



US 20050048582A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0048582 A1**

Cornelis Schreuder et al. (43) **Pub. Date: Mar. 3, 2005**

(54) **METHOD FOR THE DETECTION OF PRION DISEASES**

(76) Inventors: **Bram Edward Cornelis Schreuder**,
Lelystad (NL); **Lucius Johannes
Mattheus Van Keulen**, Bunnik (NL);
**Maria Elisabeth Wilhelmina
Vromans**, Lelystad (NL); **Johannes
Pieter Maria Langeveld**, Harderwijk
(NL); **Marinus Adrianus Smits**,
Harderwijk (NL)

Correspondence Address:
**TRASK BRITT
P.O. BOX 2550
SALT LAKE CITY, UT 84110 (US)**

(21) Appl. No.: **10/949,880**

(22) Filed: **Sep. 24, 2004**

Related U.S. Application Data

(63) Continuation of application No. 09/155,794, filed on
May 20, 1999.

(30) **Foreign Application Priority Data**

Apr. 3, 1996 (EP) 96200917.1

Publication Classification

(51) **Int. Cl.⁷** **G01N 33/53**

(52) **U.S. Cl.** **435/7.1**

(57) **ABSTRACT**

The invention provides methods for the detection of prion diseases, such as scrapie of sheep, bovine spongiform encephalopathy of cattle, Creutzfeld-Jacob disease of man, whereby aberrant proteins or prion proteins are detected in tissues which can be sampled from live animals.

Figure 1 Multiple sequence allignment of prion proteins of various origin.

Perfectly conserved: '+'	Disulfide-bond: '\$'	
Well conserved: '.'	N-Glycosylation: '#'	
	-----sign-----	
SHPRP	MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPPGN	50
BTPRP	MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPPGN	50
MINKPRP	MVKSHIGSWLLVLFVATWSDIGFCCKRKPKPGGGWNTGGSRYPGQGSPPGN	50
GORPRP	M--ANLGYWMLVLFVATWSDLGLCKKRPKPGG-WNTGGSRYPGQGSPPGN	47
HSPRP	M--ANLGCWMLVLFVATWSDLGLCKKRPKPGG-WNTGGSRYPGQGSPPGN	47
MAPRP	M--ANLSYWLLALFVAMWTDVGLCKKRPKPGG-WNTGGSRYPGQGSPPGN	47
MMPRP	M--ANLGYWLLALEVTMWTDVGLCKKRPKPGG-WNTGGSRYPGQGSPPGN	47
RRPRP	-----GGWNTGGSRYPGQGSPPGN	19+
	+*****	
SHPRP	RYPPQGGGGWQPHGGGWQPHGGGWQPHGGGWQPHGGG-----G	92
BTPRP	RYPPQGGGGWQPHGGGWQPHGGGWQPHGGGWQPHGGGWQPHGGG	100
MINKPRP	RYPPQGGGGWQPHGGGWQPHGGGWQPHGGGWQPHGGGWQPHGGG-----G	92
GORPRP	RYPPQGGGGWQPHGGGWQPHGGGWQPHGGGWQPHGGG-----G	88
HSPRP	RYPPQGGGGWQPHGGGWQPHGGGWQPHGGGWQPHGGG-----G	88
MAPRP	RYPPQGGGTWQPHGGGWQPHGGGWQPHGGGWQPHGG-----G	88
MMPRP	RYPPQGG-TWQPHGGGWQPHGGGWQPHGGGWQPHGG-----G	87
RRPRP	RYPPQSGGTWQPHGGGWQPHGGGWQPHGGGWQPHGG-----G	60+
	***** . * .***** .***** .***** .*****	
SHPRP	WGQGG-SHSQWNKPSKPKTNMKHVAGAAAAGAVVGGGLGGYMLGSAMSRPL	141
BTPRP	WGQGG-THGQWNKPSKPKTNMKHVAGAAAAGAVVGGGLGGYMLGSAMSRFL	149
MINKPRP	WGQGGSHGQWGKPSKPKTNMKHVAGAAAAGAVVGGGLGGYMLGSAMSRPL	142
GORPRP	WGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGGLGGYMLGSAMSRPI	138
HSPRP	WGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGGLGGYMLGSAMSRPI	138
MAPRP	WGQGGGTHNQWNKPSKPKTNLKHVAGAAAAGAVVGGGLGGYMLGSAMSRPM	138
MMPRP	WGQGGGTHNQWNKPSKPKTNLKHVAGAAAAGAVVGGGLGGYMLGSAMSRPM	137
RRPRP	WSQGGGTHNQWNKPSKPKTNLKHVAGAAAAGAVVGGGLGGYMLGSAMSRPM	110+
	*.*** .*.*** .***** .*.*** .***** .***** .***** .*****	
	§ #	
SHPRP	IHFNDYEDRYRENMYRYPNQVYYRFPVDQYSNQNNFVHDCVNIIVKQHT	191
BTPRP	IHFSDYEDRYRENMYRYPNQVYYRFPVDQYSNQNNFVHDCVNIIVKQHT	199
MINKPRP	IHFNDYEDRYRENMYRYPNQVYYRFPVDQYSNQNNFVHDCVNIIVKQHT	192
GORPRP	IHFSDYEDRYRENMYRYPNQVYYRPMDEYSNQNNFVHDCVNIIVKQHT	188
HSPRP	IHFSDYEDRYRENMYRYPNQVYYRPMDEYSNQNNFVHDCVNIIVKQHT	188
MAPRP	MHFNDWEDRYRENMYRYPNQVYYRFPVDQYNNQNNFVHDCVNIIVKQHT	188
MMPRP	IHFNDWEDRYRENMYRYPNQVYYRFPVDQYSNQNNFVHDCVNIIVKQHT	187
RRPRP	LHFNDWEDRYRENMYRYPNQVYYRFPVDQYSNQNNFVHDCVNIIVKQHT	160+
	***** .***** .***** .***** .***** .***** .***** .*****	
	# §	
SHPRP	VTTTTKGENFTETDIKIMERVVEQMCITQYQRESQAYYQ--RGASVILFS	239
BTPRP	VTTTTKGENFTETDIKIMERVVEQMCITQYQRESQAYYQ--RGASVILFS	247
MINKPRP	VTTTTKGENFTETDMKIMERVVEQMCITQYQRESEAYYQ--RGASAILFS	240
GORPRP	VTTTTKGENFTETDVKIMERVVEQMCITQYERESQAYYQ--RGSSMVLFS	236
HSPRP	VTTTTKGENFTETDVKIMERVVEQMCITQYERESQAYYQ--RGSSMVLFS	236
MAPRP	VTTTTKGENFTETDIKIMERVVEQMCITQYQKESQAYYDGRSSA-VLFS	237
MMPRP	VTTTTKGENFTETDVKIMERVVEQMCITQYQKESQAYYDGRSSSTVLFS	237
RRPRP	VTTTTKGENFTETDVKIMERVVEQMCITQYQKESQAYYDGRSSA-VLFS	209+
	***** .***** .***** .***** .***** .***** .***** .*****	

Figure 1, continued

```

---GPI-
SHPRP      SPPVILLISFLIFLIVG  256
BTPRP      SPPVILLISFLIFLIVG  264
MINKPRP    PPPVILLISLLILLIVG  257
GORPRP     SPPVILLISFLIFLIVG  253
HSPRP      SPPVILLISFLIFLIVG  253
MAPRP      SPPVILLISELIFELMVG  254
MMPRP      SPPVILLISFLIFLIVG  254
RRPRP      SPPVILLISFLIFLIVG  226+
            .*****+.***+.***
    
```

