別紙 3-3

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一般的名称			研究報告の公表状況	Scrapie agent (strain 263K) can transmit disease via the oral route after persistence in soil over years. Seidel, B. et al., PLoS One, Issue 5, May 2007.	公表国 ドイツ	
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研究報告の概要	使用上の注意記載状況・その他参考事項等
ヒツジのスクレイピー自然伝播において，汚染された土壌が最も可能性の高い感染源の 1 つであると長い間疑われている。本試験では，環境における病原性のプリオン蛋白（PrP）の経時的な残留程度を測定し，シリアンハムスターにおける経口経路の伝播性をバイオアッセイにより検討した。これを実施するため，スクレイピー感染ハムスターの脳ホモジネートを混ぜた土壌検体をガーゼのバッグに入れ，15〜20 cm の深さに埋めた。埋めた後に様々な時点において検体を掘り出し，ウェスタンブロット法により蛋白質分解酵素耐性 PrP^{Sc} の有無を調べた。その結果，異常な PrP は土壌中に残留し，29 ヵ月経過後にウェスタンブロット法によって検出されたことが示されたが，抽出量は経時的に低下した。21 ヵ月間土壌中に存在した折りたたみ異常プリオン蛋白は，蛋白折りたたみ異常反復増幅（PMCA）反応により蛋白分解酵素耐性 PrP の増幅を促進することができた。抽出された PrP はその生物学的活性を保持しており，131 日以内の感染土壌を摂餌した全てのシリアンハムスターおいてスクレイピーが誘発した。死亡時，これらの動物の脳には大量の異常な PrP が含まれていたことが示された。汚染された土壌の水溶性の抽出物も一部の動物でスクレイピーを引き起こしたが，この場合の潜伏期間は長かった。土壌から抽出された病原性 PrP に対して PMCA 反応を用いることができたことにより，環境におけるプリオン検出に新たな道が開かれると考えられる。	BYL-2007-0288

報告企業の意見	今後の対応
スクレイピー物質は土壌中では長期にわたり比較的安定しており，ハムスターでの経口感染の可能性が明らかになっている。さらに，このメカニズムはシカの慢性消耗性疾患などその他のプリオン病の自然伝播にも関連している可能性がある。様々な感染源から，PMCA 反応は異常 PrP の検出に非常に有用であることが証明できるかもしれない。	現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。

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Scrapie Agent (Strain 263K) Can Transmit Disease via the Oral Route after Persistence in Soil over Years

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The persistence of infectious biomolecules in soil constitutes a substantial challenge. This holds particularly true with respect to prions, the causative agents of transmissible spongiform encephalopathies (TSEs) such as scrapie, bovine spongiform encephalopathy (BSE), or chronic wasting disease (CWD). Various studies have indicated that prions are able to persist in soil for years without losing their pathogenic activity. Dissemination of prions into the environment can occur from several sources, e.g., infectious placenta or amniotic fluid of sheep. Furthermore, environmental contamination by saliva, excrements or non-sterilized agricultural organic fertilizer is conceivable. Natural transmission of scrapie in the field seems to occur via the alimentary tract in the majority of cases, and scrapie-free sheep flocks can become infected on pastures where outbreaks of scrapie had been observed before. These findings point to a sustained contagion in the environment, and notably the soil. By using outdoor lysimeters, we simulated a contamination of standard soil with hamster-adapted 263K scrapie prions, and analyzed the presence and biological activity of the soil-associated PrP^{Sc} and infectivity by Western blotting and hamster bioassay, respectively. Our results showed that 263K scrapie agent can persist in soil at least over 29 months. Strikingly, not only the contaminated soil itself retained high levels of infectivity, as evidenced by oral administration to Syrian hamsters, but also feeding of aqueous soil extracts was able to induce disease in the reporter animals. We could also demonstrate that PrP^{Sc} in soil, extracted after 21 months, provides a catalytically active seed in the protein misfolding cyclic amplification (PMCA) reaction. PMCA opens therefore a perspective for considerably improving the detectability of prions in soil samples from the field.

