Workshop of National Reference Laboratories for Parasites

Istituto Superiore di Sanità, Rome, Italy, 23-24 May, 2019

Proficiency testing on

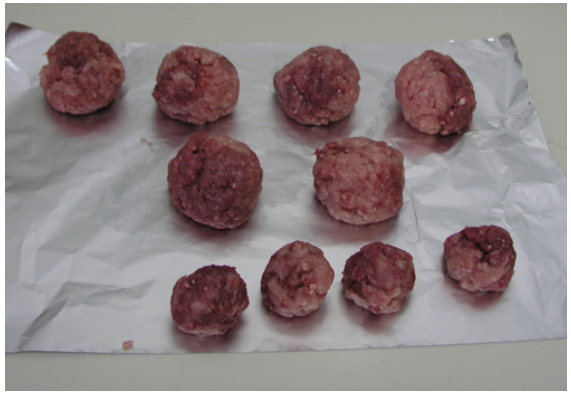
"Digestion method to detect *Trichinella* larvae in meat samples according to the EU directive 2015/1375"

Materials and methods

Participants: 32 NRLs (including 4 outside EU)

PT sample panel composition

3 positive samples
4 larvae for each sample



Results

Analyzed samples types

	Pork 100 g	Pork 35 g	Horse meat 100 g	Horse meat 35 g
N° of laboratories	26	5	1	0

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

Qualitative results

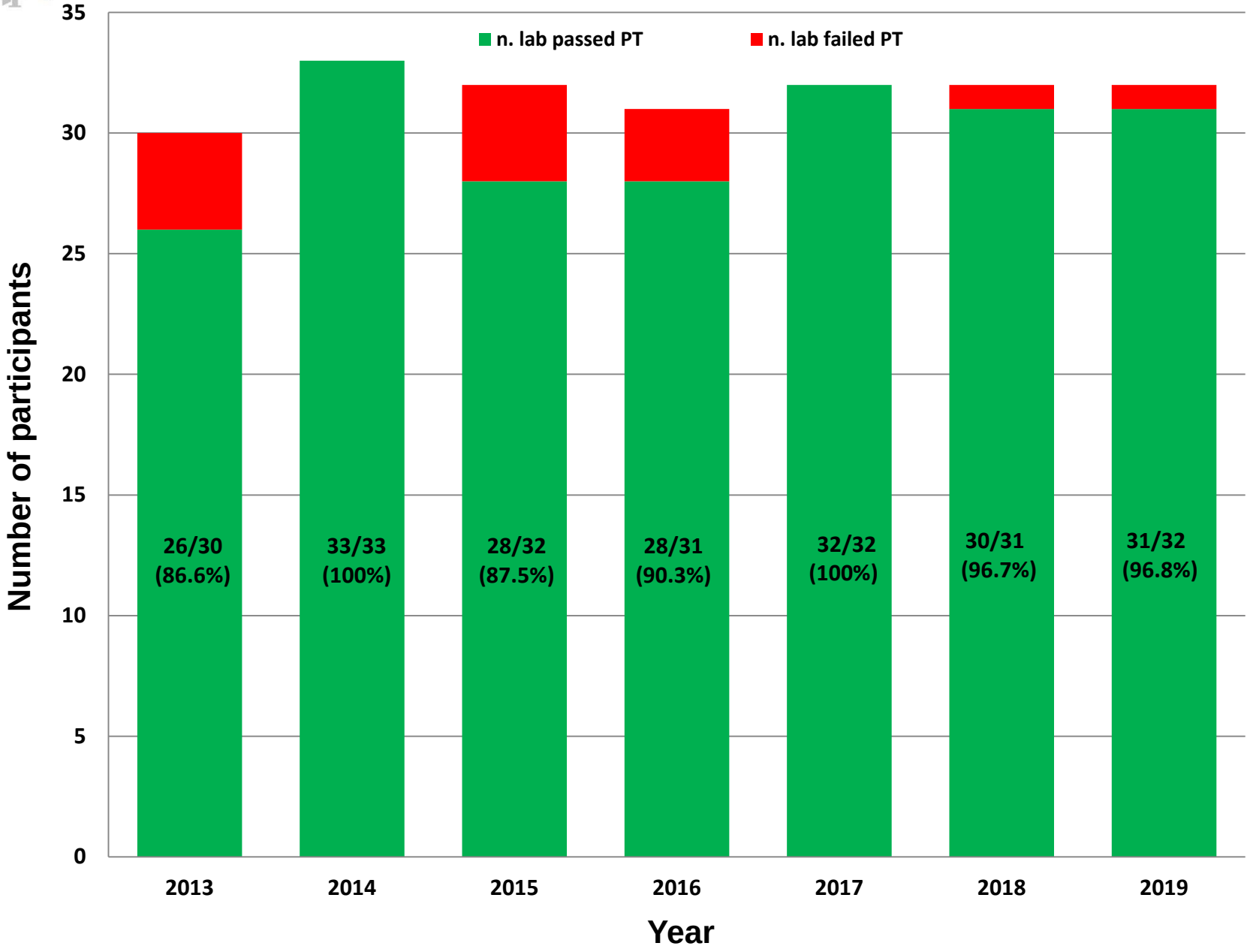
Lab code	False negatives	False positives	PT final evaluation
NRL1	0	0	positive
NRL2	0	0	positive
NRL3	0	0	positive
NRL4	0	0	positive
NRL5	0	0	positive
NRL6	0	0	positive
NRL7	0	0	positive
NRL8	0	0	positive
NRL9	0	0	positive
NRL10	0	0	positive
NRL11	0	0	positive
NRL12	0	0	positive
NRL13	0	0	positive
NRL14	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	0	0	positive
NRL24	0	0	positive
NRL25	0	0	positive
NRL26	2	0	negative
NRL35	0	0	positive
NRL40	0	0	positive
NRL41	0	0	positive
NRL42	0	0	positive
NRL47	0	0	positive
TLE6	0	0	positive