Dictionary of the sequences which have been aligned
 =====

- [1] SHPRP
 DE PROTEINSEQ OF SHPRP NT 72-839
 Size: 256 residues.
- [2] MINKPRP
 DE MINK TRANSL BY ALX 41-814
 Size: 257 residues.
- [3] GORPRP
 DE GORPRP TRANSL FROM 1-762 BY ALX
 OS GORILLA
 Size: 253 residues.
- [4] MAPRP
 DE MAPRP TRANSL FROM 11-733 BY ALX AA MANLSYWLLALFVA ADDED
 OS SYRIAN GOLDEN HAMSTER
 Size: 254 residues.
- [5] BTPRP
 DE BOVINE PRP GENE FOR A PRION-PROTEIN.
 OS BOS TAURUS (CATTLE)
 Size: 264 residues.
- [6] HSPRP
 DE HOMO SAPIENS PRP GENE TRANSL FROM 50-811 BY ALX
 OS HOMO SAPIENS
 Size: 253 residues.
- [7] MMPRP
 DE MMPRP TRANSL FROM 107-871 BY ALX
 OS MURINE PRP
 Size: 254 residues.
- [8] RRPRP
 DE RAT PRION-RELATED PROTEIN (PRP) MRNA TRANSL <1? TO 678 FRAME 1
 (ALX)
 OS RATTUS RATTUS (RAT)
 Size: 226 residues.

METHOD FOR THE DETECTION OF PRION DISEASES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation of co-pending application Ser. No. 09/155,794, filed May 20, 1999, U.S. Pat. No. _____, which is the national stage entry of PCT/NL97/00166, Apr. 2, 1997, published in English as WO 97/37227 on Oct. 9, 1997, the entire contents of the entirety of which is incorporated by this reference.

TECHNICAL FIELD

[0002] The invention relates generally to biotechnology and more particularly to the field of prion diseases, also called spongiform encephalopathies (SEs), such as scrapie of sheep, bovine spongiform encephalopathy (BSE, mad-cow disease) of cattle, Creutzfeld-Jacob disease (CJD) and kuru of man. Prion diseases are transmissible via among others ingestion of or inoculation with prion proteins, can occur iatrogenically, but can also happen occasionally or on a hereditary basis without evidence of transmission.

BACKGROUND

[0003] Prion diseases are a focal point of public interest, recently fuelled by the detection of unexpected cases of CJD in teenagers and in farmers, both in Great Britain, where transmission of prion proteins from cattle to humans via meat consumption is postulated, thus indicating the transmission of BSE to humans, thereby causing CJD.

[0004] Several factors enhance public concern:

[0005] a) the nature of the causative agent, the so called prion protein, of SEs is unknown or at least controversial,

[0006] b) whatever its nature, the agent is highly resistant to procedures that eliminate other infectious agents (e.g., heating);

[0007] c) therapeutical interventions are apparently not possible, once symptoms occur;

[0008] d) SEs have an extremely long incubation period;

[0009] e) practical, sensitive and specific diagnostic methods to be used during the preclinical phase are not available. This all adds to the general feeling of "living with a time-bomb". Not only the possible presence of prion proteins in meat and neat products poses a health threat, also the possible presence of prion proteins in blood and blood products used in transfusion, the presence in pharmaceutical products of animal origin, in cosmetics of animal origin, in sera used for cell culture, in short, in an extensive array of products of animal origin, pose possible threats to human and animal health.

[0010] Until now, confirmatory diagnosis of scrapie and also other transmissible spongiform encephalopathies depended on histological examination of the brain, collected during post-mortem examination from animals or humans with clinical signs of the disease. Deposits of an aberrant or altered protein (PrP^{Sc}, prion protein) can be detected in the

brain of diseased animals. This protein is very insensitive to methods, such as proteinase K digestion, that otherwise denature, lyse or remove normal proteins. The aberrant protein is considered central in the pathogenesis of prion disease. Albeit not infectious in a classical microbiological way due to the absence of specific nucleic acid, the aberrant protein itself is seen as the causal agent, and when a susceptible animal obtains such an aberrant protein in its body (i.e. by ingestion, inoculation or via mutation of the gene of the normal version of the PrP protein, PrP^C) a chain reaction may start that ultimately will lead to a clinical manifestation of prion disease. The chain reaction entails the formation of more aberrant proteins formed out of the normal protein present in the animal's body. Normal and aberrant forms will interact in such a way that more aberrant forms are produced. Since the aberrant form is very resistant to proteolysis, deposits of the converted prion protein will be formed, especially in the brain and other parts of the central nervous system (CNS), giving rise to the spongiform encephalopathy and thus clinical manifestations of brain disease.

[0011] As SE-infected or affected animals and man lack a disease-specific immune response, identifying individuals before they develop clinical signs (which can take years) has been practically impossible so far. No biochemical, haematological, or gross pathological abnormalities are consistently associated with SEs. The diagnosis of SEs, therefore, depends on the recognition of clinical signs, electro-encephalography or magnetic resonance imaging techniques (both used only in human patients), or the more invasive method of taking brain-biopsies. The final diagnosis is made during autopsy, by histological examination of the brain. The neuro-pathological lesions, consisting of vacuolation (spongiform change) of the grey matter associated with gliosis and neuronal loss, are generally sufficiently characteristic. Further confirmation is possible by demonstrating scrapie associated fibrils (SAFS) in brain extracts, or by demonstrating the presence of its constituent protein, PrP^{Sc}. PrP^{Sc} is associated with the disease and is an aberrant form of the host encoded prion protein (PrP), the aberrant form is induced by a conformational change. PrP^{Sc} can be detected by immunological techniques such as Western blotting or immunohistochemistry. The latter technique is gradually becoming more and more accepted as a reliable diagnostic tool for clinical cases, in both the human and veterinary SE field.

[0012] The search for a practical preclinical diagnostic test has been and continues to be a main topic of research. This generally focuses on the detection of infectivity using a bio-assay, or the detection of the disease associated PrP^{Sc}. The bio-assay, in spite of being the most sensitive detection method, is far too cumbersome and time-consuming to ever become a practical diagnostic method: test results might become available long after the patient has passed away.

[0013] Most researchers have therefore focused on techniques to detect PrP^{Sc}. Although not all researchers agree with the statement that PrP^{Sc} is the causative agent, most, if not all, agree that the association of the presence of PrP^{Sc} and disease has been firmly established. Detection of PrP^{Sc} in tissues outside the CNS would allow sampling through less invasive methods than brain biopsies, thereby brightening prospects for a practical preclinical diagnostic technique substantially. Various tissues have been used in an

attempt to develop an early detection technique: blood, urine, tissue fibroblasts, and, particularly in the animal field, lymphoid tissue. A short summary of the most promising and striking ones is given here (for an extensive review see Schreuder, 1994a, 1994b).

[0014] Blood: In human SEs, there is the often disputed experimental transmission of CJD from buffy coat samples of human CJD-patients to rodents (Muaramoto et al., 1993), but there is little or no indication that blood and specifically, buffy coat contains any infectivity in animals affected naturally with scrapie, either in clinical or in preclinical stages (Fraser and Dickinson, 1978; Hadlow et al., 1982). Interesting results have recently been reported by Meiner et al. (1992) who detected PrP^{Sc} in peripheral tissues, both in cultured fibroblasts and in monocytes, in a group of eight CJD patients carrying the codon 200 mutation and suffering from clinical disease. These authors used both Western blotting and immunocytochemistry techniques. Their publication, however, appears to have had no follow-up and even if these results could be confirmed, the chances for a reliable blood test seem remote, at least in the case of animal SEs and given the number of negative reports from literature (reviewed in Brown, 1995).