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INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) comprise a group of fatal neurodegenerative diseases such as bovine spongiform encephalopathy (BSE) in cows [1], chronic wasting disease (CWD) in deer (*Odocoileus* spp.) and elk (*Cervus elaphus nelsoni*) [2–4], scrapie in sheep and goats [5–7] and Creutzfeldt-Jakob disease (CJD) in humans [1]. The exact molecular pathomechanisms underlying TSEs have not yet been fully elucidated but it is generally accepted that a pathologically misfolded and/or aggregated isoform of the normal cellular prion protein (PrP), referred to as PrP^{Sc} and PrP^{C}, respectively, is the key pathogenic factor for this group of diseases [1].

Among the known TSEs, only scrapie and CWD are contagious diseases which show horizontal transmissibility under natural conditions [2,4,8]. CWD is the only TSE known to affect free-ranging animals [3]. The regular occurrence of scrapie in affected areas [7] and the spread of CWD in North America and Korea [9,10] among mule deer, white-tailed deer and elk indicates that a contagion in the environment is responsible for the occurrence of these TSEs [4,11–14], and even raises the possibility of a cross-species transmission under natural conditions. Recent findings demonstrated that saliva from deer with CWD harbours infectivity and can transmit this TSE upon peroral uptake [15]. Other studies pointed to transmission of scrapie among sheep by vectors like mites, fly larvae or other ectoparasites [16–19]. It has also been hypothesized that sporadically occurring TSEs may be induced by insecticides or by a disproportion of manganese and copper in soil leading to an enrichment of manganese in animals [20,21]. Alternatively, an influence of the Fe/Mn ratio in forage has been discussed in connection with TSEs [22]. However, on the balance of all evidence available so far, contaminated soil appears as one of the most likely sources of infection in the natural transmission of scrapie and possibly also CWD. It has been known for decades that sheep can become infected with scrapie while grazing on pastures where infected sheep have been kept before, and scrapie occurs often in areas where it has already occurred previously [7,8,23]. Furthermore, Brown and Gajdusek found that scrapie agent remains infectious after persisting in soil for 3 years as evidenced by intracerebral bioassay in Syrian hamsters [24]. The putative transmission of scrapie and CWD via soil is also corroborated by recent studies showing that prion infectivity binds to soil components with high affinity [25,26], thereby retaining its pathogenic biological activity [25]. Thus, soil-associated TSE agents in the environment represent a potential hazard. This holds true not least since prion infectivity exhibits an unusually pronounced resistance against both physical and

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chemical methods of inactivation, as described in detail elsewhere [27–37].

The contamination of soil with TSE infectivity can occur from several sources. Since recent studies could demonstrate that scrapie infectivity is present in various tissues and body fluids of infected animals [38,39], it has been assumed that the persistent prion protein enters the environment by contaminated excrements, birth-related tissues such as placenta, or even whole carcasses. While a similar excretion pattern appears conceivable for the CWD agent [40] which has also been found in saliva [15], the present knowledge about the BSE pathology in cattle does not argue for a significant shedding of the infectivity via faeces, urine, or during birth [41,42]. However, small ruminants infected with BSE could supposedly spread the BSE agent throughout the environment in a similar manner as known from scrapie-affected sheep or CWD-affected animals [43]. Moreover, unconventional conditions like for example the burial of animal carcasses at larger numbers as practiced for example during the foot and mouth disease outbreak in the UK in 2001 [44], may have fostered a dissemination of BSE agent in the soil or ground water. Once deposited there, all three TSE agents, BSE, scrapie, and CWD, must be assumed to persist in an infectious state for long periods of time.

