

21st Annual Meeting of the TSE EURL Rome 13th-15th May 2024



Can the use of Real-Time Quaking-Induced Conversion Assay improve the surveillance of animal TSEs?

*Maria Mazza
IZSPLV, Turin*



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Istituto Superiore di Sanità - Rome

Italian Ministry of Health

Project Code RF-2019-12369570

Alive and QuICking: early and *intra-vitam* detection of prions using Real-Time Quaking-Induced Conversion (RT-QuIC) assay as individual and flock test, to improve Scrapie and CWD surveillance in Italy

Project Leader

- ❑ IZS PLV – P.L. Acutis

Partners

- ❑ Istituto Superiore di Sanità (ISS) – Romolo Nonno
- ❑ IZS Sardegna – Ciriaco Ligios



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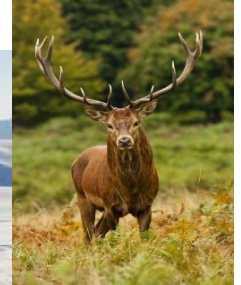
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CLASSICAL SCRAPIE IN SMALL RUMINANTS



CHRONIC WASTING DISEASE (CWD)

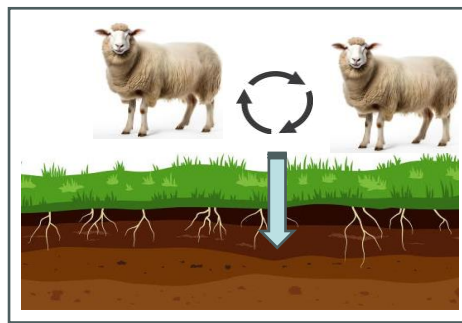


Scrapie and CWD are highly infectious due to the dissemination of prions in peripheral tissues.

Environmental contamination

Horizontal transmission among animals





prions are shed by infected hosts via urine, feces, and saliva

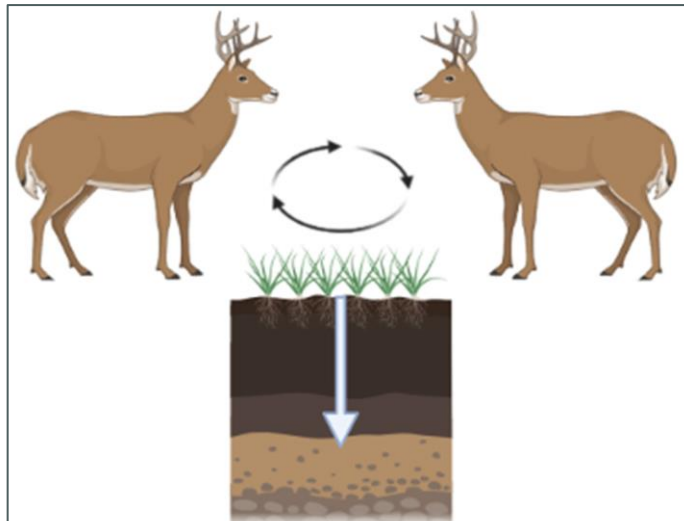


soils, soil minerals, plant materials, other environmental surfaces



Inter-species transmission

Risk for other species



Risk for humans?



CWD
surveillance
and diagnosis
has become an
important issue
in Europe

risk for humans???



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Animal to animal transmission causes difficult-to-control epidemics in domestic and wild ruminants

IN ITALY

CLASSICAL SCRAPIE IN SMALL RUMINANTS



> 1000 outbreaks identified in Italy
outbreaks with high disease prevalence



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Animal to animal transmission causes difficult-to-control epidemics in domestic and wild ruminants

CHRONIC WASTING DISEASE (CWD)

Widespread in North America and Canada



Since 2016 in Europe

- ❖ Norway (reindeer, moose and red deer)
- ❖ Finland (moose)
- ❖ Sweden (moose)