[0015] Urine: Only once has a claim been made that infectivity in urine was demonstrated in a case of CJD, by transmitting it to mice. The same author was, however, unable to repeat this experiment (Brown, 1995). A totally different approach was reported recently (Brugere et al., 1991). Urine from scrapie affected and control animals was tested in a voltametric method by repeated capillary micro-electrolysis, which allowed discrimination of these two groups. This approach appeared promising, but, its value in detecting preclinical stages of in particular BSE could not be confirmed.

[0016] Lymphoid tissue: Lymphoid tissue has apparently not been used in the field of diagnosing human SEs, it has, however, in the veterinary field. The already classical work by Hadlow has shown that in the lymphoid tissue of naturally infected scrapie sheep, infectivity was detectable by bio-assay as early as 10-14 months of age. This was before any infectivity in the CNS was found (Hadlow et al., 1980). Western blotting has revealed the presence of PrP^{Sc} in the spleen of scrapie-infected mice (Diringer et al., 1983; Doi et al., 1988), in some cases PrP^{Sc} was detected as early as 4 weeks after experimental infection. Pooled lymph nodes from these mice also contained PrP^{Sc}. Similarly, also using Western blotting, PrP^{Sc} was detected fairly consistently in a group of naturally injected sheep showing clinical signs of scrapie, in samples from the CNS, spleen, and lymph nodes (Ikegami et al., 1991). The value of this Western blotting technique was, at least for clinical cases, confirmed by other groups. The results, however, from a group of experimentally infected sheep that were killed at 16, 18 and 21 months after inoculation but before clinical signs developed, were inconsistent and difficult to evaluate: PrP^{Sc} was detected in spleen samples of only 3 out of 12 supposedly positive animals, with lymph node samples only weak or doubtful results, but no positive results were found, illustrating the insensitivity of this technique. Therefore, using Western blotting techniques in pre-clinical diagnoses of TSE give erratic and not reliable results.

[0017] The reason for these erratic results can be found in the method to prepare the PrP^{Sc} protein (present in the

affected tissues) and dissociate or separate it from the normal cellular isoform PrP protein that is also immunoreactive with the same antisera used for the Western blotting.

[0018] Ikegami et al. (1991) and Muramatsu et al. (1993) need to prepare the samples for Western blot analysis by various steps. They first enrich the samples by preparing tissue extracts containing fractions relatively enriched for both PrP^{Sc} and PrP, after which the need to remove the PrP protein with a proteinase K treatment. This procedure entails at least 10 separate incubation and separation steps in which the absolute amount of the proteins to be detected in the sample is reduced at every step. Although this protocol works very well for the diagnosis of the clinical phase of SE's, where an abundance of PrP^{Sc} is present in relation to the normal cellular isoform PrP, in the preclinical phase of TSE, the absolute amount of PrP^{Sc} is so small that it usually gets lost during the preparation.

[0019] In BSE, the situation differs from that of scrapie: on the one hand, results from mice-transmission experiments using different tissues of BSE affected cattle, may indicate that distribution of the BSE agent tissues outside the CNS is not as extensive as in the case of scrapie in sheep, on the other hand it may be that the mice used in the bio-assays are far less sensitive for BSE than for scrapie. Experimental transmission of BSE to mice only succeeded when brain material was used (Fraser et al., 1988; Fraser et al., 1990); mice inoculated with other materials, including spleen, semen, buffy coat, muscle, bone marrow and placenta remained healthy.

[0020] However, all above techniques other than bio-assays have in common that diagnosis of SEs can only be established in the clinical phase of the disease, often at autopsy only. Considering the fact that bio-assays are very slow, due to the very slow progress of the disease in the experimental animal that is used for the bio-assay as such, no methods are currently available that offer immediate diagnoses of SEs in a pre-clinical phase of the disease. Thus, although the average expert in diagnostic test development has currently a wealth of diagnostic techniques available to detect all kinds of proteins in biological samples, using monoclonal or polyclonal antisera in enzyme- or label-linked immunoassays, using techniques with or without enriching methods for the protein under study, no gold-standard is available to give guidance to the development of those diagnostic techniques that would be applicable in the case of pre-clinical diagnosis of prion disease. In other words, methods to establish sophisticated diagnostic tests are currently well known to the general expert in the field; the expert lacks, however, methods to establish the sensitivity and specificity of those sophisticated diagnostic tests due to the lack of a "gold standard".

DISCLOSURE OF THE INVENTION

[0021] We have now found a reliable and fast diagnostic method for pre-clinical diagnosis of prion diseases or SE's. The invention offers a method for pre-clinical diagnosis in sheep scrapie but also for other SEs like BSE and CJD. We used scrapie in sheep as a model to study SEs. Knowledge of the group of SEs, which includes the human forms such as CJD and Kuru, has been largely obtained from studies with scrapie. Scrapie is a progressive and fatal neurological disease of sheep and goats and is considered the "archetype"

of the group of SEs and the probable cause for the BSE epidemic in the United Kingdom. The control and sanitary measures taken during the outbreak of BSE in the UK were also largely based on what was known about scrapie. Taking into account the above mentioned data of Hadlow on the presence of infectivity in various peripheral tissues, we concluded that among others lymphoid tissue would be a candidate for the development of a preclinical test based on detection of PrP^{Sc}, but also other tissues, such as but not limited to retina, alveolar macrophages or monocytes, where PrP infectivity is found.

[0022] In our hands, immunohistochemistry (IHC) using the immuno-peroxidase staining method, when used on histological sections of the brains for diagnosing clinical scrapie and BSE, proved a highly reliable and practical method for detecting PrP^{Sc} (Van Keulen et al., 1995) and less cumbersome than Western blotting. Using the same IHC-technique and the same antisera, we examined a number of lymphoid tissues in a group of naturally affected, clinically-positive scrapie sheep (n=55) (Van Keulen et al., in press, see also the experimental part). We demonstrated the presence of PrP^{Sc} in the spleen, the retropharyngeal lymph node, mesenteric lymph node and the palatine tonsils, in all but one of the animals (98%). Of all examined lymph nodes, tonsils were found having the highest PrP^{Sc} deposition rate that could be detected per number of follicles: in all positive cases, more than 60% of the tonsil follicles stained positive and in 95% of these cases this was even more than 80%. To assess the applicability of this method in the pre-clinical phase of scrapie, we embarked upon a study involving sequential biopsy taking of tonsils in sheep, tonsils were chosen while the experimental availability for sequential studies is guaranteed, however, using other tissues can as well be contemplated for pre-clinical diagnosis. We have detected the scrapie associated PrP^{Sc} in tonsils of 10 month old sheep, which is at less than half-way the incubation period as the sheep under study are expected to develop scrapie when approximately 25 months old. In sheep that are expected to develop scrapie at a much later stage or stay healthy during their whole life span, we did not detect this PrP^{Sc} protein.

[0023] With regard to scrapie, future control programs could profit from these findings. Control programs in several breeds could consist of a combination of breeding programs that make use of the established linkage between PrP genotype and increased scrapie-susceptibility or -resistance, and the above described method that detects the pathognomonic presence of PrP^{Sc} in tonsils of susceptible animals in the preclinical stage of the disease.