So far, the oral transmission efficacy of long-term prion contaminations in soil have not been investigated. Therefore, we have studied the persistence of PrP^{Sc} in the environment over time and measured its oral transmissibility by bioassay in Syrian hamsters. With outdoor lysimeter experiments we simulated the situation on pastures using soil spiked with scrapie-infected hamster brain homogenate over a period of 29 months and analyzed the fate of the prion proteins by sensitive Western blotting and, in part, also by protein misfolding cyclic amplification (PMCA). The infectivity of such contaminated soil samples and the respective aqueous soil extracts was tested in the hamster bioassay.

RESULTS

Extraction and Recovery

In the first phase of the study several buffers – known as standard buffers for protein extraction from mammalian cells – and other solutions were tested in order to find out the optimal extraction method for prion protein from contaminated soil (see Materials and Methods). The obtained results lead us to use 1% SDS (sodium dodecyl sulphate) in sterilized water (figure 1a), which allowed detection of PrP^{Sc} in samples of German standard soil containing an amount of PrP^{Sc} that corresponded to 1.25 µg of infectious brain material (figure 1b).

Soil Incubation Experiments

The findings from our lysimeter experiments indicated a remarkable persistence of PrP^{Sc} in soil by clearly showing that - even after an incubation for 29 months - PrP^{Sc} could be still extracted from soil and detected by Western blotting. These results were confirmed by using two alternative highly sensitive Western blotting techniques. As shown in figure 2a, PrP^{Sc}, in the form of its protease-resistant core of the pathological prion protein PrP^{Sc} (PrP27-30), is still detectable in soil after 29 months. However, a decrease in the extractable concentration is obvious. The strongest decrease can be seen during the first month of incubation in the soil (figure 2a, lane 2 and 3). After three months the extractable amount of PrP27-30 remained almost constant until month 21, with small variations at month 12 and month 18 (figure 2a, lane 6 and 7). In Fig. 2a, a slightly stronger signal for

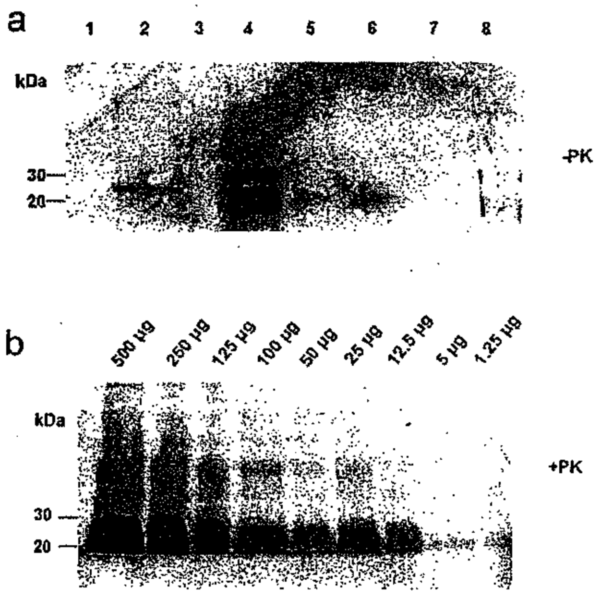

Figure 1. Western blot analysis of short-time incubation experiments. a) Western blot detection of PrP^{C} extracted from soil mixed with non-infectious brain homogenate (5% pork brain in German standard soil). Several different buffers and solutions were used for extraction. Lane 1: water; lane 2: Triton X-100; lane 3: 1% urea; lane 4: 1% SDS; lane 5: Zwittergent; lane 6: RIPA buffer; lane 7: NP-40; lane 8: Na-sarcosyl. b) Western blot detection of PrP27-30, the proteinase K-resistant core of PrP^{Sc}, extracted by using 1% SDS from soil contaminated with 263 K scrapie brain homogenate from hamsters after 1 h of incubation (dilution series). PrP^{Sc} could be detected in soil samples containing 1.25 µg or higher amounts of scrapie brain tissue after extraction with 1% SDS-solution. Samples were digested with proteinase K prior to Western blotting.
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PrP27-30 was found for 21 months (lane 8) as compared to 18 months (lane 7). Both samples were taken from the same soil bag, however, the location from where the sampling was performed may have differed. Thus, the finding possibly reflects minor inhomogenities either in the original load of contamination or in the degradation of PrP^{Sc} depending on the microenvironment of the examined sample. On the other hand, it has to be noted that the extraction yield and Western blotting efficiency inevitably may also vary in certain ranges from sample to sample. A further decrease was observed after 26 and 29 months (figure 1a, lane 9 and 10), however, the PrP27-30 specific bands remain clearly visible. Where performed, analyses of samples from different soil bags produced consistent results. In order to verify that the detected bands originated from PrP^{Sc} a control experiment was performed: After deglycosylation with PNGase F the PrP27-30 bands extracted from soil after 21 and 18 month showed an electrophoretic shift towards a single band at about 20 kDa, the molecular mass to be expected for deglycosylated PrP27-30 from 263K hamster scrapie (figures 2 b, lanes 3 and 4, respectively).

