DEPARTMENT
INFECTIOUS DISEASES

XIX Workshop of National Reference Laboratories for Parasites
Istituto Superiore di Sanità
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Proficiency testing on *Trichinella* larvae identification at species level by a molecular method

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Purpose and participants

Purpose: to test the capacity of NRLs to identify *Trichinella* larvae at the species level

Participants: **23 NRLs** (including 4 outside EU)

Test material

PT item: *Trichinella* muscle larvae preserved in 96% ethanol

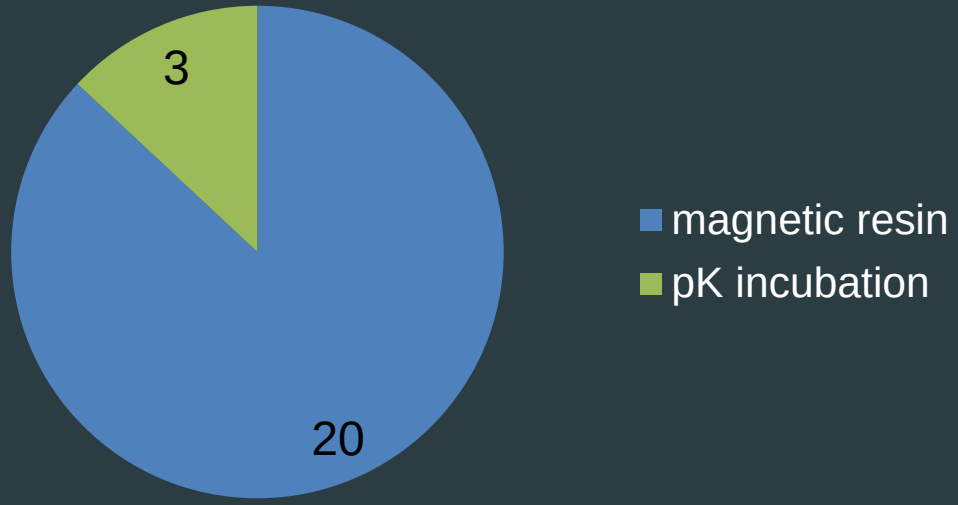
PANEL COMPOSITION (4 samples)		
Species	**Larvae per vial**	**Evaluation criteria**
T. spiralis	10	Correct identification of all four species
T. nativa	10	
T. britovi	10	
T. pseudospiralis	10	

Test method:

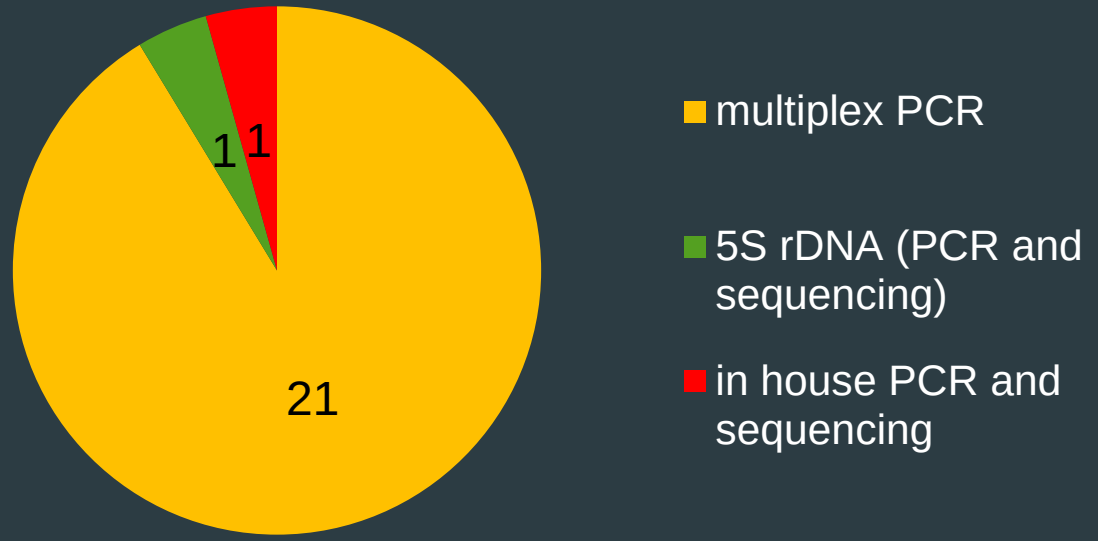
- Any molecular method able to discriminate the *Trichinella* species or genotype was allowed
- Possibility to analyse larvae singularly or as pool, depending on the sensitivity of the method used or on the experience of the technical staff

