

## Workshop of National Reference Laboratories for Parasites Istituto Superiore di Sanità, Rome, Italy, 24-25 May, 2018

### Proficiency testing on

“Digestion method to detect *Trichinella* larvae in meat samples according to the EU directive 2015/1375”



# Materials and methods

Participants: 31 NRLs (including 4 outside EU)

## PT sample panel

<b>N° of samples</b>	<b>1</b>	<b>1</b>	<b>1</b>
<b>N° of larvae in the sample</b>	<b>0</b>	<b>4</b>	<b>4</b>



## Analyzed samples types

	<b>Pork 100 g</b>	<b>Pork 35 g</b>	<b>Horse meat 100 g</b>	<b>Horse meat 35 g</b>
N° of laboratories	26	3	1	1

- 26 labs used the magnetic stirred digestion method
- 1 labs used the mechanically assisted digestion method (Stomacher)
- 4 labs used the automatic digestion method (Trichomatic 35)



# Qualitative results

Lab code	False negatives	False positives	PT final evaluation
NRL1	0	0	positive
NRL2	0	0	positive
NRL3	0	0	positive
NRL4	0	0	positive
NRL5	0	0	positive
NRL6	0	0	positive
NRL7	0	0	positive
NRL8	0	0	positive
NRL9	0	0	positive
NRL10	0	0	positive
NRL11	0	0	positive
NRL12	0	0	positive
NRL13	0	0	positive
NRL14	0	0	positive
NRL15	0	0	positive
NRL16	0	0	positive
NRL17	0	0	positive

Lab code	False negatives	False positives	PT final evaluation
NRL18	0	0	positive
NRL19	0	0	positive
NRL20	0	0	positive
NRL21	0	0	positive
NRL22	0	0	positive
NRL23	1	0	negative*
NRL24	0	0	positive
NRL24	0	0	positive
NRL26	0	0	positive
NRL35	0	0	positive
NRL40	0	0	positive
NRL41	0	0	positive
NRL42	0	0	positive
TLE6	0	0	positive