Amplification of PrP^{Sc} extracted from soil

In order to check whether PrP^{Sc} extracted from soil can be used for the amplification of protease resistant prion protein by the protein misfolding cyclic amplification (PMCA) reaction [45,46], soil extracts from month 21 were used as test seeds. As shown in figure 2c, a strong increase of the signal for PrP^{Sc} was obtained with this method: while no signal for PrP^{Sc} can be detected in

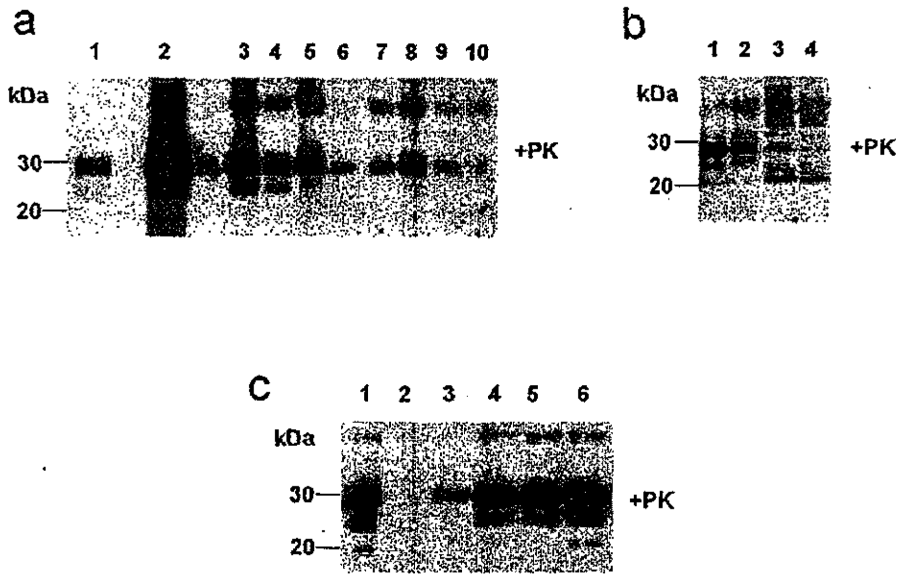

Figure 2. Western blot analysis of long-time incubation experiments. a) Western blot detection of PrP27-30 extracted from prion-contaminated soil after different time periods. Lane 1: PK-digested 263K scrapie hamster brain homogenate containing 5×10^{-7} g of brain tissue (positive control); lanes 2–10: PrP27-30 extracted at time point 0 (lane 2), after 1 month (lane 3), after 3 months (lane 4), after 6 months (lane 5), after 12 months (lane 6), after 18 months (lane 7), after 21 months (lane 8), after 26 months (lane 9) and after 29 months (lane 10). **b)** Deglycosylated PrP27-30 extracted from prion contaminated soil. Lane 1: PK-digested 263K scrapie hamster brain homogenate containing 5×10^{-7} g of brain tissue (positive control); lane 2: soil-extracted PrP27-30 after 21 months; lanes 3 and 4: deglycosylated soil-extracted PrP27-30 after 21 months (lane 3) and after 18 months (lane 4). **c)** PMCA amplification of PrP^{Sc} extracted from contaminated soil. Lane 1: PK-digested 263K scrapie hamster brain homogenate containing 5×10^{-7} g of brain tissue (positive control); lanes 2–6: sample signals after 0, 40, 80, 120 and 160 cycles of PMCA, respectively.
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samples without PMCA treatment (lane 2), clear signals became visible after 40 cycles of PMCA (lane 3) and even more intense after 80, 120 and 160 cycles of PMCA (lanes 4, 5 and 6, respectively).