Overtime comparison

NRL code	2013	2014	2015	2016	2017	2018	2019
1	positive	positive	positive	negative	positive	positive	positive
2	positive	positive	positive	positive	positive	positive	positive
3	positive	positive	positive	positive	positive	positive	positive
4	positive	positive	positive	positive	positive	positive	positive
5	positive	positive	positive	positive	positive	positive	positive
6	positive	positive	positive	positive	positive	positive	positive
7	positive	positive	positive	positive	positive	positive	positive
8	positive	positive	positive	positive	positive	positive	positive
9	positive	positive	positive	positive	positive	positive	positive
10	positive	positive	positive	positive	positive	positive	positive
11	positive	positive	positive	positive	positive	positive	positive
12	positive	positive	positive	positive	positive	positive	positive
13	positive	positive	positive	positive	positive	positive	positive
14	positive	positive	positive	positive	positive	positive	positive
15	negative	positive	positive	negative	positive	positive	positive
16	negative	positive	positive	positive	positive	positive	positive
17	positive	positive	positive	positive	positive	positive	positive
18	negative	positive	positive	positive	positive	positive	positive
19	positive	positive	negative	positive	positive	positive	positive
20	positive	positive	positive	positive	positive	positive	positive
21	positive	positive	positive	positive	positive	positive	positive
22	positive	positive	positive	positive	positive	positive	positive
23	positive	positive	negative	positive	positive	negative	positive
24	positive	positive	positive	positive	positive	positive	positive
25	positive	-	negative	positive	positive	positive	positive
26	positive	positive	positive	negative	positive	positive	negative
34	positive	positive	positive	positive	positive	positive	positive
35	-	positive	negative	positive	positive	positive	positive
40	positive	positive	positive	positive	positive	positive	positive
41	positive	positive	positive	-	positive	positive	positive
42	negative	positive	positive	positive	positive	positive	positive
43	-	positive	-	-	-	-	-
44	-	positive	-	-	-	-	-
47	-	-	-	-	-	-	positive
TLE6	-	positive	positive	positive		positive	

Overtime comparison



Conclusions

- 31 out of 32 laboratories (96,8%) passed the PT
- One lab reported in one sample more larvae than expected, the origin of the extra larvae recovered is still under investigation

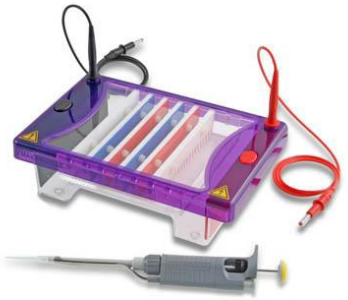
Will we ever be able to maintain 100% positive results overtime?

Could the sporadic cases of PT failure be related to staff turnover?

Workshop of National Reference Laboratories for Parasites
Istituto Superiore di Sanità, Rome, Italy, 23-24 May, 2019

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: 23 NRLs (including 2 outside EU)
- PT item: pool of 10 larvae of 4 different species
- Methods: any molecular method able to discriminate the *Trichinella* species

Materials and methods

PANEL COMPOSITION (4 samples)			
Species	**N° of vials**	**N° of larvae for each vial**	**Evaluation criteria**
T. spiralis	1	10	Correct identification of the 4 species
T. nativa	1	10	
T. britovi	1	10	
T6 genotype	1	10	

Possibility to analyse 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	Correct id.	Incorrect id.	Final evaluation
NRL1	Multiplex PCR	4	0	positive
NRL2	Multiplex PCR	4	0	positive
NRL3	Multiplex PCR	3	1	negative
NRL6	5S sequencing	4	0	positive
NRL7	Multiplex PCR	4	0	positive
NRL8	Multiplex PCR	4	0	positive
NRL10	Multiplex PCR	4	0	positive
NRL12	Multiplex PCR	4	0	positive
NRL13	Multiplex PCR	4	0	positive
NRL14	Multiplex PCR	2	2	negative
NRL16	Multiplex PCR	4	0	positive
NRL17	Multiplex PCR	4	0	positive