Different CWD strains in Europe than in the US



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Animal to animal transmission causes difficult-to-control epidemics in domestic and wild ruminants

CHRONIC WASTING DISEASE (CWD)

IN ITALY



A survey was made of different species of cervids from October 2016 until early 2023

- ✓ Cervids found dead in the wild or on farms
- ✓ Cervids suffering from dementia or nervous symptoms shot on the farm or during hunting

Targets: brain stem and mid retropharyngeal lymph nodes



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HerdChek BSE/Scrapie Antigen Kit, Idexx



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IN ITALY

2016 – 2022

a total of 3218 cervids were analyzed

Anno di prelievo	Motivo del prelievo						TOTALE test CWD
	1.Capo selvatico abbattuto per ragioni sanitarie (es. defedato, con sintomatologia sospetta, ecc.)	2.Capo selvatico rinvenuto morto, presumibilmente a seguito di incidente stradale (N.B.: età minima >18 mesi)	3.Capo selvatico rinvenuto morto, ragionevolmente per causa diversa da incidente stradale	4.Capo allevato abbattuto per ragioni sanitarie (es. defedato, con sintomatologia sospetta, ecc.)	5.Capo allevato rinvenuto morto	Motivo del prelievo mancante	
2016	8	40	24	0	0	2	74
2017	64	316	138	4	24	23	569
2018	43	403	144	4	24	0	618
2019	40	404	127	1	6	0	578
2020	25	310	100	1	8	0	444
2021	30	308	126	4	8	1	477
2022	29	293	121	1	14	0	458
Totale	239	2.074	780	15	84	26	3.218

wild found dead (road kill) ↗ ↖

↗ ↖ wild found dead

No positive cases were identified



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ADOPTED: 15 March 2023

doi: [10.2903/j.efsa.2023.7936](https://doi.org/10.2903/j.efsa.2023.7936)

Monitoring of chronic wasting disease (CWD) (IV)

EFSA Panel on Biological Hazards (BIOHAZ).

Konstantinos Koutsoumanis, Ana Allende, Avelino Alvarez-Ordoñez, Declan Bolton, Sara Bover-Cid, Marianne Chemaly, Robert Davies, Alessandra De Cesare, Lieve Herman, Friederike Hilbert, Roland Lindqvist, Maarten Nauta, Luísa Peixe, Panagiotis Skandamis, Elisabetta Suffredini, Michael W Miller, Atle Mysterud, Maria Nöremark, Marion Simmons, Michael A Tranulis, Gabriele Vaccari, Hildegunn Viljugrein, Angel Ortiz-Pelaez and Giuseppe Ru



Italian Ministry of Health established monitoring only for the *Cervus elaphus* species



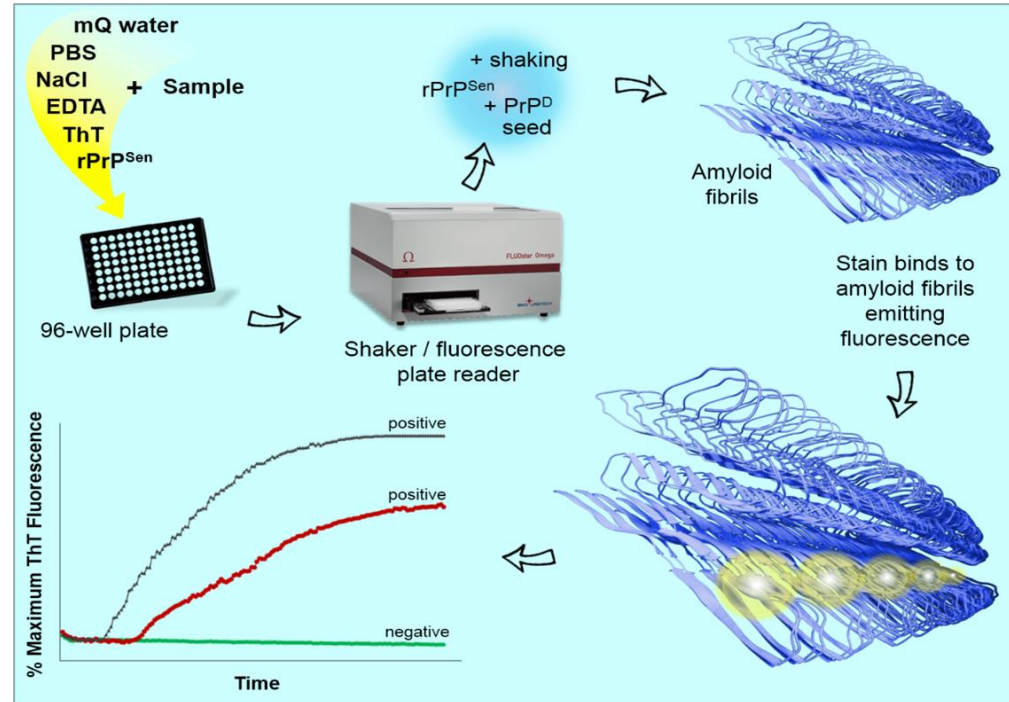
Diagnostic methods highly sensitive:

