Real-Time Quaking-Induced Conversion: Detection of Pathological Prion Protein in Milk Samples from Scrapie Naturally Infected Sheep

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BACKGROUND

One of the major challenges in managing Transmissible Spongiform Encephalopathies (TSEs) in animals is the lack of validated and sensitive intra-vitam assays. The misfolded isoform of the prion protein, PrPSc, is a specific marker of prion diseases and is widely distributed in the central nervous system (CNS), lymphoreticular system (LRS) tissues, and body fluids in both clinically and preclinically Scrapie-affected sheep. Although milk has been demonstrated to play a role in prion transmission [1-2], its potential use as a biological matrix for diagnosis has been only partially investigated [3]. The high sensitivity of Real-Time Quaking-Induced Conversion (RT-QuIC, Figure 1) assay in amplifying PrPSc across various biological matrices [4] suggests it is an ideal method for ante-mortem TSE diagnosis. However, it remains ineffective on blood and impractical for cerebrospinal fluid (CSF) due to collection challenges at the flock level. Here, we developed RT-QuIC conditions for the detection of PrPSc in milk collected from naturally Scrapie-infected sheep.

MATERIALS AND METHODS

FIGURE 1 - Real-Time Quaking-Induced Conversion (RT-QuIC) assay

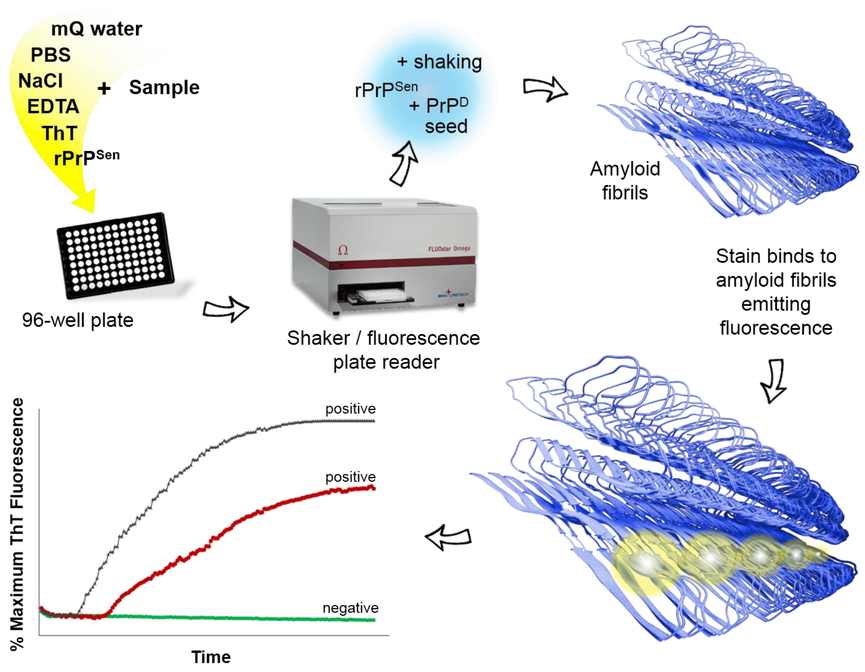

TABLE 1 - Epidemiological data and biochemical results

ID Farm	ID Animal	Date of Birth	Genotype	Sintomatology	EIA rapid test
IT066OR011	IT095001283007	01/12/2019	ARQ/ARQ	Yes	Positive
IT066OR011	IT095001283013	02/12/2019	ARQ/ARQ	No	Positive
IT066OR011	IT095001282869	04/12/2019	ARQ/ARQ	No	Negative

