demonstrate the zoonotic risk of BSE as the result of a combination of favourable circumstances (sudden emergence of a new animal disease, then of a new human disease, both associated with unique phenotype characteristics) and numerous studies; it is impossible to say whether such circumstances and studies would occur for other agents of TSEs.

The epidemiological studies aiming to establish a link between the onset of cases of CJD and exposure to animals or animal products carry little weight. This is the result of a combination of several parameters of which the principal ones are: the limitations and possible bias of surveillance or detection systems, the very low prevalence of these diseases, the lack of precision in measurements of exposure (especially dietary) related to the very long incubation period, poor knowledge of the genetic parameters of susceptibility, the difficulty of taking full account of exposure specific to human subpopulations as a function of their professional or leisure activities, and the diversity of the forms of TSE in both animals and humans.
However, a slight but recurrent relationship between human TSE and exposure to animals and/or animal products appears in certain studies and could indicate an epidemiological link between animal and human TSEs, without allowing identification of the type of TSE and the human pathology.

In the current state of knowledge and methodologies, the evaluation of the zoonotic risk associated with agents of TSE can only be based on the examination of a cluster of indirect elements, none of which taken individually could constitute formal proof for either affirming or denying that an agent of animal TSE is capable of being transmitted to man. Among these different elements, the ability of an agent of TSE to propagate in primates or in transgenic animals expressing the coding gene for the PrP protein seems the most pertinent.

AFSSA therefore reaffirms that it is not scientifically pertinent to consider that no agent of animal TSEs, other than classic BSE, presents a zoonotic risk. The recent demonstration of the capacity of BSE-L to propagate more efficiently than classic BSE in experimental models with primates and transgenic 'humanised' mice clearly shows that this new strain presents a potential zoonotic risk.
These uncertainties justify retaining the precautionary measures that aim to reduce human exposure to agents of TSE circulating in animal populations.

The Director-General

MARC MORTUREUX

KEYWORDS

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