Presence of PrP^{Sc} in surrounding soil samples

For analyzing the fate of the prion protein in more detail, soil samples in the vicinity of the gauze bag, as well as the gauze bag itself were analyzed by Western blotting. As shown in figure 3a, no PrP^{Sc} specific signal could be detected in the surrounding soil samples. This is indicative of PrP^{Sc} being immobilized after binding to soil compartments. In some approaches, a very faint PrP^{Sc} specific signal was visible when analyzing aliquots of SDS solution in which the gauze bag had been washed, however, it could be shown that this positive prion signal was a result of adherent soil particles (figure 3, lane 3, arrow).

Bioassay Experiments

For a detailed risk assessment of scrapie-contaminated soil it was of major importance to analyze whether the detectable PrP^{Sc} in the soil extracts still exhibited oral infectivity after incubation times up to 29 months. Therefore, a bioassay with Syrian hamsters was performed by feeding the animals with contaminated soil or aqueous soil extracts that had been collected after soil incubation for 26 and 29 months, respectively. Hamsters fed with contaminated soil exhibited first scrapie-associated symptoms at 131 ± 6 days [mean$\pm$SD] after the first application. The hamsters reached the terminal stage of scrapie at 162 ± 12 days after the first feeding (tables 1 & 2). This indicates substantial amounts of persistent infectivity in soil that had been incubated for 26 and 29 months. After reaching the terminal stage of scrapie the animals were sacrificed and analyzed for the presence of PrP^{Sc} in their brains. As shown in figure 4a (lanes 2 and 3 depicting two hamsters exemplarily), the brains of all animals in this group contained high amounts of PrP^{Sc}. In addition, comparison of the electrophoretic and glycosylation profiles of the brain samples from these hamsters (figure 4b, lanes 4 and 5) with those of hamsters challenged with different TSE isolates (figure 4b, ME7-H, lane 1; BSE-H, lane 2, 263K, lane 3) confirmed, that strain specific biochemical properties of the pathological prion protein

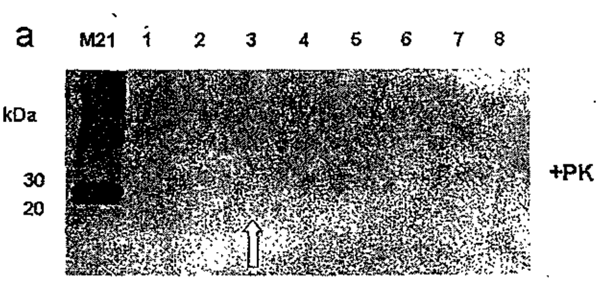

Figure 3. Western blot detection of PrP27-30 extracted from prion-contaminated soil after 21 months (M21) and respective surrounding samples. M21: contaminated soil sample inside the gauze bag; lane 1: soil sample collected outside of the steel cage; lane 2: soil sample collected directly over the gauze bag; lane 3: analysis of the empty gauze bag; lane 4: soil sample collected underneath the steel cage; lane 5: soil sample collected directly next to the gauze bag; lane 6: roots collected next to the gauze bag; lane 7: soil sample collected underneath the gauze bag; lane 8: non-contaminated soil. Arrow head at lane 3 indicates a faint PrP27-30 signal, resulting from residual soil particles that remained attached to the gauze bag.
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Table 1. Feeding schedule for bioassay in Syrian hamsters