- ~50 ml of milk were collected from lactating sheep belonging to an Italian outbreak of classical Scrapie with positivity for Maedi-Visna virus (Table 1).
- TSE diagnosis was performed on obex by rapid test (IDEXX HerdChek BSE-scrapie antigen test kit EIA rapid test) and western blot.
- Sheep brain tissues affected by Scrapie resuspended at 10% (weight/volume) in homogenization buffer were used for preliminary spiking experiments in milk collected from uninfected scrapie sheep and as positive controls in RT-QuIC reactions.
- Milk samples (1 or 10 mL) were added with an equal volume of isopropanol/butanol (1:1 v/v) and centrifuged at 13,000 g for 30 min. Supernatants were discarded and the pellets were suspended in 0.1% of SDS/PBS solution or in lysis buffer (N-lauroyl sarcosine) and assayed neat or diluted from 1:10 to 1:1000.
- RT-QuIC tests were performed according to the protocol previously reported by Favole et al.[5].

RESULTS AND CONCLUSIONS

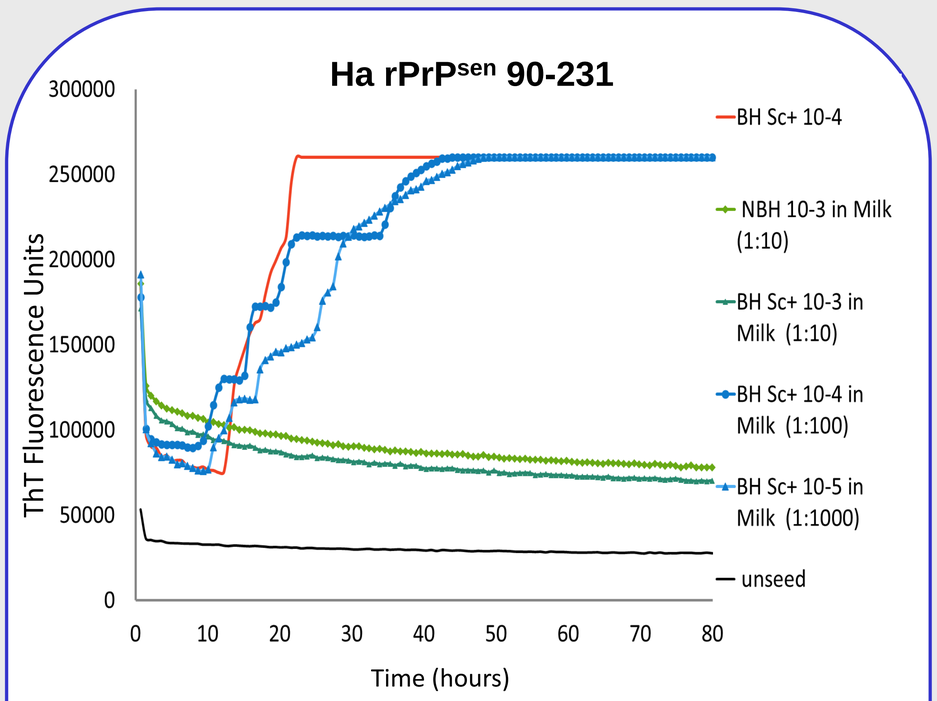

Figure 2. Detection of PrPSc from Scrapie brain homogenates spiked in diluted milk. Recombinant hamster PrPSen 90-231 or Bank vole 23-230 substrates were used to detect PrPSc. Normal control brain homogenates (NBH, green) and unseeded reactions (black) showed no response. Data are represented as the average fluorescence intensity of quadruplicate seeded reactions.