Lab code	Method used	Correct id.	Incorrect id.	Final evaluation
NRL18	Multiplex PCR	4	0	positive
NRL20	Multiplex PCR	4	0	positive
NRL21	Multiplex PCR	4	0	positive
NRL22	Multiplex PCR	4	0	positive
NRL23	Multiplex PCR	4	0	positive
NRL24	Multiplex PCR	4	0	positive
NRL25	Multiplex PCR	4	0	positive
NRL35	Multiplex PCR	4		positive
NRL40	5S sequencing	3	1	negative
NRL47	In house pcr and sequencing	4	0	positive
TLE6	Multiplex PCR	4	0	positive

Incorrect identifications

- *T. nativa* identified as *T. paupae*

Lab reported too many PCR bands than expected, probably non-specific reaction

- Incorrect identification of T6 genotype that was reported as *T. nativa* (identification based on 5S and COI seq)

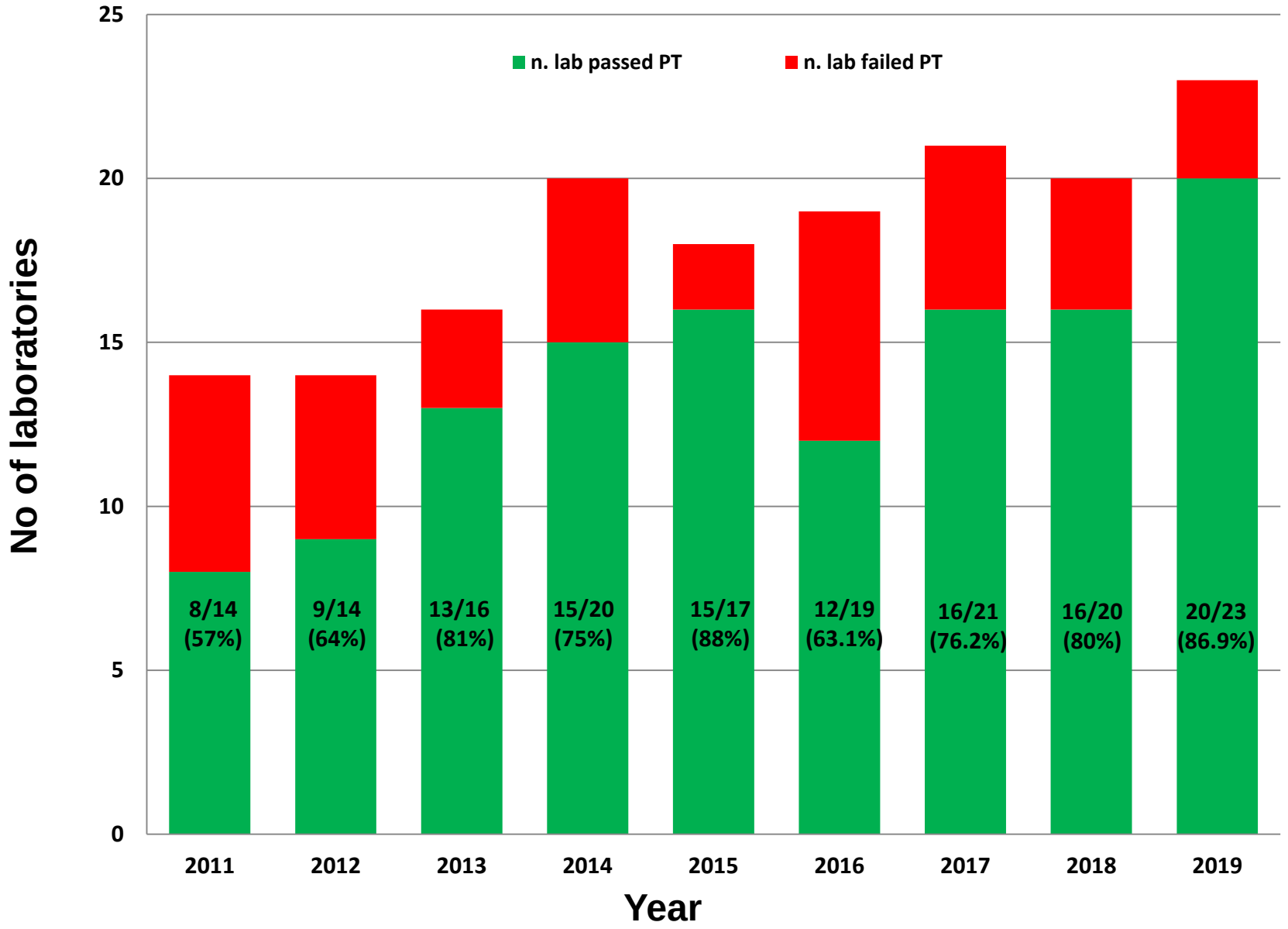
- Incorrect identification of *T. nativa* and T6, both reported as *T. britovi*

The lab obtained missed or aspecific amplifications even with positive controls

	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



Conclusions

- The most part of participants demonstrated to be able to correctly identify the *Trichinella* species working with pool or single larvae
- The multiplex PCR continue to be the most used method probably because of the low requirements in term of cost and staff experience comparing to PCR and sequencing approach
- Biomolecular techniques requires a fine tuning before their use in routine analysis and this is particularly true for the multiplex PCR, because of the presence of several couples of primers in the same reaction

Thanks for your attention