Date	amount of soil or aqueous extract administered orally*	Collection time of soil sample
31/01/06	100 mg or 100 µl	month 26
07/02/06	100 mg or 100 µl	month 26
14/02/06	100 mg or 100 µl	month 26
21/02/06	100 mg or 100 µl	month 26
28/02/06	100 mg or 100 µl	month 26
08/03/06	100 mg or 100 µl	month 26
14/03/06	100 mg or 100 µl	month 26
21/03/06	50 mg or 50 µl	month 26
28/03/06	100 mg or 100 µl	month 29
06/04/06	100 mg or 100 µl	month 29
11/04/06	100 mg or 100 µl	month 29
20/04/06	100 mg or 100 µl	month 29

*mg relates to soil sample; µl relates to aqueous extract

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Table 2. Infection rate in hamster after oral challenge

Group	No. of animals infected with scrapie	Terminal stage of scrapie after the first oral application/Mean incubation period with the respective standard deviation in days
Control	0/5	
Control soil	0/5*	–
Control aqueous extract	0/5*	–
Contaminated soil	12/12	162 +/− 12
Contaminated aqueous soil extract	4/11*	256 +/− 41

*In the marked groups one animal each died at the beginning of the bioassay because of digestive disorders unrelated to scrapie disease

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were maintained in the animals fed with soil that had been incubated with 263K scrapie brain homogenate.

Remarkably, not only the hamsters fed with contaminated soil but also four hamsters of the group fed with aqueous soil extracts developed terminal scrapie at 256±41 days after the first application (with incubation times of individual animals ranging from 201 to 321 days). Since this bioassay is ongoing, further animals of this group might still develop scrapie in the future.

DISCUSSION

The results of this research project show for the first time that the scrapie strain 263K remains persistent in soil over a period of at least 29 months and remains highly infectious after oral application to Syrian hamsters. It has to be pointed out that the key results of our time-course study on the fate of PrP^{Sc} in soil have been validated, in part by examining blinded samples, at independent laboratories.

Only a few studies have addressed the question of a persistence of prions in soil so far [24–26,47], and the results from these studies are in principle in accordance with our observations. A pioneering study was published by Brown and Gajdusek in 1991 [24] showing that an aqueous extract from scrapie-contaminated soil remains infectious even after an incubation period of three years as confirmed by hamster bioassay. However, the infectivity studies were conducted by intracerebral injection and not by oral application. Furthermore, the PrP^{Sc} concentration was not analyzed in this study, so that no data are available about the proteins absorption behavior to soil particles and about the corresponding degradation kinetics. Most recently, PrP^{Sc} has been shown to bind to soil minerals [25] but only short-time incubation experiments of maximal one week were conducted and, again, bioassays were performed by the intracerebral route.

In this study we show by Western blotting a strong decrease in the amount of extractable PrP^{Sc} over an incubation period of 29 months in soil. It is not yet clear whether this decrease resulted from a molecular degradation of PrP^{Sc} or a tighter binding to soil particles. Stronger binding of molecules to soil particles with increasing incubation time is a well-known phenomenon in soil chemistry – the so called "aging" – and influences bioavailability and re-mobilization significantly [48,49].

Upon feeding hamsters with scrapie contaminated soil which had been incubated for over two years in outdoor lysimeters all animals developed terminal scrapie after relatively short in-