- to implement an *intra-vitam* TSEs diagnosis
- to improve actual surveillance systems (Scrapie and CWD)



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REAL-TIME QUAKING- INDUCED CONVERSION (RT-QuIC)



Italian Ministry of Health
Project Code RF-2019-12369570

WP1 - Aim 1

To evaluate the performances of RT-QuIC to detect PrP^{Sc} seeding associated activity on biological fluids of animals naturally infected with classical scrapie, for an *intra-vitam* diagnosis

WP2 - Aim 2

To apply RT-QuIC assay on bulk milk and feces, in order to detect classical scrapie agent at the flock level

WP3 - Aim 3

To improve the accuracy of the diagnostic system for CWD, through the application of RT-QuIC as screening test and the evaluation of antibodies against PrP^{CWD}



WP1 - Aim 1

To evaluate the performances of RT-QuIC to detect PrP^{Sc} seeding associated activity on biological fluids of animals naturally infected with classical scrapie, for an *intra-vitam* diagnosis

WP2 - Aim 2

To apply RT-QuIC assay on bulk milk and feces, in order to detect classical scrapie agent at the flock level

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Sheep samples collected

	N. cases	Pos. rapid test*	Neg. rapid test*
asymptomatic	37	15	22
symptomatic	6	5	1
Total cases	43	20	23

*HerdChek BSE/Scrapie Ag kit (Idexx)



Tissues/fluids/excreta	N. cases	Pos. rapid test*	Neg. rapid test*
Brain stem, blood, retroph. lymph nodes, tonsils, feces	43	20	23
Milk	12	7	5
CSF	30	14	16



Milk

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To develop RT-QuIC conditions for the detection of PrP^{Sc} in milk collected from naturally Scrapie-infected sheep.