[0024] With regard to BSE, and SEs in general, changes and adjustments of the technique used can now be made to adjust to the specific circumstances and conditions of BSE, and SE, diagnosis. Those changes can be guided by specific knowledge about homologies and heterologies in the amino acid sequences of prion proteins from different species (for a selection of known sequences see FIG. 1). Also, guidance may be found in selecting specific antisera by selecting for reactivity of selected continuous or discontinuous peptide sequences of those prion proteins. First of all, the IHC-technique may be further refined for use in BSE and in peripheral lymph nodes in particular. This could require adaptations of the protocol in use for immuno-staining of brain sections. PrP^{Sc} detection in lymphoid tissues has been tried only using immuno-blotting methods and in clinical cases (Mohri et al., 1992). These results were negative, indicating a detection problem with regard to sensitivity. No

serious efforts have been made to detect PrP^{Sc} in preclinical stages of BSE. The technique of taking tonsillar biopsies in live cattle is feasible and even easier than in sheep, as cattle can do with a light sedation (Xylazine (Rompun)). The possibility of an early diagnosis in case of BSE could alleviate the need for certain draconian measures proposed today with regard to the cattle population in the UK.

[0025] Far reaching implications of our invention lie in the field of human SEs. Also here the applicability of the IHC technique in the preclinical phase can now be established. In literature, we did not find any reference to the examination of lymphoid tissues in this context. With tonsils being more readily accessible and with almost always access to a pathologic-anatomical laboratory, the above described technique, applied in human SEs, could contribute to an early diagnosis of suspected cases of SEs. This allows the possibility of detecting individuals harboring the disease at a moment early in the incubation period; at least considerably long before clinical signs appear, which in turn would allow certain therapeutic measures to be applied for specific groups at risk (at least interventions that delay the progression of the disease, such as the use of amphotericin B).

[0026] The present invention thus provides methods for or the detection of prion disease whereby aberrant proteins are detected in various tissues, such as but not limited to lymphoid or tonsillar tissue, which can be sampled from live animals, in particular from farm animals or humans or other mammals. The invention also provides methods that distinguish between aberrant and normal protein, by removing the normal protein with methods that proteolyse, hydrolyse or denature the normal protein, or by immunologically detecting the aberrant protein. Immunological detection entails any method currently known by the expert in diagnostic test development, all methods employing immunological detection with enzyme- or label-linked or non-linked antibodies, even Western blotting techniques, may now be developed into sensitive and specific techniques, due to the fact that a gold-standard for pre-clinical diagnosis of prion disease has now become available. These methods may also be developed into diagnostic tests or test kits comprising the necessary elements of any of the above methods. The invention further provides use of any of the above methods, tests or test kits in the diagnosis of prion disease, in disease control programs, in the selection of meat fit for consumption and in the selection of blood or blood products.

BRIEF DESCRIPTION OF THE FIGURE

[0027] FIG. 1 is a multiple sequence alignment of prion proteins of various origins.

DETAILED DESCRIPTION OF THE INVENTION

EXPERIMENTAL

[0028] Immunohistochemical detection of prion protein in lymphoid tissues of sheep with clinical cases of natural scrapie

[0029] Materials and Methods

[0030] Sheep. Sixty seven sheep with nervous disorders resembling those of a scrapie infection were purchased. Fifty-five sheep were diagnosed with scrapie by histopathological and immunohistochemical examination of the brain. (van Keulen et al., 1995). One animal suffered from both a scrapie infection and a concurrent meningo-encephalitis

probably caused by *Listeria monocytogenes*. Scrapie-positive sheep originated from 30 different flocks. The group consisted of 54 females and one male ranging in age from 2 to 5 years and comprised eight different breeds and cross-breeds. Twelve sheep did not show any histopathological signs of a scrapie infection nor did they display any PrP^{Sc} immunostaining in the brain. Five of these sheep were diagnosed with meningo-encephalitis, one had intramyelinic edema of unknown cause, and 6 sheep showed no histopathological abnormalities. Scrapie negative sheep were all females from 10 different flocks and two different breeds and crossbreeds, ranging in age from 1 to 5 years.

[0031] Necropsy. Necropsy was performed within 36 hours after natural death or immediately after killing the animal by intravenous injection of sodium pentobarbital and exsanguination. The brain was removed from each sheep for scrapie diagnosis as described previously (van Keulen et al., 1995). Samples were taken from several lymphoid tissues including spleen, palatine tonsil, superficial cervical lymph node (prescapular lymph node), subiliac lymph node (prefemoral lymph node), medial retropharyngeal lymph node, tracheobronchial lymph node, mesenteric lymph node, and ileum.

[0032] Histological and immunohistochemical procedures. Tissue samples were immediately immersed for 24 hours in periodate-lysine-paraformaldehyde fixative (PLP) containing 2% paraformaldehyde (Merck, Darmstadt, Germany). Samples were then trimmed to a maximum thickness of 2 mm and fixed for another 24 hours in freshly prepared PLP. After fixation, tissue samples were washed in water, routinely dehydrated and embedded in paraffin. Three sections of 5 μ m were cut, mounted on 3-aminoalkyltriethoxysilane-coated glass slides (Sigma, St. Louis Mo., USA), dried for at least 48 hours at 60° C. and deparaffinized. The first section was stained with hematoxylin-eosin (HE). Second and third sections were immunostained with anti-peptide serum directed against the ovine prion protein and pre-immune serum respectively according to the following procedure; after 30 minutes immersion in 98% formic acid (Merck), sections were washed and autoclaved immersed in water for another 30 minutes at 121° C. in a pressure cooker. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol (Merck). Incubation at room temperature for 1 hour with anti-peptide antiserum or pre-immune serum, diluted 1:1500 in phosphate-buffered saline (pH 7.2) containing 1% bovine serum albumin (Sigma), was followed by incubation, first with biotin-conjugated goat-anti-rabbit IgG and then with streptavidin-peroxidase for 10 and 5 minutes respectively (Dakopatts, Glostrup, Denmark). As substrate we used aminoethylcarbazole (Zymed Laboratories Inc., San Francisco Calif., USA) because its red color could easily be differentiated from the yellow-brownish ceroid/lipofuscin and hemosiderin pigment which was often present in the lymphoid tissues. Between the various steps, sections were thoroughly rinsed in phosphate-buffered saline containing 0.05% Tween-20 (Merck). Sections were counterstained with Mayers hematoxylin for 30 seconds and mounted in Glycergel (Dakopatts). With every immunohistochemical staining, a section of the medulla oblongata of a confirmed scrapie-affected sheep was simultaneously stained for PrP to check correct immunostaining procedures.

[0033] Peptide synthesis and anti-peptide antisera. Five peptides with sequences derived from the ovine prion protein (PrP 94-105, 100-111, 126-143, 145-177, 223-234)

were synthesized and used to raise anti-peptide antisera in rabbits following previously published procedures (van Keulen et al., 1995). Antisera were confirmed to be specific for PrP (both undigested and after proteinase K treatment) on western blots of partially purified prion protein from scrapie-affected sheep brain according to established procedures (Hilmert and Diringer, 1984). Pre-immune sera were collected before immunization and served as negative control sera.

[0034] The sera used have advantages which are based on a mixture of empirical, theoretical and analytical values the combination of which makes them invaluable in the diagnostic application. The preparation of the sera has been described in a publication of van Keulen et al. 1995. The immunochemical properties of these sera are partly published. The specific sera used in this example have been designed for scrapie diagnosis, however, guidance can be found in the below given indications for the development of sera that are applicable in diagnosis of the other SEs, provided one selects the sequences as corresponding to the species specific sequence of the prion protein. When needed one may select other animals than rabbits to generate the specific sera.

[0035] 1: the sera have been induced with synthetic peptides with sequences based on the sequence of PrP protein.

[0036] 2: the sera have been induced in rabbits.

[0037] 3: the peptides sequences have such differences with the rabbit PrP sequence that they induced not only antibodies which recognized these peptides but also the authentic PrP protein.