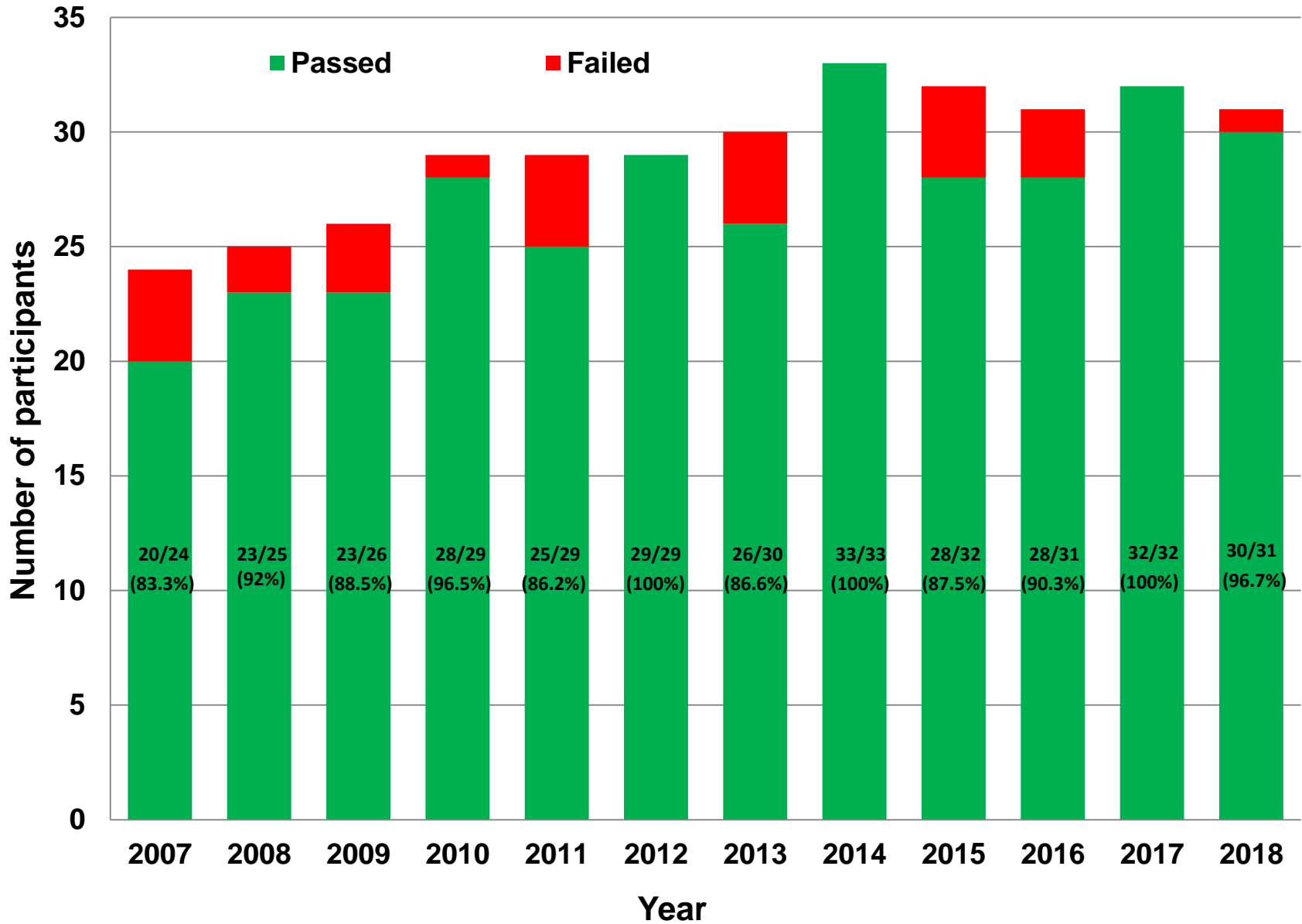
\*Positive result in EQA scheme

# Qualitative results

## Overtime comparison

NRL code	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
1	positive	positive	positive	positive	positive	positive	positive	positive	positive	negative	positive	positive
2	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
3	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
4	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
5	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
6	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
7	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
8	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
9	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
10	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
11	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
12	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
13	negative	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
14	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
15	negative	positive	negative	positive	positive	positive	negative	positive	positive	negative	positive	positive
16	positive	positive	positive	positive	negative	positive	negative	positive	positive	positive	positive	positive
17	negative	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
18	positive	positive	positive	positive	negative	positive	negative	positive	positive	positive	positive	positive
19	positive	positive	positive	positive	positive	positive	positive	positive	negative	positive	positive	positive
20	-	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
21	positive	positive	positive	positive	negative	positive	positive	positive	positive	positive	positive	positive
22	negative	negative	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
23	positive	positive	positive	positive	positive	positive	positive	positive	negative	positive	positive	negative
24	positive	positive	negative	positive	positive	positive	positive	positive	positive	positive	positive	positive
25	positive	negative	negative	positive	negative	positive	positive	-	negative	positive	positive	positive
26	-	-	-	positive	positive	positive	positive	positive	positive	negative	positive	positive
34	-	-	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
35	-	-	-	negative	positive	positive	-	positive	negative	positive	positive	positive
40	-	-	-	-	-	positive	positive	positive	positive	positive	positive	positive
41	-	-	-	-	-	-	positive	positive	positive	-	positive	positive
42	-	-	-	-	-	-	negative	positive	positive	positive	positive	positive
43	-	-	-	-	-	-	-	positive	-	-	-	-
44	-	-	-	-	-	-	-	positive	-	-	-	-
TLE6	-	-	-	-	-	-	-	positive	positive	positive	-	positive

# Qualitative results



# Conclusions

- All but one participant passed the PT
- No false positives have been reported
- Future task: to be able to maintain 100% positive results over time

## Workshop of National Reference Laboratories for Parasites Istituto Superiore di Sanità, Rome, Italy, 24-25 May, 2018

### Proficiency testing on

“*Trichinella* larvae identification at species level by a molecular method”





# Introduction

- Purpose: to test the capacity of NRLs to identify *Trichinella* muscle larvae at the species level
- Participants: 22 NRLs (including 3 outside EU)
- PT item: pool of 10 larvae of 4 different species
- Methods: any test method able to discriminate the *Trichinella* species



# Materials and methods

## POOL OF LARVAE SET (4 samples)

Species	N° of vials	N° of larvae for each vial	Evaluation criteria
<i>T. nativa</i>	1	10	Correct identification of the 4 species
<i>T. britovi</i>	1	10	
<i>T. pseudospiralis</i>	1	10	
<i>T. zimbabwensis</i>	1	10	

Possibility to analyze larvae singularly or as pool, depending on the sensitivity of the method used as well as on the experience of the technical staff