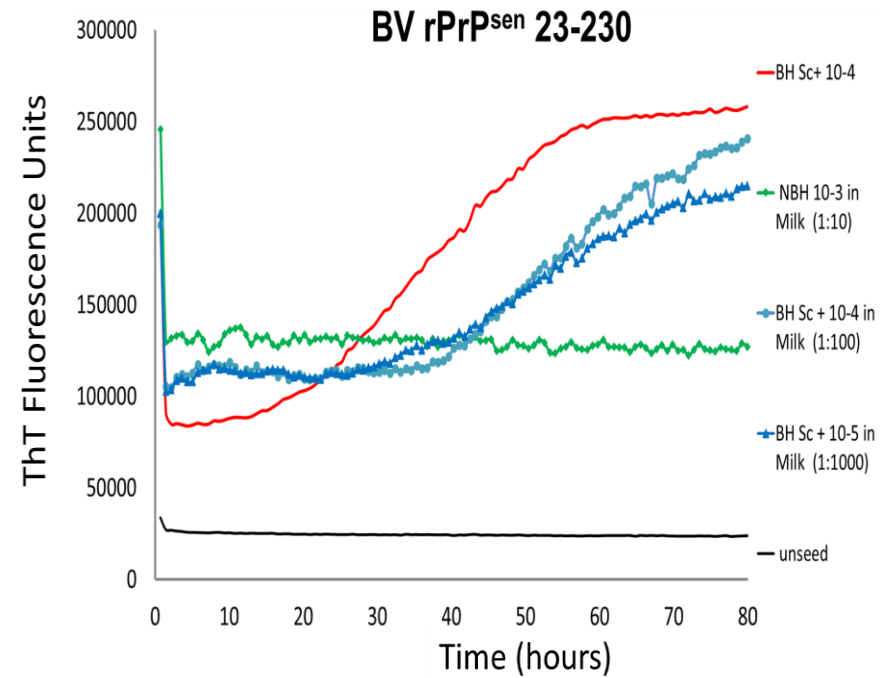
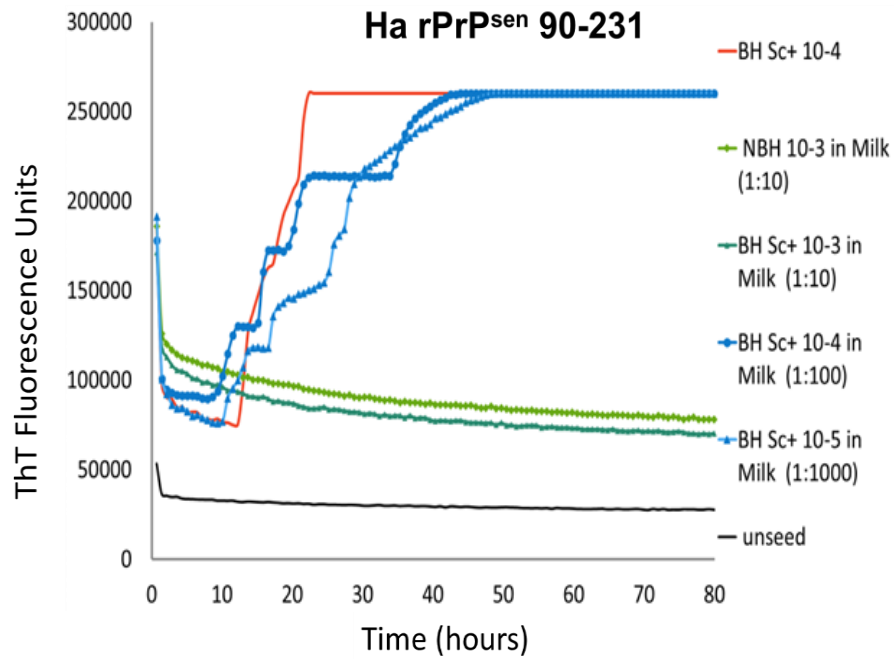
ID Farm	ID Animal	Date of Birth	Genotype	Sintomatology	EIA rapid test
IT066OR011	IT095001283007	01/12/2019	ARQ/ARQ	Yes	Positive
IT066OR011	IT095001283013	02/12/2019	ARQ/ARQ	No	Positive
IT066OR011	IT095001282869	04/12/2019	ARQ/ARQ	No	Negative

~50 ml of milk were collected from lactating sheep belonging to an Italian outbreak of classical Scrapie with positivity for Maedi-Visna virus

Technical details concerning the preparation of milk samples analysed with RT-QuIC are described in the poster presented by my colleague Alessandra Favole, which you can see during the poster session.