[0038] 4: the peptides used for immunization are kept short (12 mers); this shortness is supposed to have a critical role in the high specificity for the scrapie forms of PrP and thus in the binding in the tissue sections even after harsh denaturing and degradative treatments.

[0039] 5: the sequences used for immunization and yielding the specific scrapie PrP staining were selected from the protease K resistant domain of the PrP^{Sc}.

[0040] 6: the sera of use in the diagnostic IHC are also well reactive in other immunochemical tests such as: Western blotting of both PrP^C and PrP^{Sc}, ELISA with PrP protein, PEPSCAN with 12 mer peptides with overlapping sequences of sheep PrP.

[0041] 7: the peptides selected have properties (hydrophilicity, flexibility, surface occurrence) which are—when used for immunization—advantageous for eliciting antibodies with binding to the antigen on which the sequences have been based.

[0042] 8: the antisera elicited show the right specificity when analyzed in PEPSCAN with 12 mer peptides. The addition of a foreign dimeric glycine at either the N-terminus or the C-terminus of these peptides does not decrease the specificity of the peptides but more probably does make the immunization more effective, supposedly because it makes the peptides stand out farther away from the carrier

protein and makes them more flexible on the carrier protein properties which are important determinants in antigenicity.

[0043] 9: the sequences selected for peptide synthesis and immunization represent domains which have a low tendency to form secondary structure (α -helix or β -sheet) and are not part of the four regions described in the literature a being able to form β -sheet as synthetic peptides.

[0044] Results

[0045] Immunohistochemical Testing of Antipeptide Antisera.

[0046] An identical and distinct immunolabelling pattern was detected with all anti-peptide antisera in the lymphoid tissues of scrapie-affected sheep. Because the five antisera were directed against different epitopes of the PrP protein, cross-reactivity of the anti-peptide antisera with another protein can be excluded. We therefore classified the immunolabelled protein as PrP. We further defined this PrP as scrapie-associated PrP (PrP^{Sc}), because no PrP immunoreactivity was seen in any of the lymphoid tissues of scrapie-negative sheep. Replacing the anti-peptide antisera with pre-immune sera did not result in any immunolabelling.

[0047] Localization of PrP^{Sc} in the Lymphoid Tissues.

[0048] PrP^{Sc} was located within the primary and secondary lymphoid follicles of the spleen, palatine tonsil, lymph nodes, and solitary follicles or Peyer's patches of the ileum. The PrP^{Sc} immunolabelling pattern consisted of a reticular network in the center of the lymphoid follicle which varied in staining intensity. Apart from this network, fine to coarse granules of PrP^{Sc} were seen in the cytoplasm of non-lymphoid cells within the follicle. Several of these cells were identified as macrophages because of the simultaneous presence of ceroid/lipofuscin pigment in their cytoplasm. No immunolabelling of the B lymphocytes in the lymphoid follicle was seen.

[0049] Occasionally, additional immunolabelling was found in specific cells and regions of the lymphoid tissues. In the spleen, individual cells in the periarterial lymphatic sheath (PALS) and the marginal zone surrounding the splenic corpuscles contained granules of PrP^{Sc} sometimes combined with ceroid/lipofuscin pigment within the cytoplasm. No PrP^{Sc} was seen in the red pulp of the spleen. In the palatine tonsil and ileum, branches or granules of PrP^{Sc} were found interspersed between the lymphocytes of the dome area between the follicles and the crypt epithelium. In the lymph nodes, granules of PrP^{Sc} were seen between the lymphocytes of the paracortex.

[0050] Distribution of PrP^{Sc} in Lymphoid Tissues.

[0051] PrP^{Sc} was detected in 54 (98%) of the 55 scrapie-affected sheep in the spleen, tonsil, retropharyngeal lymph node and mesenteric lymph node. In the tracheobronchial, prefemoral and prescapular lymph node, PrP^{Sc} was seen in a slightly lower percentage of the sheep (table 1). PrP^{Sc} was found in solitary lymphoid follicles or Peyer's patches of the ileum in 24 (89%) of the 27 sheep in which lymphoid tissue was present in the sections of the ileum. In only 1 of the 55 scrapie-affected sheep, PrP^{Sc} could not be detected in any of the lymphoid tissues.

[0052] The percentage of lymphoid follicles that contained PrP^{Sc} was estimated for the sections of the spleen, tonsil and lymph nodes. In the palatine tonsil of 98% of the scrapie-affected sheep, over 60% of the lymphoid follicles contained PrP^{Sc}. In the tonsils of 93% of the sheep with scrapie the percentage of PrP^{Sc}-positive lymphoid follicles even exceeded 80%. In the spleen or lymph nodes, PrP^{Sc} accumulation in more than 60% of the lymphoid follicles was only present in less than 30% of the sheep.

[0053] Immunohistochemical detection of prion protein in lymphoid tissues of sheep with pre-clinical cases of natural scrapie.

[0054] Material and Methods

[0055] Sheep.

[0056] We selected a group of 10 purposely bred lambs, six of them homozygous for the PrP allele with valine (V) at position 136 and glutamine (Q) at position 171. In several breeds, this PrP^{VQ} allele is significantly associated with an increased susceptibility for scrapie (Belt et al., 1995). The remaining four lambs were heterozygous and possessed one PrP^{VQ} allele and one PrP^{AR} allele (alanine at position 136 and arginine at position 171). The PrP^{AR} allele is significantly associated with increased resistance of sheep for scrapie. In a flock with natural scrapie we observed that sheep with the genotype PrP^{VQ/VQ} died from scrapie at approximately 25 months of age and that the majority of the sheep with the genotype PrP^{VQ/AR} were still healthy at 70 months of age. Since we expected that the PrP^{VQ/VQ} sheep would almost certainly develop clinical signs of scrapie within approximately 25 months after birth and that the PrP^{VQ/AR} sheep would stay healthy, we regarded these two groups of sheep as a suitable model to study changes at known stages of the incubation period. All 10 sheep were born and raised on the same farm, in an environment where scrapie has been occurring for several years. They were kept here until six months old, when they were transferred to our Institute, to a paddock where various scrapie positive animals had spent their last days.

[0057] Sampling and Testing of Tonsils of the Live Animal

[0058] Tonsil biopsies were collected under general anaesthesia, which was achieved by intravenous application of a combination of Ketalar (Ketamine-HCl) 4 mg/kg, Xylazine (Rompun) 0.05 mg/kg and Atropine 0.1 mg/kg. We used a mouth gag, a laryngoscope, and a biopsy forceps with a head of approximately 4 mm in diameter. Tonsils in sheep are not as readily accessible as in some other species, such as man, where they often protrude into the pharyngeal lumen. In sheep, they are hidden, surrounding a small cavity. It proved, however, sufficient to take a biopsy of the edge of the entrance to this cavity, the fossa tonsillaris, thereby collecting in general sufficient material (follicles!) to allow examination. Some experience in the technique was obtained by collecting, just before the animals were euthanized, tonsillar biopsies from 11 sheep, among them clinically affected scrapie sheep. Histological procedures included immunostaining with specific (anti-PrP^{Sc}) anti-peptide-sera, as described above and in Van Keulen et al., 1995. From the 11 sheep, eight proved to be scrapie positive while three turned out negative, as was confirmed histologically and by IHC of brain tissue during post mortem examination. The tonsillar biopsies of all eight positive

animals showed a positive immune-staining in the IHC, whereas no immuno-staining could be detected in the three negative cases.

[0059] In the actual experiment, we planned to take tonsillar biopsies sequentially, at regular intervals and starting at an age of six months. For logistic reasons this was delayed. We collected biopsies from both groups for the first time at approximately 10 months after birth, when none of the sheep showed clinical signs of scrapie. The youngest sheep were nine-and-half months, the oldest sheep was 10 months and one week.

