Extraction and decontamination of scrapie prions

Paula Ariadna Marco Lorente[1], Nerea Larrañaga[1], Fernando López[1], Marina Betancor[1], Sonia Pérez Lázaro[1], Rosa Bolea[1], Juan J. Badiola[1], Alicia Otero[1]

[1]Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Facultad de Veterinaria, Universidad de Zaragoza — IA2, Zaragoza, España

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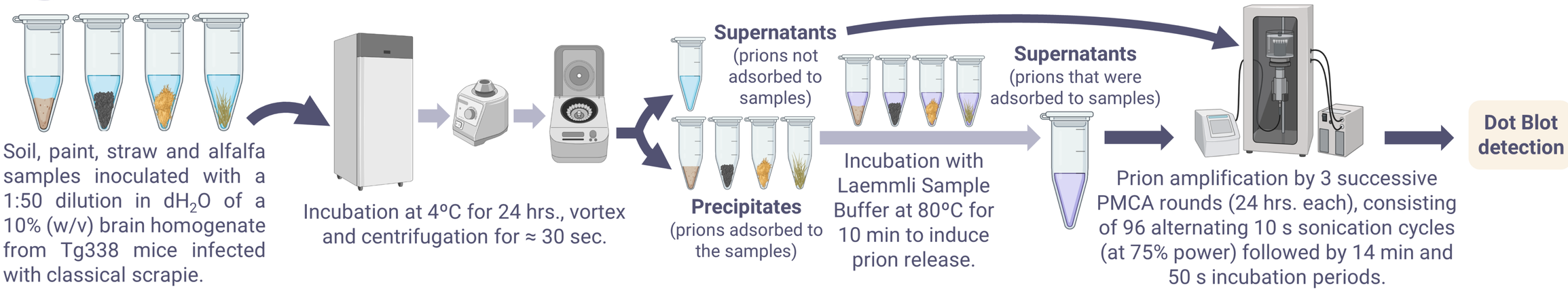
Introduction

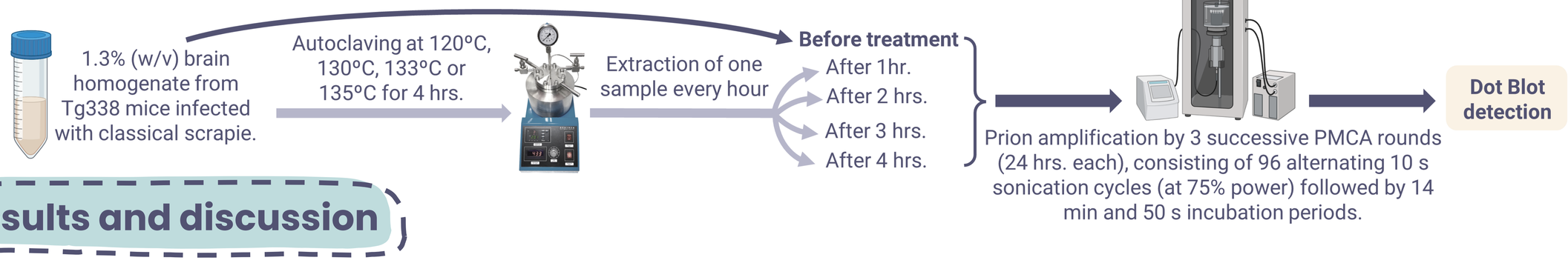
Scrapie prions can persist and retain their pathogenicity in the environment for years due to their high resistance to degradation and decontamination treatments. In fact, it has been shown that sheep can become infected with classical scrapie through contamination of the environment (pens, bedding, soil, feed, etc.), which acts as a reservoir for the pathology. However, there is a shortage of effective techniques for the extraction and detection of prions present in this type of material and there is no fully effective decontamination method available. Therefore, we decided to carry out two studies. In the first one, we developed a protocol for the extraction and amplification by Protein Misfolding Cyclic Amplification (PMCA) of classical scrapie prions adsorbed on different materials from the herd environment (soil, paint, straw, and alfalfa). On the other hand, in the second study, we tested the decontamination effectiveness of autoclaving at different times and temperatures.