# Results

Lab code	Method used	N. correct identification	N. incorrect identification	N. missed identification	Final evaluation
NRL1	Multiplex PCR	4	0	0	positive
NRL2	Multiplex PCR	4	0	0	positive
NRL3	Multiplex PCR	4	0	0	positive
NRL6	5S sequencing	4	0	0	positive
NRL7	Multiplex PCR	4	0	0	positive
NRL8	Multiplex PCR	4	0	0	positive
NRL10	Multiplex PCR	4	0	0	positive
NRL12	Multiplex PCR	4	0	0	positive
<b>NRL14</b>	<b>Technical problem</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>negative</b>
NRL16	Multiplex PCR	4	0	0	positive
NRL17	Multiplex PCR	4	0	0	positive
<b>NRL18</b>	<b>results not submitted</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>NRL20</b>	Multiplex PCR	3	<b>1</b>	0	<b>negative</b>
NRL21	Multiplex PCR	4	0	0	positive
NRL22	Multiplex PCR	3	<b>1</b>	0	<b>negative</b>
NRL23	Multiplex PCR	4	0	0	positive
NRL24	Multiplex PCR	4	0	0	positive
NRL25	Multiplex PCR	4	0	0	positive
NRL40	5S sequencing	4	0	0	positive
<b>NRL42</b>	<b>results not submitted</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
NRL46	Multiplex PCR	4	0	0	positive
<b>TLE6</b>	Multiplex PCR	3	<b>1</b>	0	<b>negative</b>

# Results

The three labs that failed the PT did not recognize the *T. zimbabwensis* specific band that was mistaken for that of *T. pseudospiralis*

	T. spiralis	T. nativa	T. britovi	T. pseudospiralis	T. murrelli	Trichinella T6	T. nelsoni	T. papuae	T. zimbabwensis
ESV	173	127	127	310-350	127	127	155	240	264
ITS1-T. britovi			253						
ITS1-T6						210			
ITS2-T. murrelli					316				
ITS2-T. nelsoni							404		