[0060] Results

[0061] After IHC-staining we found clear, already extensive, PrP^{Sc} staining in the tonsillar biopsies of all six susceptible PrP^{VQ/VQ} sheep, whereas no immune-staining was detectable in the tonsillar biopsies of any of the resistant PrP^{VQ/AR} sheep. We have thus detected the scrapie associated PrP^{Sc} in tonsils of 10 months old sheep, which is at less than half-way the incubation period as they are expected to develop scrapie when approximately 25 months old. In sheep that are expected to develop scrapie at a much later stage or stay healthy during their whole life span, we did not detect this PrP^{Sc} protein. We conclude that IHC-staining and related methods provide the possibility for pre-clinical diagnosis in sheep scrapie as well as for other SEs like BSE -and CJD.

REFERENCES

- [0062] Belt, P. B. G. M., I. H. Muileman, B. E. C. Schreuder, J. Bos-de Ruijter, A. L. J. Gielkens, and M. A. Smits. 1995. Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. *J. Gen. Virol.* 76:509-517.
- [0063] Fraser, H. and A. G. Dickinson. 1978. Studies of the lymphoreticular system in the pathogenesis of scrapie: the role of spleen and thymus. *J. Comp. Pathol.* 88:563-573.
- [0064] Hadlow, W. J., R. C. Kennedy, and R. E. Race. 1982. Natural infection of Suffolk sheep with scrapie virus. *J. Infect. Dis.* 146:657-664.
- [0065] Hilmert, H. and H. Diringer. 1984. A rapid and efficient method to enrich SAF protein from scrapie brains of hamsters. *Biosci. Rep.* 4:165-170.
- [0066] Ikegami, Y., M. Ito, H. Isomura, E. Momotani, K. Sasaki, Y. Muramatsu, N. Ishiguro, and M. Shinagawa. 1991. Pre-clinical and clinical diagnosis of scrapie by detection of PrP protein in tissues of sheep. *Vet. Rec.* 128:271-275.
- [0067] Mohri, S., C. F. Farquhar, R. A. Somerville, M. Jeffrey, J. Foster, and J. Hope. 1992. Immunodetection of a disease specific PrP fraction in scrapie-affected sheep and BSE-affected cattle. *Vet. Rec.* 131:537-539.
- [0068] Muramoto, T., T. Kitamoto, J. Tateishi, and I. Goto. 1993. Accumulation of abnormal prion protein in mice infected with Creutzfeldt-Jakob disease via intraperitoneal route: a sequential study. *Am. J. Pathol.* 143:1470-1479.
- [0069] van Keulen, L. J. M., B. E. C. Schreuder, R. H. Meoien, G. Mooij-Harkes, M. Poelen-van den Berg, M. E. W. Vromans, and J. Langeveld. 1995. Immunohistochemical detection and localization of prion protein in brain tissue of sheep with natural scrapie. *Vet. Pathol.* 32:299-308.
- [0070] Brown, P. Current opinion in Hematology 2, 472-477 (1995)
- [0071] Brugère H., Banissi C., Brugere-Picoux J., Chatelain J. and Buvet R. Recherche d'un témoin biochimique urinaire de l'infection du mouton par la tremblante. *Bull acad vet de France* 1991; 64: 139-45.
- [0072] Diringer H., Gelderblom H., Hilmert H, Ozel M., Edelblut C., and Kimberlin R H. Scrapie infectivity, fibrils and low molecular weight protein. *Nature* 1983; 306: 476-8.
- [0073] Doi S., Ito M., Shinagawa M., Sato G., Isomura H, and Goto H. Western blot detection of scrapie-associated fibril protein in tissues outside the central nervous system from preclinical scrapie-infected mice. *J gen Virol* 1988; 69:955-60.
- [0074] Fraser H. Bruce M E, and McConnell I. Murine scrapie strains, BSE models and genetics. In: Sub-Acute spongiform Encephalopathies. Procs of a Seminar in CEC Agricultural Research Programme, Brussels, 12-14 November 1990 (Bradley R., Savey M., and Marchant B A, eds.). Kluwer Academic Publishers, Dordrecht, The Netherlands 1991; 131-6
- [0075] Hadlow W J, Kennedy R C and Race R E. Natural infection of Suffolk sheep with scrapie virus. *J InfDis* 1982; 146: 657-64
- [0076] Hadlow W J, Kennedy R C, Race R E and Eklund C M. Virologic and histologic findings in dairy goats affected with natural scrapie. *Vet Pathol* 1980; 17: 187-99.
- [0077] Fraser H, McConnell I, Wells GAH, and Dawson M. Transmission of bovine spongiform encephalopathy to mice. *Vet Rec* 1988; 123: 472.
- [0078] Meiner Z., Halimi M., Polakiewicz R D, Prusiner S B and Gabizon R. Presence of prion protein in peropheral tissues of Libyan Jews with Creutzfeld-Jacob disease. *Neurology* 1992; 42, 1355-1360
- [0079] Schreuder B. Animal spongiform encephalopathies—an update. Part 1. Scrapie and lesser known animal spongiform encephalopathies. *Vet Quart.* 1994; 16, 174-181
- [0080] Schreuder B. Animal spongiform encephalopathies—an update. Part 2. Bovine spongiform encephalopathy. *Vet Quart.* 1994; 16, 182-192
- [0081] Muramatsu Y., Onodera A., Horiuchi M., Ishigano N. and Shinagawa M. Detection of PrP^{Sc} in sheep at the preclinical stage of scrapie and its significance for diagnosis of insidious infection. *Arch. Virol* 134; 427-432 (1993)

 SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 8

<210> SEQ ID NO 1

<211> LENGTH: 256

<212> TYPE: PRT

<213> ORGANISM: *Ovis aries*

<400> SEQUENCE: 1

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
 1 5 10 15

Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
 20 25 30

Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
 35 40 45

Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Trp Gly Gln Pro His
 50 55 60

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
 65 70 75 80

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly
 85 90 95

Gly Ser His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met
 100 105 110

Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu
 115 120 125

Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe
 130 135 140

Gly Asn Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr
 145 150 155 160

Pro Asn Gln Val Tyr Tyr Arg Pro Val Asp Arg Tyr Ser Asn Gln Asn
 165 170 175

Asn Phe Val His Asp Cys Val Asn Ile Thr Val Lys Gln His Thr Val
 180 185 190

Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys Ile
 195 200 205

Met Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln Tyr Gln Arg Glu
 210 215 220

Ser Gln Ala Tyr Tyr Gln Arg Gly Ala Ser Val Ile Leu Phe Ser Ser
 225 230 235 240

Pro Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
 245 250 255

<210> SEQ ID NO 2

<211> LENGTH: 264

<212> TYPE: PRT

<213> ORGANISM: *Bos taurus*

<400> SEQUENCE: 2

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
 1 5 10 15

Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
 20 25 30

Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
 35 40 45

-continued

Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His
 50 55 60
 Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
 65 70 75 80
 Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
 85 90 95
 Gly Gly Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys
 100 105 110
 Pro Ser Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala
 115 120 125
 Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala
 130 135 140
 Met Ser Arg Pro Leu Ile His Phe Gly Ser Asp Tyr Glu Asp Arg Tyr
 145 150 155 160
 Tyr Arg Glu Asn Met Tyr Arg Tyr Pro Asn Gln Val Tyr Tyr Arg Pro
 165 170 175
 Val Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His Asp Cys Val Asn
 180 185 190
 Ile Thr Val Lys Glu His Thr Val Thr Thr Thr Thr Lys Gly Glu Asn
 195 200 205
 Phe Thr Glu Thr Asp Ile Lys Met Met Glu Arg Val Val Glu Gln Met
 210 215 220
 Cys Ile Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr Gln Arg Gly
 225 230 235 240
 Ala Ser Val Ile Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser
 245 250 255
 Phe Leu Ile Phe Leu Ile Val Gly
 260

<210> SEQ ID NO 3
 <211> LENGTH: 257
 <212> TYPE: PRT
 <213> ORGANISM: Mustela sp.