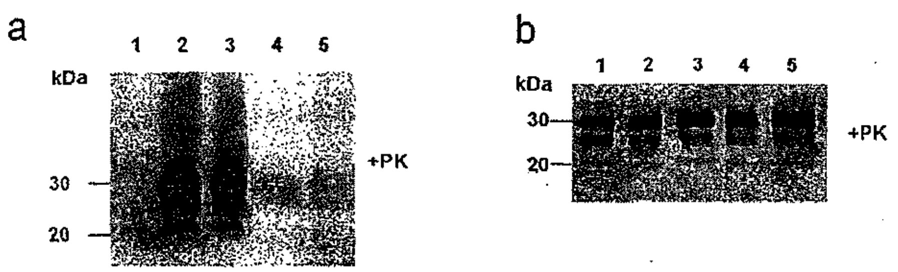

Figure 4. Western blot analysis of hamsters orally challenged with contaminated soil and western blot typing of PrP27-30. a) Western blot showing PrP^{Sc} in the brains of hamster orally challenged with contaminated soil samples and in the soil samples used for the bioassay. Lane 1: negative control hamster; lane 2: hamster H19 fed 12 times with aqueous extracts of soil samples from month 26 and 29; lane 3: hamster H4B fed 12 times with soil samples from month 26 and 29; lane 4: scrapie-contaminated soil (18 months); lane 5: scrapie-contaminated soil (21 months); lane 6: negative soil sample. b) Western blot typing of electrophoretic mobilities and glycosylation characteristics of PrP27-30 from different hamster-adapted TSE reference isolates, and from hamsters perorally challenged with 263K scrapie-contaminated soil. Lane 1: ME7-H scrapie agent; lane 2: hamster-adapted BSE-isolate (BSE-H); lane 3: 263K scrapie agent, lane 4: H10 (hamster 10, fed 12 times with soil samples from months 26 and 29); lane 5: H7 (hamster 7 fed 12 times with soil samples from month 26 and 29).

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cubation times (162 dpi). In other studies it has been well established that pure 10% (w/v) brain homogenates from 263K scrapie hamsters cause terminal scrapie in perorally challenged hamsters after mean incubation times of about 155–165 days with an attack rate of 100% [50–53]. This indicates that scrapie-contaminated soil may represent a potential TSE hazard for ruminants in the environment. While a considerable excretion of infectivity has to be assumed for scrapie or BSE infected sheep and CWD infected deer [40,43,54], it is generally acknowledged that the potential environmental contamination risk represented by BSE infected cattle is marginal, if at all present [41,42]. On the other hand, the burial of bovine carcasses [44] might have accidentally led to a spill of BSE prions into the environment. Furthermore, the fact that even feeding of aqueous extracts from scrapie-contaminated soil induced a terminal scrapie infection in four hamsters so far suggests that surface water or groundwater from pastures of scrapie-affected flocks may provide a potential source of scrapie infectivity.

However, the relevance of the results obtained in this study for the field situation should be interpreted with some caution, since only one soil type was used and only a limited number of animals were challenged in the bioassay. Therefore, other soil types and a larger number of animals have to be tested in future studies to allow for a robust risk assessment. Furthermore the exact binding properties and degradation kinetics of PrP^{Sc} should be subject to further research. In addition, all published studies addressing the persistence of prion infectivity in soil were performed with scrapie prions while TSE agents causing BSE and especially CWD have not been analyzed so far.

An intensified monitoring of PrP^{Sc} (and possibly also prion infectivity) in the soil appears mandatory for a more precise assessment of the risks emanating for humans and animals from prions in the environment. As shown in this report, PrP^{Sc} extracted from soil can be used as a catalytically active seed in the protein misfolding cyclic amplification (PMCA) reaction. This opens a promising perspective for considerably improving the detectability of prions in the environment.

MATERIALS AND METHODS

Biological Safety

Assays with scrapie-infected hamster brain were performed under laboratory conditions according to bio safety level 3** and in protected outdoor lysimeters, respectively.

Extraction of Brain Material

To identify a suitable buffer for extracting prion infectivity and PrP^{Sc} from soil, German standard soil (Lufa 2.2 and Borstel) was mixed with scrapie-infected hamster brain (strain 263K) provided by the TSE-Resource-Centre, Berkshire, Great Britain. To test the efficiency of this method the following solutions and buffers - especially non-ionic and ionic detergents - have been tested:

a) sterile water, b) 1% urea in sterile water c) 1% SDS in sterile water, d) 1% Zwittergent 3-08 in sterile water, e) 1% Triton X-100 sterile water, f) 10% Na-sarcosylate in sterile water, g) RIPA-buffer (0.25% Na-deoxycholate, 0.9% NaCl, 1% NOP-40, 0.8% Tris-HCl in sterile water (Carl Roth GmbH, Karlsruhe, Germany), h) 10% NOP-40 in sterile water, (Sigma-Aldrich, Steinheim, Germany).