Material and methods

1 Protocol for prion extraction from environmental samples



2 Autoclave decontamination tests



Results and discussion

1 Extraction protocol results

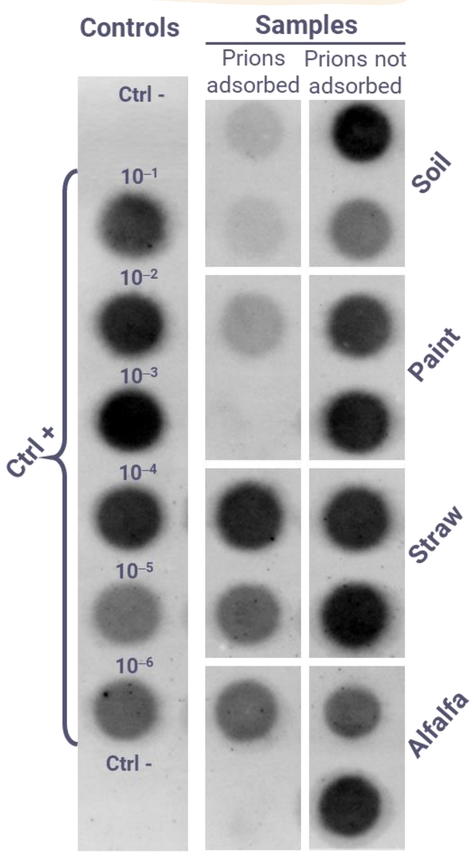


Figure 1. Detection by Dot Blot of adsorbed and non-adsorbed scrapie prions in environmental samples after amplification by PMCA. Each sample was analyzed twice. Serial dilutions (10^{-1}–10^{-6}) of a classical scrapie isolate (Dawson strain) were used as a positive control. A negative mouse brain homogenate was used as a negative control.

- The amount of non-adsorbed prions is higher than the amount of adsorbed prions in environmental samples.
- Straw and alfalfa are the materials with the highest capacity for prion adsorption and release, as they manifest the highest signal intensity. This could be due to their highly absorbent nature, which would have facilitated both the entry and accumulation of prions and their subsequent removal.
- Given the low signal intensity, the amount of prions adsorbed to the soil and paint seems to be lower. However, it is possible that the amount of adsorbed prions is higher but could not be extracted due to the strong adsorption to the sample.

