



Rijksinstituut voor Volksgezondheid
en Milieu
*Ministerie van Volksgezondheid,
Welzijn en Sport*

NRL for Parasites the Netherlands

Results and challenges in the covid
pandemic years



Tasks of NRL-P NL

- Organize PTs 2 times per year
- On-site audits: one round of all five/six labs per year
- Provide training to slaughterhouse (SL) lab technicians on request
- Provide advise to Competent Authority (CA)
 - Yearly report on PTs and audits
 - Report on introduction schedule and -results of new private labs
 - ad hoc matters regarding food safety
- Confirmation of findings in SLs
- Maintain knowledge base on food-borne parasites requested by CA



Proficiency Testing

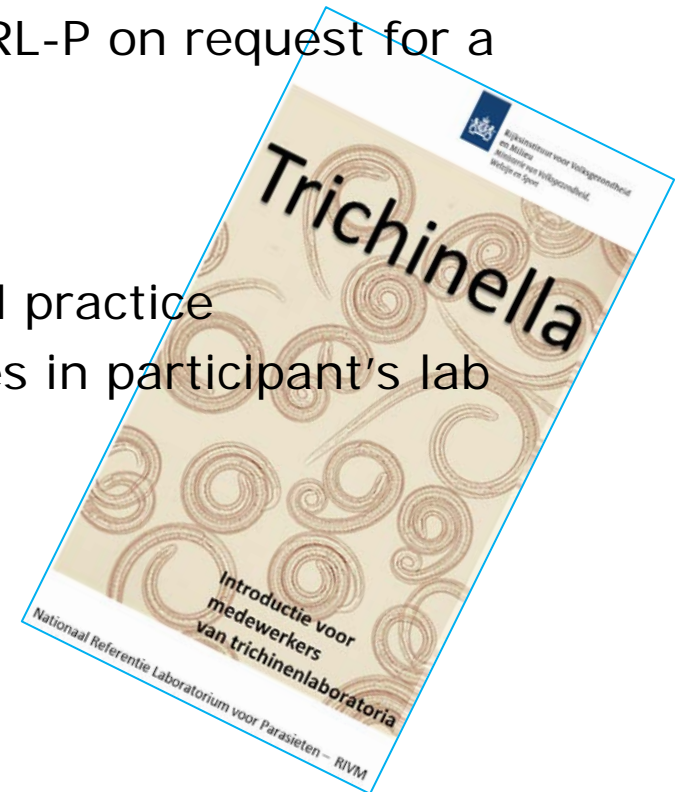
- NRL-P NL organized two rounds of PTs for 5 SL and our own lab
- Analysis results reported per technician
- Results to be reported within 5 working days by participating labs
- Each lab should provide correct identification of positive and negative samples
- Evaluation of each technician's quantitative results
- After 3 years of correct performance the frequency of obligatory PT participation reduced to 1 per year

Technician	Sample	Positive	Negative	% Correct
A	1	4	0	100%
		0	4	100%
	2	4	0	100%
		0	4	100%
	3	4	0	100%
		0	4	100%
B	1	4	0	100%
		0	4	100%
	2	4	0	100%
		0	4	100%
	3	4	0	100%
		0	4	100%
C	1	4	0	100%
		0	4	100%
	2	4	0	100%
		0	4	100%
	3	4	0	100%
		0	4	100%



Training

- Training of SL technicians is provided by NRL-P on request for a maximum of 4 persons at a time
- Training consists of
 - theoretical background and microscopical practice
 - digestion and quantification of PT samples in participant's lab

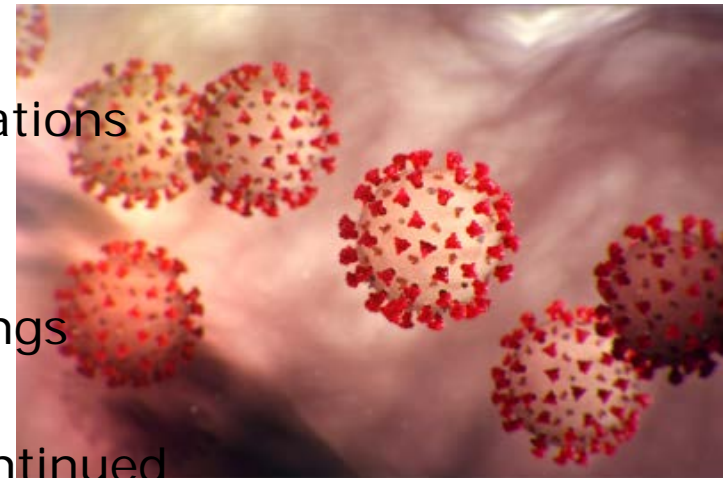




Covid-19 pandemic (1)