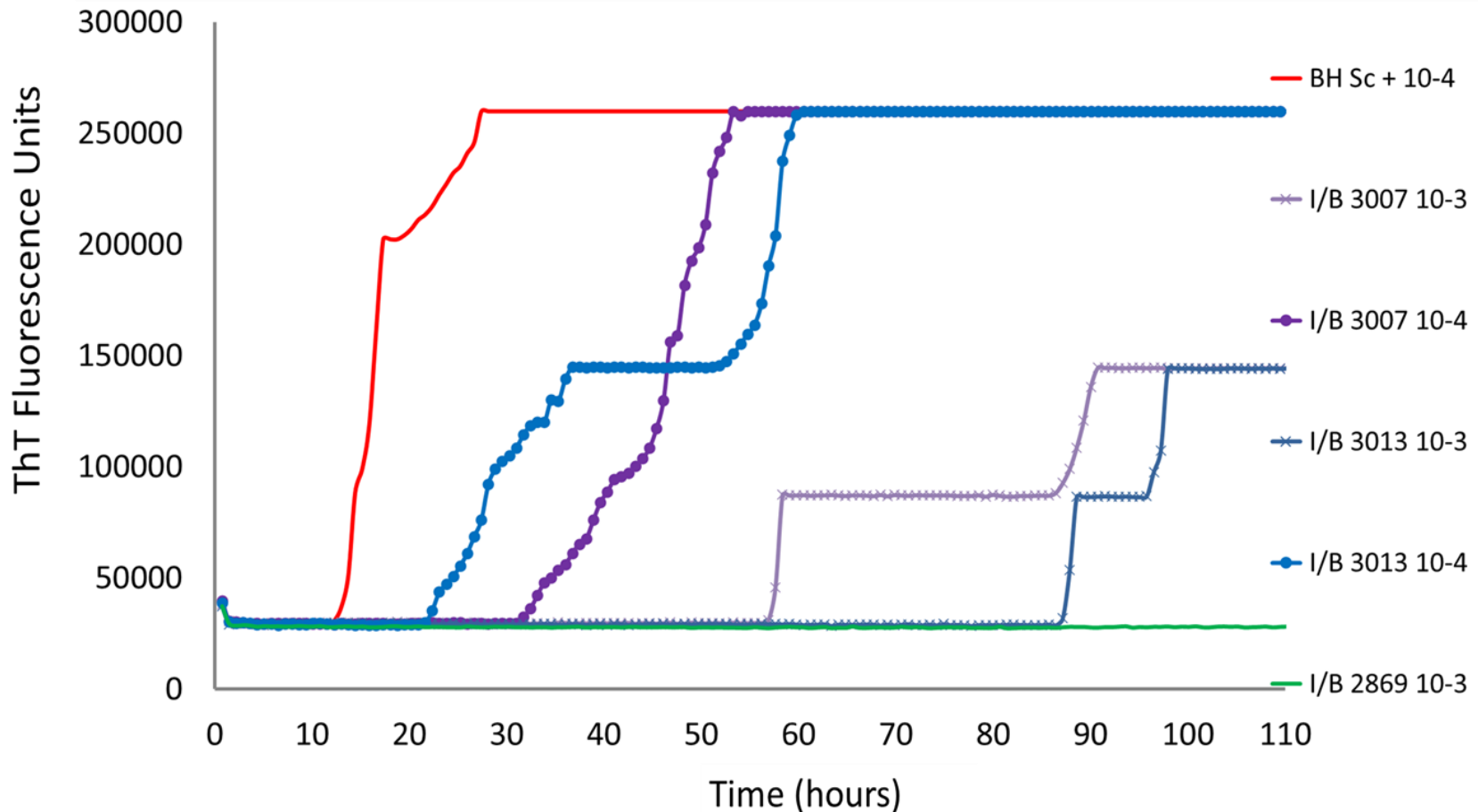
Detection of PrP^{Sc} from Scrapie brain homogenates spiked in diluted milk



Serial dilution of Scrapie-infected and uninfected milk samples precipitated with isopropanol/butanol (I/B)



Ha rPrP^{sen} 90-231



REMARKS



Ha 90-231 and BV 23-230 rPrP^{Sen} substrates can sensitively detect Scrapie PrP^{Sc} spiked in diluted milk