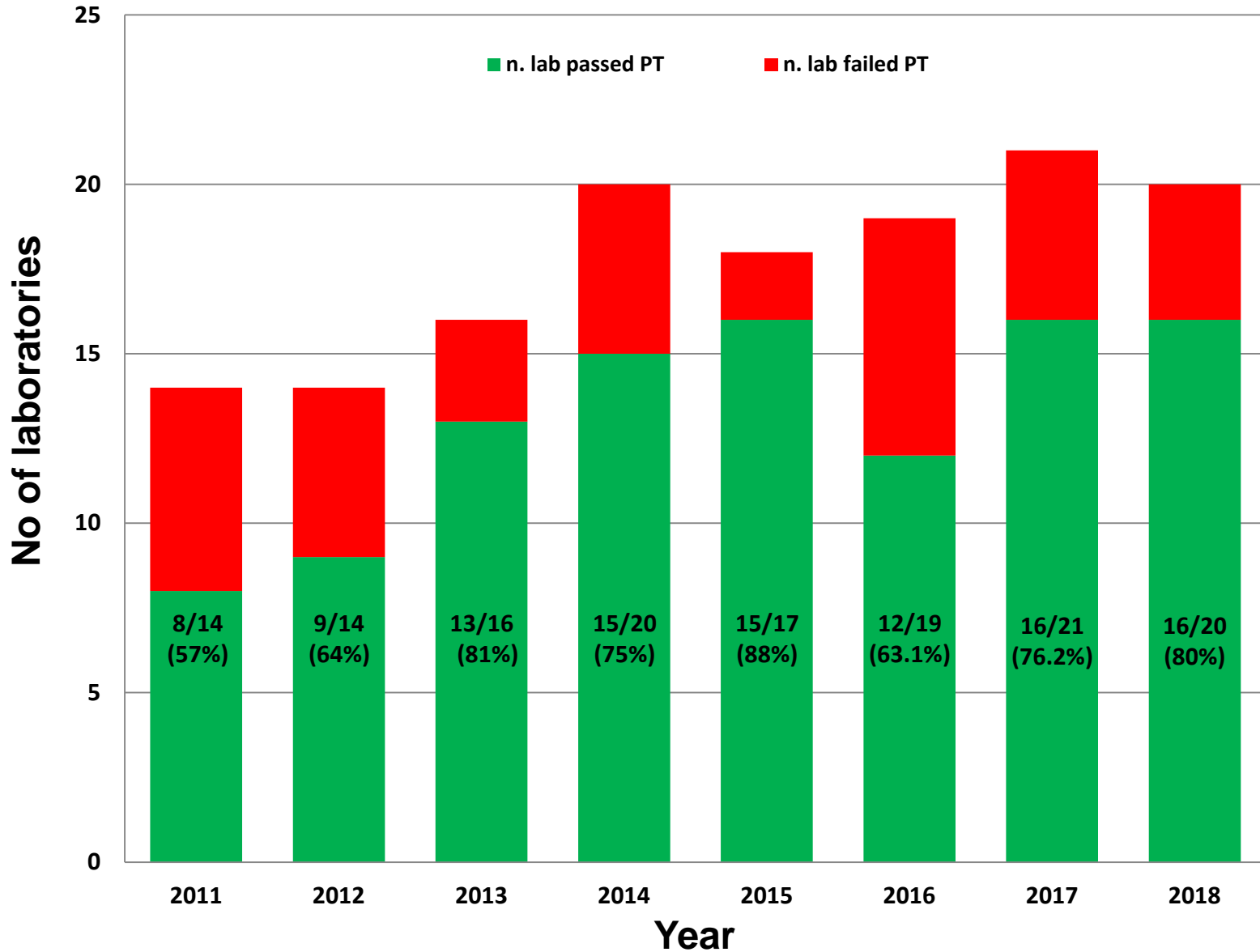
# Results

## Overtime comparison

Laboratory code	PT 2011	PT 2012	PT 2013	PT 2014	PT 2015	PT 2016	PT 2017	PT 2018
NRL1	positive	positive	positive	positive	positive	negative	negative	positive
NRL2	-	-	-	negative	-	-	positive	positive
NRL3	negative	-	positive	positive	positive	positive	negative	positive
NRL4	positive	positive	positive	positive	-	positive	-	-
NRL6	positive	positive	negative	positive	negative	positive	positive	positive
NRL7	-	negative	negative	positive	positive	positive	positive	positive
NRL8	positive	positive	positive	positive	positive	positive	positive	positive
NRL9	negative	-	negative	-	-	-	-	-
NRL10	negative	positive	-	positive	positive	positive	positive	positive
NRL11	positive	negative	positive	positive	-	-	-	-
NRL12	-	-	positive	-	positive	positive	positive	positive
NRL14	-	-	-	-	-	negative	negative	negative
NRL16	positive	positive	positive	positive	positive	positive	negative	positive
NRL17	-	-	-	positive	positive	negative	positive	positive
NRL18	-	-	-	-	-	-	positive	-
NRL20	-	-	-	-	-	-	-	negative
NRL21	positive	negative	positive	positive	positive	negative	positive	positive
NRL22	-	positive	negative	negative	positive	positive	positive	negative
NRL23	-	positive	positive	positive	positive	positive	positive	positive
NRL24	negative	-	positive	positive	positive	positive	positive	positive
NRL25	-	-	positive	positive	positive	negative	positive	positive
NRL34	negative	negative	positive	positive	positive	negative	-	-
NRL35	negative	negative	-	negative	positive	negative	positive	positive
NRL40	-	positive	-	-	-	-	positive	positive
NRL42	-	-	-	negative	-	-	negative	-
NRL44	-	-	-	negative	-	-	-	-
NRL45	-	-	-	-	negative	-	-	-
NRL46	-	-	-	-	-	-	positive	-
TLE6						positive	positive	negative

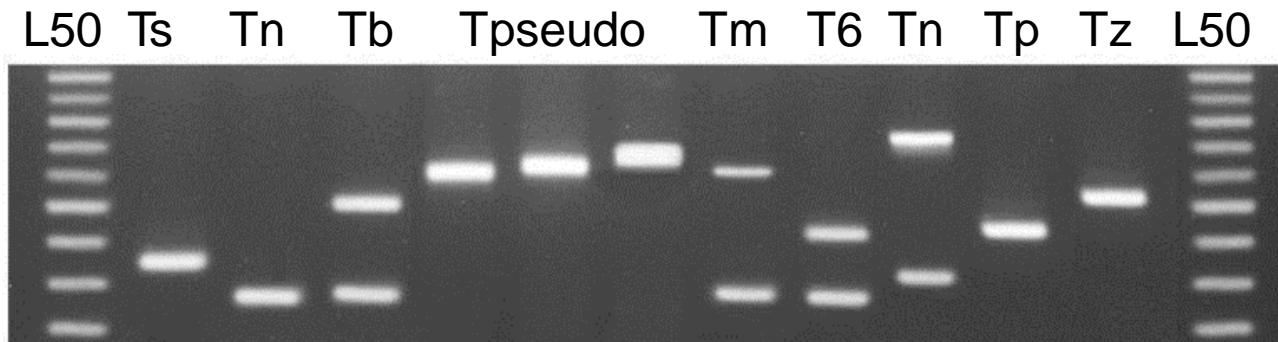
# Results

## Overtime comparison



# Conclusions

The main problem continue to be the wrong interpretation of the electrophoretic band patter due to the lack of experience (especially for the non European species)



2% agarose gel

# Thanks for your attention