NRL-P recognized as critical process: at RIVM access to the labs guaranteed during lock-down periods

- In general: access to the lab restricted to 2 persons at a time
- In training situations:
 - 1 trainer and 2 trainees
 - keep at least 1 m distance
 - use face shields for demonstration situations
 - lab blocked for all other users
- This meant doubling the number of trainings
- Confirmation/evaluation of SL findings continued





Covid-19 pandemic (2)

Situation in the slaughterhouses

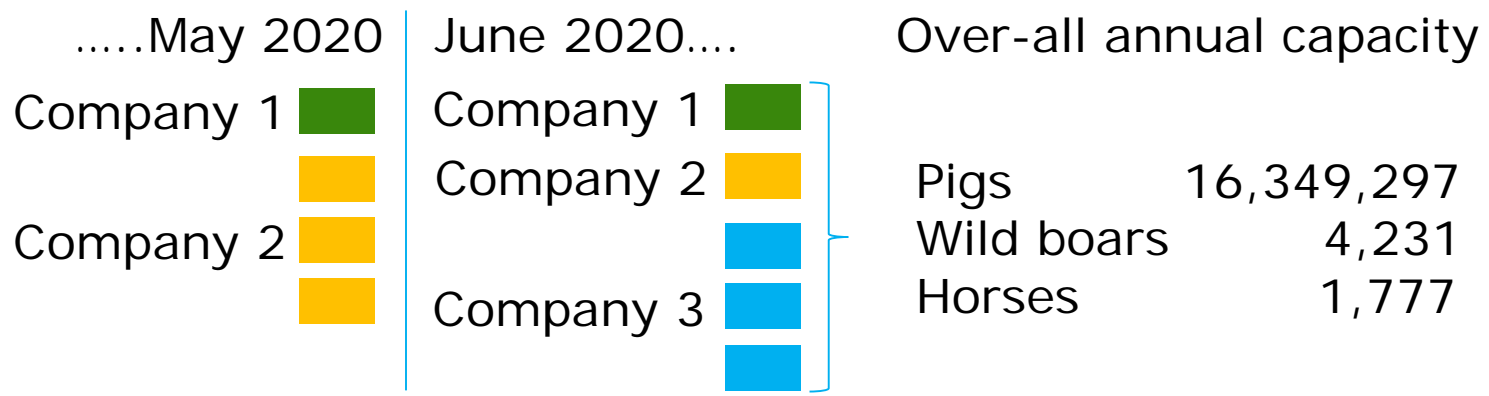
- Full slaughter process continued
- Two SLs temporarily closed due to covid-19 circulating among workers in May-June 2020
- All employees checked daily at the gate from that time onward
- Restrictions for all visitors
- NRL-P NL was allowed to perform on site audits





Covid-19 pandemic (3)

- Until May 2020: 2 private companies with 1 and 3 labs respectively
- May 2020 a third private company entered the market
- In April 2022 yet another competitor requested to enter the market

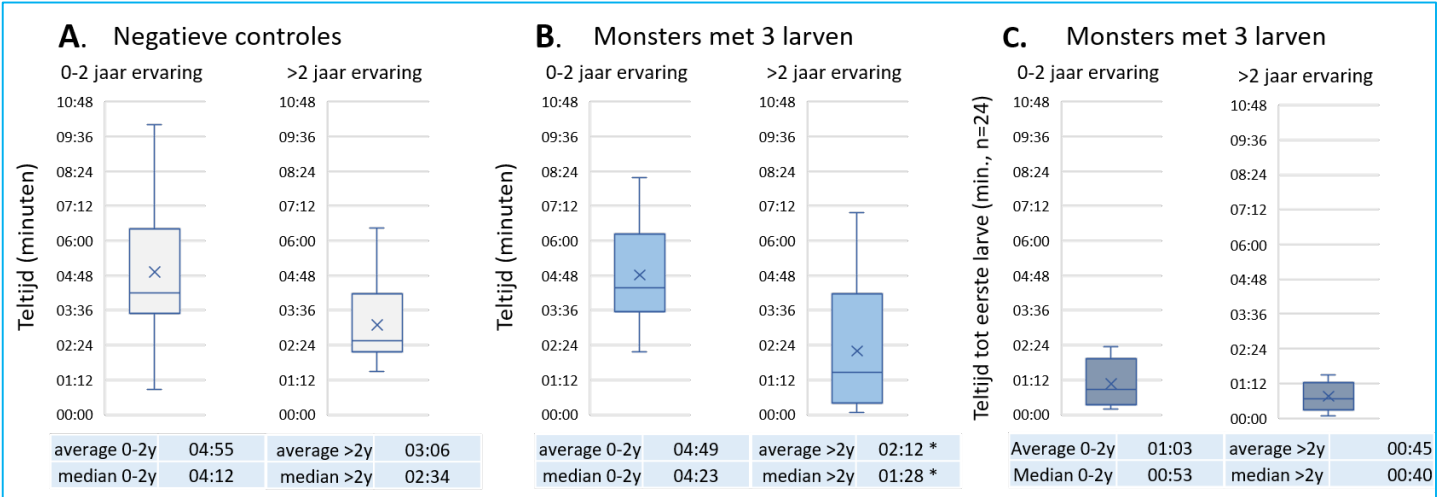


- NRL-P NL was able to perform entrance-training, PT and entrance-audit throughout 2020-2022, although hindered by the lock-down of one slaughterhouse in May/June 2020, exactly in the middle of the transition period