These data confirm the secretion of prions within milk during the early stages of disease progression and a role for milk in prion transmission

The application of RT-QuIC to milk samples provides a non-invasive approach to detect classical Scrapie agent in the pre-clinical/sub-clinical phase

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WP3 - Aim 3

To improve the accuracy of the diagnostic system for CWD, through the application of RT-QuIC as screening test



Evaluate the diagnostic performances of commercially available rapid tests authorized by the EC as well as of Immunoblotting confirmatory methods to detect European CWD cases

Italian Ministry of Health

Project Code RF-2019-12369570

Evaluate the diagnostic performances of commercially available rapid tests authorized by the EC as well as of Immunoblotting confirmatory methods to detect European CWD cases



Three rapid test

- TeSeE SAP Combi kit (Bio-Rad)
- TeSeE sheep/goats kit (Bio-Rad)
- HerdCheck BSE/Ag kit (Idexx)

Two WB methods

- TeSeE Western Blot (Bio-Rad)
- SAF-IB (9A2, 6H4, L42, SAF84, SHA31)





Veterinærinstituttet
Norwegian Veterinary Institute



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PLOS ONE

RESEARCH ARTICLE

Are rapid tests and confirmatory western blot used for cattle and small ruminants TSEs reliable tools for the diagnosis of Chronic Wasting Disease in Europe?

Maria Mazza, Linh Tran, Daniela Loprevite, Maria C. Cavarretta, Daniela Meloni, Luana Dell'Atti, Jørn Våge, Knut Madslie, Tram T. Vuong, Elena Bozzetta, Sylvie L. Benestad

Published: August 30, 2023 <https://doi.org/10.1371/journal.pone.0286266>



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PLOS ONE

August 30, 2023 <https://doi.org/10.1371/journal.pone.0286266>

What this study revealed...

All rapid tests are able to identify the different strains of CWD circulating in the Nordic countries but with different analytical sensitivity. HerdChek BSE-Scrapie Ag test resulted the most sensitive

The analytical sensitivity of both western blot methods was similar or higher to that of rapid tests, validating their ability to confirm CWD cases identified by the screening



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WP3 - Aim 3

To improve the accuracy of the diagnostic system for CWD, through the application of RT-QuIC as screening test

Evaluate the ability of RT-QuIC to detect the European CWD cases



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Evaluate the ability of RT-QuIC to detect the European CWD cases

12 Scandinavian CWD samples

- 3 moose from Sweden
- 3 moose from Finland
- 5 moose from Norway
- 1 rein deer from Norway