<400> SEQUENCE: 3

Met Val Lys Ser His Ile Gly Ser Trp Leu Leu Val Leu Phe Val Ala
 1 5 10 15
 Thr Trp Ser Asp Val Gly Phe Cys Lys Lys Arg Pro Lys Pro Gly Gly
 20 25 30
 Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
 35 40 45
 Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His
 50 55 60
 Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
 65 70 75 80
 Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly
 85 90 95
 Gly Gly Ser His Ser Gln Trp Gly Lys Pro Ser Lys Pro Lys Thr Asn
 100 105 110
 Met Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly
 115 120 125
 Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His

-continued

35			40			45									
Tyr	Pro	Pro	Gln	Gly	Gly	Gly	Thr	Trp	Gly	Gln	Pro	His	Gly	Gly	Gly
	50					55						60			
Trp	Gly	Gln	Pro	His	Gly	Gly	Gly	Trp	Gly	Gln	Pro	His	Gly	Gly	Gly
	65				70					75					80
Trp	Gly	Gln	Pro	His	Gly	Gly	Gly	Trp	Gly	Gln	Gly	Gly	Gly	Thr	His
				85					90					95	
Asn	Gln	Trp	Asn	Lys	Pro	Ser	Lys	Pro	Lys	Thr	Asn	Met	Lys	His	Met
			100					105					110		
Ala	Gly	Ala	Ala	Ala	Ala	Gly	Ala	Val	Val	Gly	Gly	Leu	Gly	Gly	Tyr
		115					120					125			
Met	Leu	Gly	Ser	Ala	Met	Ser	Arg	Pro	Met	Met	His	Phe	Gly	Asn	Asp
	130					135					140				
Trp	Glu	Asp	Arg	Tyr	Tyr	Arg	Glu	Asn	Met	Asn	Arg	Tyr	Pro	Asn	Gln
	145				150					155					160
Val	Tyr	Tyr	Arg	Pro	Val	Asp	Gln	Tyr	Asn	Asn	Gln	Asn	Asn	Phe	Val
			165						170					175	
His	Asp	Cys	Val	Asn	Ile	Thr	Ile	Lys	Gln	His	Thr	Val	Thr	Thr	Thr
			180					185					190		
Thr	Lys	Gly	Glu	Asn	Phe	Thr	Glu	Thr	Asp	Ile	Lys	Ile	Met	Glu	Arg
		195					200					205			
Val	Val	Glu	Gln	Met	Cys	Thr	Thr	Gln	Tyr	Gln	Lys	Glu	Ser	Gln	Ala
	210					215					220				
Tyr	Tyr	Asp	Gly	Arg	Arg	Ser	Ser	Ala	Val	Leu	Phe	Ser	Ser	Pro	Pro
	225				230					235					240
Val	Ile	Leu	Leu	Ile	Ser	Phe	Leu	Ile	Phe	Leu	Met	Val	Gly		
			245					250							

<210> SEQ ID NO 7

<211> LENGTH: 254

<212> TYPE: PRT

<213> ORGANISM: Murinae gen. sp.

<400> SEQUENCE: 7

Met	Ala	Asn	Leu	Gly	Tyr	Trp	Leu	Leu	Ala	Leu	Phe	Val	Thr	Met	Trp
	1			5					10					15	
Thr	Asp	Val	Gly	Leu	Cys	Lys	Lys	Arg	Pro	Lys	Pro	Gly	Gly	Trp	Asn
		20						25					30		
Thr	Gly	Gly	Ser	Arg	Tyr	Pro	Gly	Gln	Gly	Ser	Pro	Gly	Gly	Asn	Arg
		35				40						45			
Tyr	Pro	Pro	Gln	Gly	Gly	Thr	Trp	Gly	Gln	Pro	His	Gly	Gly	Gly	Trp
	50					55					60				
Gly	Gln	Pro	His	Gly	Gly	Ser	Trp	Gly	Gln	Pro	His	Gly	Gly	Ser	Trp
	65				70					75					80
Gly	Gln	Pro	His	Gly	Gly	Gly	Trp	Gly	Gln	Gly	Gly	Gly	Thr	His	Asn
			85						90					95	
Gln	Trp	Asn	Lys	Pro	Ser	Lys	Pro	Lys	Thr	Asn	Leu	Lys	His	Val	Ala
			100					105					110		
Gly	Ala	Ala	Ala	Ala	Gly	Ala	Val	Val	Gly	Gly	Leu	Gly	Gly	Tyr	Met
		115					120				125				
Leu	Gly	Ser	Ala	Met	Ser	Arg	Pro	Met	Ile	His	Phe	Gly	Asn	Asp	Trp
	130					135					140				

-continued

Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr Pro Asn Gln Val
 145 150 155 160
 Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His
 165 170 175
 Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr Thr
 180 185 190
 Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg Val
 195 200 205
 Val Glu Gln Met Cys Val Thr Gln Tyr Gln Lys Glu Ser Gln Ala Tyr
 210 215 220
 Tyr Asp Gly Arg Arg Ser Ser Ser Thr Val Leu Phe Ser Ser Pro Pro
 225 230 235 240
 Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
 245 250

<210> SEQ ID NO 8
 <211> LENGTH: 226
 <212> TYPE: PRT
 <213> ORGANISM: Rattus rattus

<400> SEQUENCE: 8

Gly Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro
 1 5 10 15
 Gly Gly Asn Arg Tyr Pro Pro Gln Ser Gly Gly Thr Trp Gly Gln Pro
 20 25 30
 His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro
 35 40 45
 His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Ser Gln Gly
 50 55 60
 Gly Gly Thr His Asn Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn
 65 70 75 80
 Leu Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly
 85 90 95
 Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Met Leu His
 100 105 110
 Phe Gly Asn Asp Trp Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg
 115 120 125
 Tyr Pro Asn Gln Val Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln
 130 135 140
 Asn Asn Phe Val His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr
 145 150 155 160
 Val Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys
 165 170 175
 Met Met Glu Arg Val Val Glu Gln Met Cys Val Thr Gln Tyr Gln Lys
 180 185 190
 Glu Ser Gln Ala Tyr Tyr Asp Gly Arg Arg Ser Ser Ala Val Leu Phe
 195 200 205
 Ser Ser Pro Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile
 210 215 220
 Val Gly
 225

What is claimed is:

1. A method for the immunological detection of prion disease, said method comprising:

extracting tissue from an animal; and

screening for prion disease, said screening comprising detecting aberrant prion proteins in the tissue extracted from the animal with an antibody induced against an epitope of said aberrant prion protein, but not an epitope of a normal prion protein.

2. A method for the immunological detection of prion disease, said method comprising:

collecting tissue from an animal;

inducing production of an antibody with specificity against an epitope of an aberrant prion protein, but not an epitope of a normal prion protein; and

screening the tissue for the presence of an aberrant prion protein with said antibody;

wherein said antibody is also reactive in an immunochemical test when analyzed with 12 mer peptides with overlapping sequences of sheep PrP.

3. A diagnostic test for performing the method according to claim 1, comprising the antibody induced against the epitope of the aberrant prion protein.