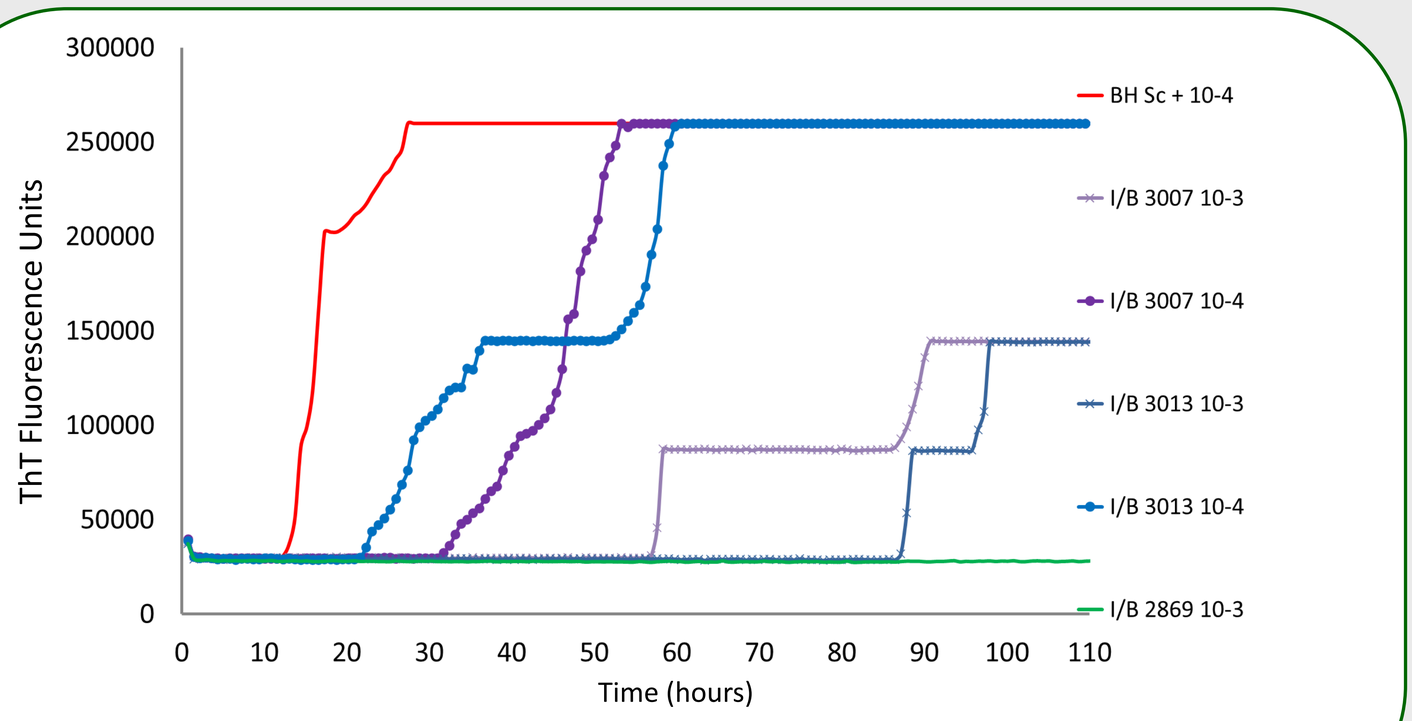

FIGURE 3. Serial dilution of Scrapie-infected and uninfected milk samples precipitated with isopropanol/butanol method were used to seed quadruplicate RT-QuIC reactions.

We found that both Ha 90-231 and BV 23-230 rPrPSen substrates can sensitively detect Scrapie PrPSc spiked in diluted milk (Figure 2). Specifically, rPrPSen Ha 90-231 exhibited rapid reactions with lag phases comparable to reactions seeded with Scrapie brain homogenates. Furthermore, the precipitation protocol using an isopropanol/butanol solution enabled the detection of seeding activity associated with the presence of PrPSc in RT-QuIC tests, with a latency phase of 20-30 hours when applied to 10 mL of individual milk samples collected from 2/2 Scrapie naturally infected sheep (Figure 3).

These data confirm the secretion of prions within milk during the early stages of disease progression and a role for milk in prion transmission [1-3]. Furthermore, the application of RT-QuIC to milk samples offers a non invasive methodology to detect Scrapie during preclinical/subclinical disease.

References

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Acknowledgements

This work was supported by grant from the Italian Ministry of Health to Pier Luigi Acutis (grants RF-2019-12369570)