Initially non-infectious pork brain was mixed with German standard soil and the efficiency of PrP^{C} extraction using the above mentioned buffers was tested. In a second step, the optimal extraction procedure was verified by applying the procedure to soil samples mixed with 263K scrapie agent and monitoring the PrP^{Sc} retrieval.

Incubation Experiments and Sampling Scheme

The outdoor experiments and all other experiments were performed using brains of terminally-diseased hamsters challenged with scrapie strain 263K.

For each approach, 1 g infectious hamster brain material was homogenized in 10 ml PBS (phosphate buffered saline) and added to 20 g of standard German sandy loom soil (Borstel). The soil/brain mixture was filled into gauze bags and buried in lysimeters filled with the same soil at a depth between 15 and 20 cm. The gauze had a mesh size of 250 µm, which enables the contact with microorganisms and meso fauna with the soil/brain mixture but avoids the contact with macro fauna derived/related organisms. To protect the gauze bags from mice, the bags were put into steel cages. At defined time points (after 0, 1, 3, 6, 12, 18, 21, 26, 29 months) the steel cages were dug out and the gauze bags as well as the surrounding soil were taken for sampling and analyzing. The analyses on the presence of residual PrP27-30 in soil samples were performed with a different number of soil bags, depending on the incubation times to be tested. For "short" incubation times of <12 months, for which detectability of residual PrP appeared most likely when the experiments were designed, samples from three different soil bags were examined. For "intermediate" incubation times of 12–18 months samples from two different bags were tested, and for "long" incubation times of >18 months one soil bag was yet available for sampling. All bags were buried in close vicinity.

Sample Preparation and Western Blot Analysis of Soil and Hamster Brain Samples

Remark: Western blot experiments were performed in independent runs at different laboratories, with each laboratory using its established techniques and procedures. The protocols are listed below. The different analytical protocols produced consistent results.

Western Protocol I

For the protein extraction from the contaminated soil as well as from surrounding soil samples, 20 ml of a 1%-SDS-solution (SDS; Sigma-Aldrich, Steinheim, Germany) were added to 20 g of testing material in a 50 ml tube. The suspension was vigorously shaken on a horizontal shaker for approx. 1 h, followed by a centrifugation step at 5,000 rpm for 20 min. 200 µl of the clear supernatant was incubated with proteinase K (50 µg/ml; 37°C; 1 h, Carl Roth GmbH, Karlsruhe, Germany) to eliminate non-resistant proteins. After digestion, the supernatants were boiled for 5 min in Laemmli's sampling buffer (50 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 50 mM ß-mercaptoethanol and 0.001% bromphenol blue) in a 1.5 ml tube and analyzed by Western blotting. The prepared samples were stored at −80°C.

For screening, Western blot samples were boiled for 5 min and separated by polyacrylamide gel electrophoresis (SDS-PAGE) by using 8–16% Tris-Glycine-SDS precast gels (i-Gels, Gradipore, LTF-Labortechnik, Wasserburg, Germany) or 4–20% precast gels (Precise Protein Gels, Perbio Science GmbH, Heidelberg, Germany) according to standard procedures as described previously [55–57]. After SDS-PAGE, the proteins were transferred to a polyvinylidene difluoride membrane (PVDF, Immobilon Millipore, Billerica, USA) using a semi-dry blotting system. Membranes were blocked in Superblock (Perbio Science GmbH, Heidelberg, Germany) with 0.05% Tween 20 over night at 4°C. Blots were incubated for 1 h in primary antibody solution (monoclonal anti-PrP 3F4, Chemicon International, Inc., California, dilution: 1:3,000 in PBS and 1.5% BSA or monoclonal anti-