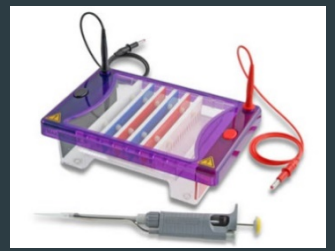
Samples and methods

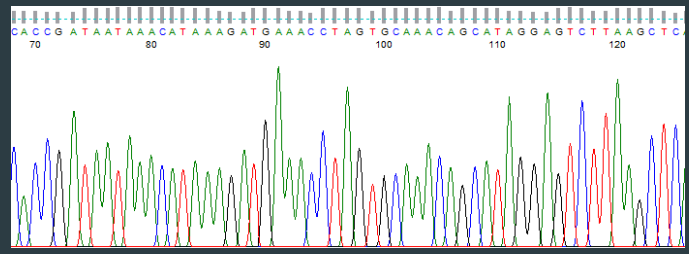
DNA purification methods



Detection methods used for larvae identification at species level







Results

Laboratory code	Right identification	Wrong identification	Missed identification	Final evaluation
TM1	4	0	0	positive
TM9	4	0	0	positive
TM10	4	0	0	positive
TM16	4	0	0	positive
TM17	3	1	0	negative
TM19	4	0	0	positive
TM20	4	0	0	positive
TM21	4	0	0	positive
TM25	4	0	0	positive
TM26	4	0	0	positive
TM29	4	0	0	positive
TM30	4	0	0	positive
TM32	4	0	0	positive
TM33	3	1	0	negative
TM34	4	0	0	positive
TM35	4	0	0	positive
TM36	4	0	0	positive
TM39	4	0	0	positive
TM40	4	0	0	positive
TM43	4	0	0	positive
TM44	4	0	0	positive
TM53	4	0	0	positive
TM54	4	0	0	positive

Incorrect identifications

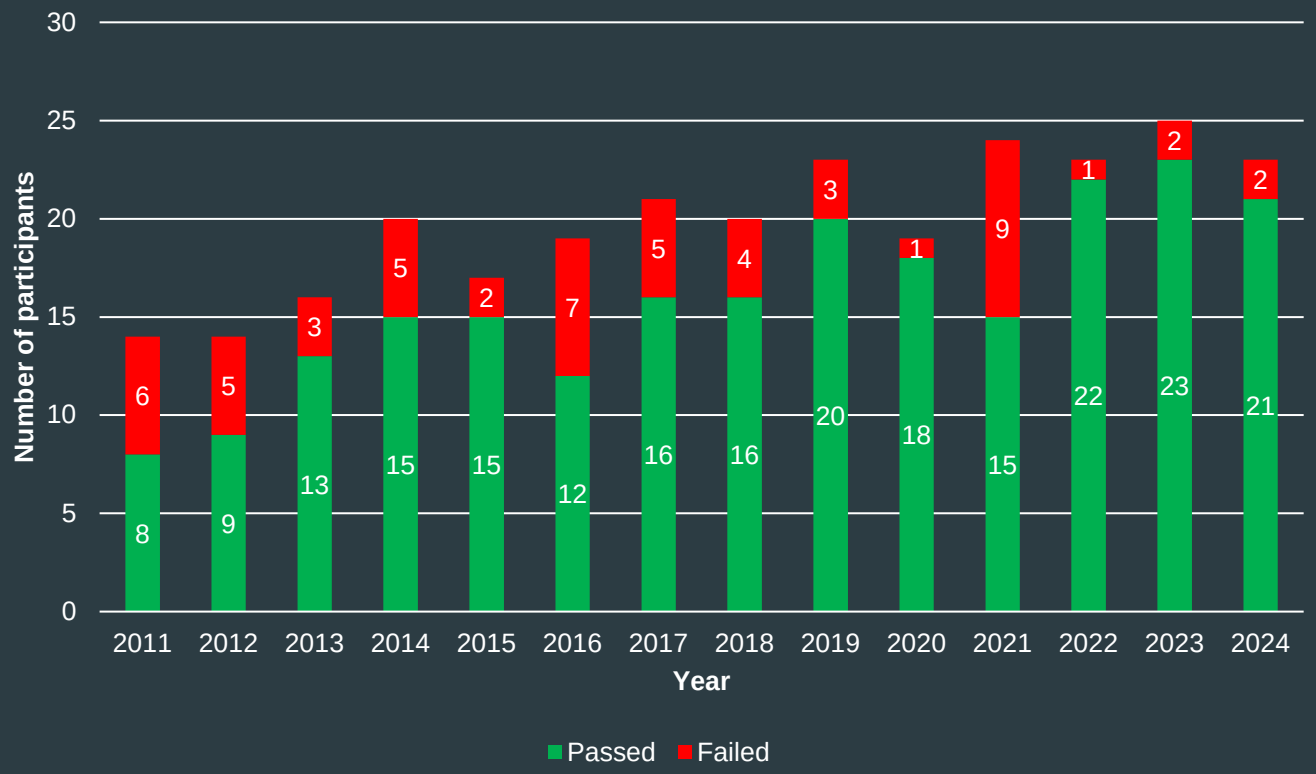
Lab	Reported species	Correct species	Causes
TM17	T6 genotype	*T. nativa*	New DNA intercalating agent + too much PCR product loaded in the gel = non-specific amplification bands
TM33	*T. nelsoni*	*T. nativa*	Suboptimal setting of PCR reaction? Problems during capillary electrophoresis run?