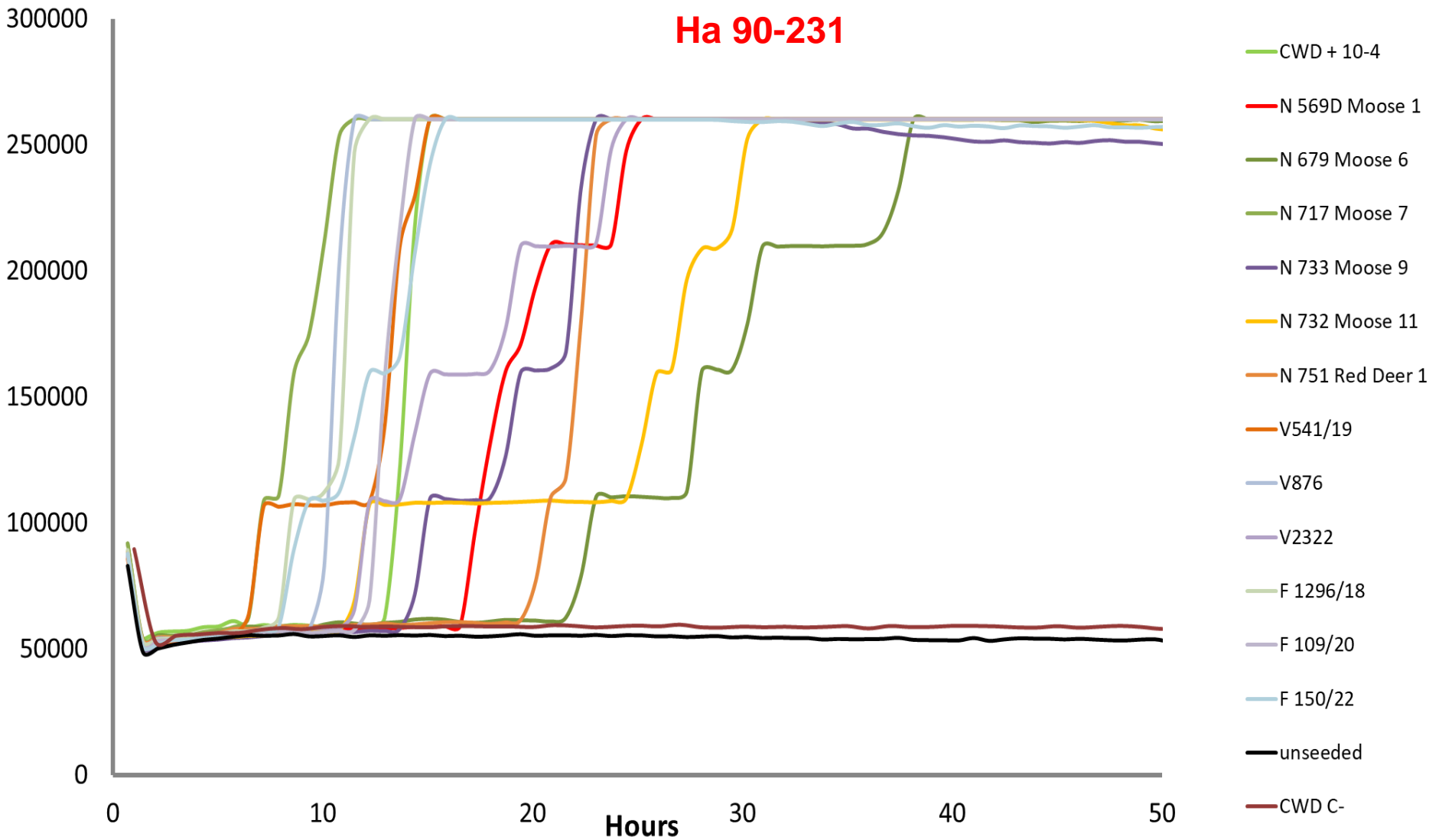
Homogenates of nervous tissue analyzed by RT-QuIC using Ha 90-231 as rPrP substrate





RT-QuIC analysis of Scandinavian CWD cases

Ha 90-231



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WP3 - Aim 3

To improve the accuracy of the diagnostic system for CWD, through the application of RT-QuIC as screening test and the evaluation of antibodies against PrP^{CWD}

Retrospective study

Apply RT-QuIC on Italian cervids samples analyzed by HerdChek BSE-Scrapie Ag test



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Retrospective study

Apply RT-QuIC on Italian cervids analyzed by HerdChek BSE-Scrapie Ag test

~100 cases of Italian cervids (mainly deer) were selected from those analysed by rapid test. Brainstem and retropharyngeal lymph nodes (not for all cases) were extracted.