* * * * *

专利名称(译)	检测朊病毒病的方法		
公开(公告)号	US20050048582A1	公开(公告)日	2005-03-03
申请号	US10/949880	申请日	2004-09-24
[标]申请(专利权)人(译)	CORNELIS SCHREUDER BRAM EDWARD VAN KEULEN LUCIUS JOHANNES MATTHEUS WILHELMINA VROMANS MARIA ELISABETH MARIA LANGEVELD JOHANNES PIETER SMITS MARINUS ADRIANUS		
申请(专利权)人(译)	CORNELIS SCHREUDER BRAM EDWARD VAN KEULEN LUCIUS JOHANNES MATTHEUS WILHELMINA VROMANS MARIA ELISABETH MARIA LANGEVELD JOHANNES PIETER SMITS MARINUS ADRIANUS		
当前申请(专利权)人(译)	CORNELIS SCHREUDER BRAM EDWARD VAN KEULEN LUCIUS JOHANNES MATTHEUS WILHELMINA VROMANS MARIA ELISABETH MARIA LANGEVELD JOHANNES PIETER SMITS MARINUS ADRIANUS		
[标]发明人	CORNELIS SCHREUDER BRAM EDWARD VAN KEULEN LUCIUS JOHANNES MATTHEUS WILHELMINA VROMANS MARIA ELISABETH MARIA LANGEVELD JOHANNES PIETER SMITS MARINUS ADRIANUS		
发明人	CORNELIS SCHREUDER, BRAM EDWARD VAN KEULEN, LUCIUS JOHANNES MATTHEUS WILHELMINA VROMANS, MARIA ELISABETH MARIA LANGEVELD, JOHANNES PIETER SMITS, MARINUS ADRIANUS		
IPC分类号	G01N33/53 G01N33/569 G01N33/68		
CPC分类号	G01N33/6896 G01N2800/2828 G01N2333/47		
优先权	1996200917 1996-04-03 EP 09/155794 1999-05-20 US		
其他公开文献	US7211401		
外部链接	Espacenet USPTO		

摘要(译)

本发明提供了检测朊病毒疾病的方法，例如绵羊的瘙痒病，牛的牛海绵状脑病，人的Creutzfeld-Jacob病，其中在可从活体动物取样的组织中检测到异常蛋白质或朊病毒蛋白质。

Figure 1 Multiple sequence alignment of prion proteins of various origin.

Perfectly conserved: '*' Disulfide-bond: 'S'
 Well conserved: '.' N-Glycosylation: 'N'
 -----sign-----

SHPRP	IVKSHIGSWILVLFVAVGVDVGLCKRRKPKGGWNTGGSRYPGGSPGGN	50
BTFRP	IVKSHIGSWILVLFVAVGVDVGLCKRRKPKGGWNTGGSRYPGGSPGGN	50
HNKPRP	IVKSHIGSWILVLFVAVGVDVGLCKRRKPKGGWNTGGSRYPGGSPGGN	50
GORPRP	M--ANLGVWLLVLFVAVGVDVGLCKRRKPKGG--WNTGGSRYPGGSPGGN	47
HSRPP	M--ANLGVWLLVLFVAVGVDVGLCKRRKPKGG--WNTGGSRYPGGSPGGN	47
NSRPP	M--ANLGVWLLVLFVAVGVDVGLCKRRKPKGG--WNTGGSRYPGGSPGGN	47
MRPRP	M--ANLGVWLLVLFVAVGVDVGLCKRRKPKGG--WNTGGSRYPGGSPGGN	47
RRPRPCGNRTGGSRYPGGSPGGN	19*

SHPRP	RVPFGGGGWCQPHGGGWQCPHGGGWQCPHGGGWQCPHGGG	90
BTFRP	RVPFGGGGWCQPHGGGWQCPHGGGWQCPHGGGWQCPHGGG	100
HNKPRP	RVPFGGGGWCQPHGGGWQCPHGGGWQCPHGGGWQCPHGGG	96
GORPRP	RVPFGGGGWCQPHGGGWQCPHGGGWQCPHGGGWQCPHGGG	88
HSRPP	RVPFGGGGWCQPHGGGWQCPHGGGWQCPHGGGWQCPHGGG	88
NSRPP	RVPFGGGGWCQPHGGGWQCPHGGGWQCPHGGGWQCPHGGG	88
MRPRP	RVPFGGG--TWQPHGGGWQCPHGGGWQCPHGGGWQCPHGGG	87
RRPRP	RVPFGGGGWCQPHGGGWQCPHGGGWQCPHGGGWQCPHGGG	60*

SHPRP	WGQGG-SHSQWPKPSKPKTMMKIVAGAAAAGAVVGLGCVHLGSAMSRPL	141
BTFRP	WGQGG-THSQWPKPSKPKTMMKIVAGAAAAGAVVGLGCVHLGSAMSRPL	149
HNKPRP	WGQGGSHGQWPKPSKPKTMMKIVAGAAAAGAVVGLGCVHLGSAMSRPL	142
GORPRP	WGQGGTSHSQWPKPSKPKTMMKIVAGAAAAGAVVGLGCVHLGSAMSRPL	138
HSRPP	WGQGGTSHSQWPKPSKPKTMMKIVAGAAAAGAVVGLGCVHLGSAMSRPL	138
NSRPP	WGQGGTSHSQWPKPSKPKTMMKIVAGAAAAGAVVGLGCVHLGSAMSRPL	138
MRPRP	WGQGGTSHSQWPKPSKPKTMMKIVAGAAAAGAVVGLGCVHLGSAMSRPL	137
RRPRP	WGQGGTSHSQWPKPSKPKTMMKIVAGAAAAGAVVGLGCVHLGSAMSRPL	110*

SHPRP	IHFSDVIEDRYVENMRYFNQVYVRFVDVYGNQNFVHDCVITIKQHT	191
BTFRP	IHFSDVIEDRYVENMRYFNQVYVRFVDVYGNQNFVHDCVITIKQHT	199
HNKPRP	IHFSDVIEDRYVENMRYFNQVYVRFVDVYGNQNFVHDCVITIKQHT	192
GORPRP	IHFSDVIEDRYVENMRYFNQVYVRFVDVYGNQNFVHDCVITIKQHT	188
HSRPP	IHFSDVIEDRYVENMRYFNQVYVRFVDVYGNQNFVHDCVITIKQHT	188
NSRPP	IHFSDVIEDRYVENMRYFNQVYVRFVDVYGNQNFVHDCVITIKQHT	187
MRPRP	IHFSDVIEDRYVENMRYFNQVYVRFVDVYGNQNFVHDCVITIKQHT	160*
RRPRP*

SHPRP	VTTTRGENFTETDIKMEHVVVEQMCITQVREGQAAYQ--RGSVILFS	239
BTFRP	VTTTRGENFTETDIKMEHVVVEQMCITQVREGQAAYQ--RGSVILFS	247
HNKPRP	VTTTRGENFTETDIKMEHVVVEQMCITQVREGQAAYQ--RGSVILFS	240
GORPRP	VTTTRGENFTETDIKMEHVVVEQMCITQVREGQAAYQ--RGSVILFS	236
HSRPP	VTTTRGENFTETDIKMEHVVVEQMCITQVREGQAAYQ--RGSVILFS	237
NSRPP	VTTTRGENFTETDIKMEHVVVEQMCITQVREGQAAYQ--RGSVILFS	237
MRPRP	VTTTRGENFTETDIKMEHVVVEQMCITQVREGQAAYQ--RGSVILFS	237
RRPRP	VTTTRGENFTETDIKMEHVVVEQMCITQVREGQAAYQ--RGSVILFS	209*