Covid-19 pandemic (4)

- NRL-P communication implementation of ISO 18743:2015
- All Dutch *Trichinella* SLs implemented the new ISO in 2021
- Annual audit series based on ISO 18743:2015 and EU Regulation 2015/1375
- Validation of counting time with 25 technicians and 4 samples involved



Maintaining the knowledge base (1)

Experimental Parasitology 224 (2021) 108099



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journal homepage: www.elsevier.com/locate/yexpr

Inactivation of *Trichinella* muscle larvae at different time-temperature heating profiles simulating home-cooking

Frits Franssen^{a,*}, Huifang Deng^a, Arno Swart^a, Axel Bonačić Marinović^a, Xiaolei Liu^b, Minglei Liu^b, Joke van der Giessen^a

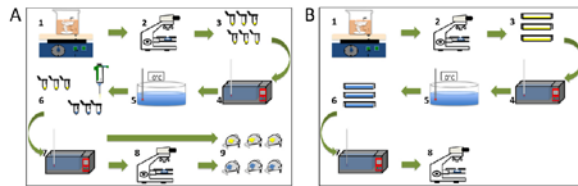


Fig. 1. Process overview of heat treatment experiments.

A: experiments A1 and A2.

1. Artificial digestion to liberate *Trichinella* muscle larvae. 2. Larvae were enumerated by microscopy. 3. Larvae were transferred to Eppendorf tubes in 100 μ l aliquots containing 100 larvae each. 4. Larvae were heat-treated in series of six samples. 5. Larvae were cooled on ice to stop the heating process. 6. Methylene blue was added to three vials of each series (Eppendorf vials with blue content) and three other samples were mock treated (vials with yellow content). 7. All vials were placed in water bath at 37 °C for 15 min. 8. Three vials contained Methylene blue

were evaluated microscopically. 9. All series were administered to inbred mice per gastric tube.

B: experiment B.
1. Artificial digestion to liberate *Trichinella* muscle larvae. 2. Larvae were enumerated by microscopy. 3. Larvae were transferred to plates in 30 ml aliquots containing 100 larvae each. 4. Larvae were heat-treated. 5. Larvae were cooled on ice to stop the heating process. 6. Methylene blue was added to the plates in 30 ml aliquots. 7. Plates were placed in a water bath at 37 °C for 15 min. 8. Plates containing larvae were evaluated microscopically.

Overall conclusion:

- Above 60 °C for 15 min no infectious larvae in mouse experiment and larval morphology predicts infectivity
- Model-predicted inactivation and infectivity correspond perfectly with observed

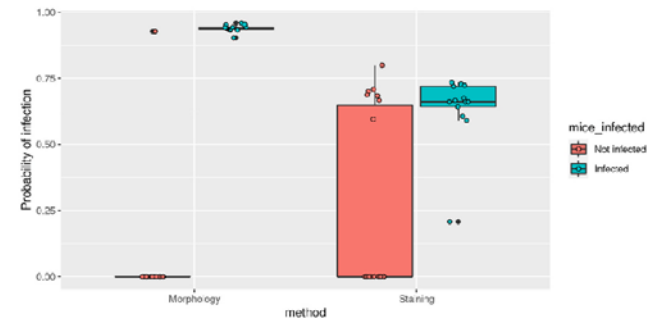


Fig. 5. Boxplots of the predicted probability of infection (based on doses according to either the MB staining or morphological examination method), split in the data for the infected and uninfected mice. Clearly, the dose obtained using the morphological examination method is more discriminating in distinguishing infection.



Maintaining the knowledge base (2)

Mining Public Metagenomes for Environmental Surveillance of Parasites: A Proof of Principle

Frits F. J. Franssen^{1*}, Ingmar Janse¹, Dennis Janssen¹, Simone M. Caccio², Paolo Vatta², Joke W. B. van der Giessen¹ and Mark W. J. van Passel^{1†}

Overall conclusion:

- Metagenomic analysis applicable for FBP
- Database analysis 3080 vs 3095 crypto reads
- Metagenomic pipeline appears sensitive
- Practical validation under evaluation in OH-EJP Project PARADISE