36 brainstem homogenates were analyzed by RT-QuIC using Ha 90-231 as rPrP substrate



To date no positive cases were identify

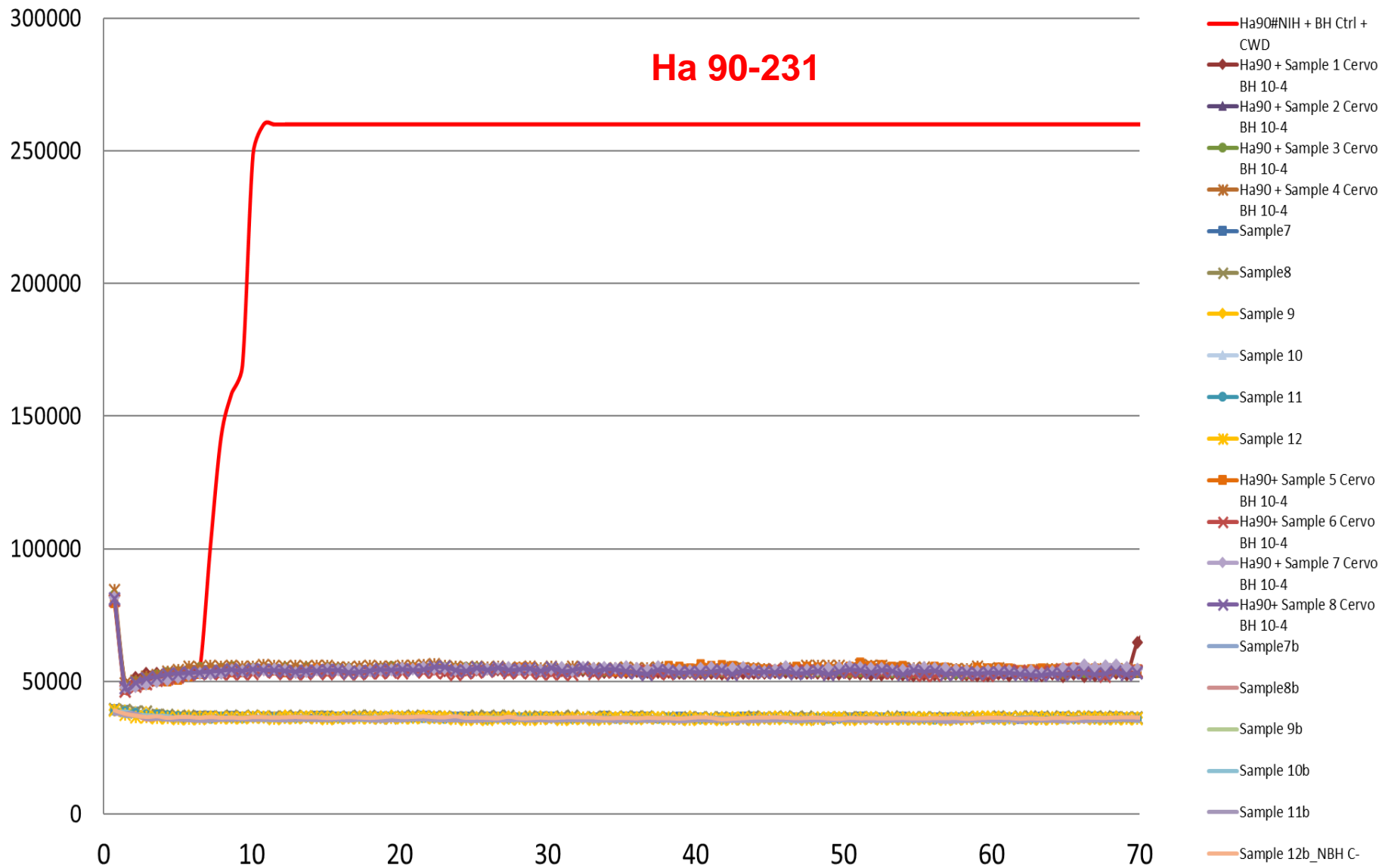
Analyses of all other samples are still in progress



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RT-QuIC analysis of Italian cervids



REMARKS

RT-QuIC

RT-QuIC protocol can sensitively detect European CWD cases

Amplification of CWD positive samples occurs within 20 hours

No differences seem to be in amplification profiles among European CWD samples





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Dolores Gavier-Widén



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"G. PEGREFFI"

Ciriaco Ligios
Davide Pintus

Thanks for your
attention!!!