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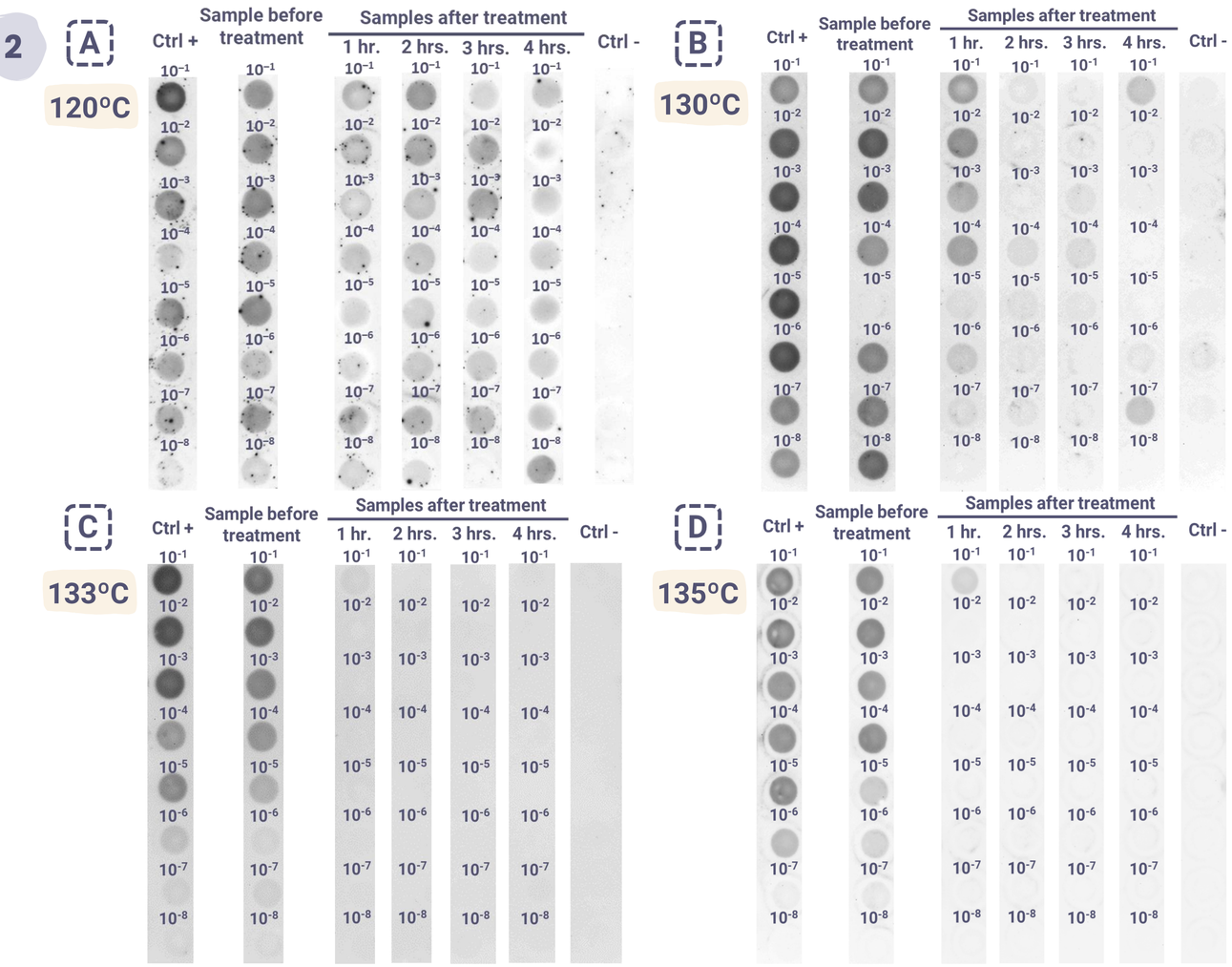

Figure 2. Dot blot detection of prions in serial dilutions (10^{-1}–10^{-8}) of samples collected before treatment and 1 hr., 2 hrs., 3 hrs. and 4 hrs. after treatment at 120ºC (A), 130ºC (B), 133ºC (C) or 135ºC (D) after PMCA amplification. Serial dilutions (10^{-1}–10^{-8}) of a classical scrapie isolate (Dawson strain) were used as a positive control. A negative mouse brain homogenate was used as a negative control.

After treatment at 120°C, no signs of decontamination were observed compared to untreated samples. However, favorable decontamination results began to be observed after 2 hours of treatment at 130ºC. Decontamination was almost total after 1 hour at 133ºC and the same result was obtained at 135ºC, so higher temperatures will be required for total decontamination.

Conclusions

- The prion extraction protocol developed was successful for the extraction and detection of prions adsorbed to all types of environmental samples, but especially in straw and alfalfa. Straw and alfalfa are the materials with the highest capacity to absorb and release prions. In contrast, soil and paint seem to have a lower adsorption capacity, although it is also possible that the prions could not be completely extracted due to strong binding to the sample.
- To achieve full decontamination, a minimum temperature between 133°C and 135°C for 2 hours is required. It will be necessary to use temperatures higher than 135°C to reduce the time required for decontamination.

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