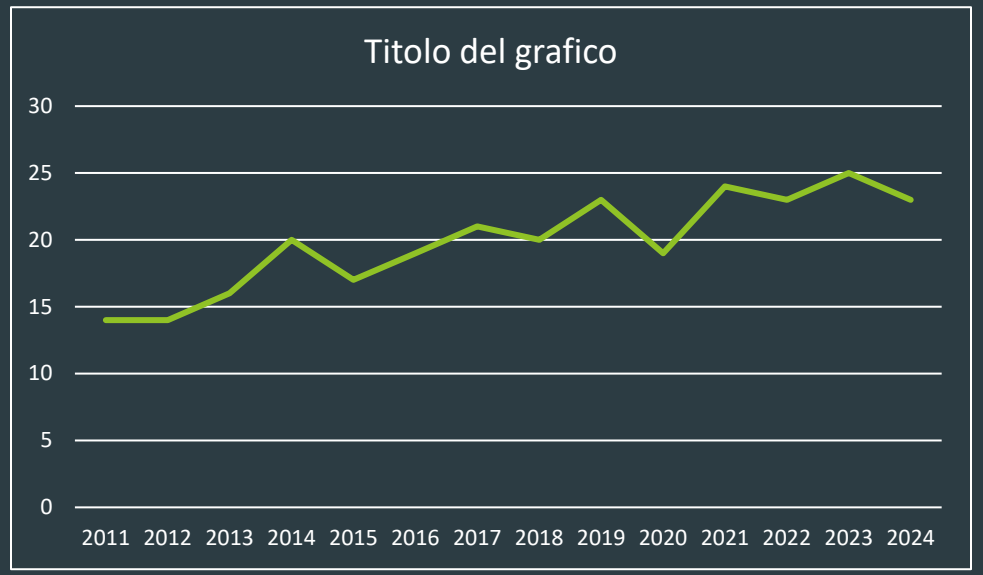
	T. spiralis	T. nativa	T. britovi	**T. pseudospiralis**	T. murrelli	Trichinella T6	T. nelsoni	T. papuae	**T. zimbabwensis**
ESV	173	127	127	**310-350**	127	127	155	240	**264**
ITS1-T. britovi			253						
ITS1-T6						210			
ITS2-T. murrelli					316				
ITS2-T. nelsoni							404		

Overtime comparison of results (last 5 years) by laboratory

Laboratory code	2020	2021	2022	2023	2024
TM1	-	positive	positive	positive	positive
TM9	-	positive	-	positive	positive
TM10	positive	positive	positive	positive	positive
TM16	-	positive	positive	positive	positive
TM17	positive	positive	positive	positive	negative
TM19	positive	negative	positive	positive	positive
TM20	positive	positive	positive	positive	positive
TM21	positive	positive	positive	negative	positive
TM25	positive	positive	positive	positive	positive
TM26	positive	positive	positive	positive	positive
TM29	positive	positive	positive	positive	positive
TM30	positive	positive	positive	positive	positive
TM32	-	positive	positive	positive	positive
TM33	-	negative	negative	negative	negative
TM34	-	negative	positive	positive	positive
TM35	positive	positive	positive	positive	positive
TM36	positive	negative	positive	positive	positive
TM39	negative	positive	positive	positive	positive
TM40	positive	negative	positive	positive	positive
TM43	positive	positive	positive	positive	positive
TM44	positive	positive	positive	positive	positive
TM53	positive	negative	positive	positive	positive
TM54	-	negative	positive	positive	positive

Overall overtime comparison by year





Increased number of participants linked to augmented number of NRLs that validated molecular method for *Trichinella* species identification

<u>Main failure reasons:</u>

- Mistakes in recognizing the specific band pattern of *Trichinella* species
- Use of suboptimal reagents
- Failure to optimize the PCR reaction or the electrophoretic run

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Conclusions

- Most part of participants used the EURLP method, based on DNA purification by magnetic beads and amplification by multiplex PCR, to identify larvae at species level

- Two laboratories failed the PT because of the misidentification of *T. nativa* probably due to lack of optimization of the PCR reaction and/or problems during the electrophoretic run

Thanks for your attention