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XVIII Workshop of National Reference Laboratories for Parasites
Istituto Superiore di Sanità
16-17 November 2023

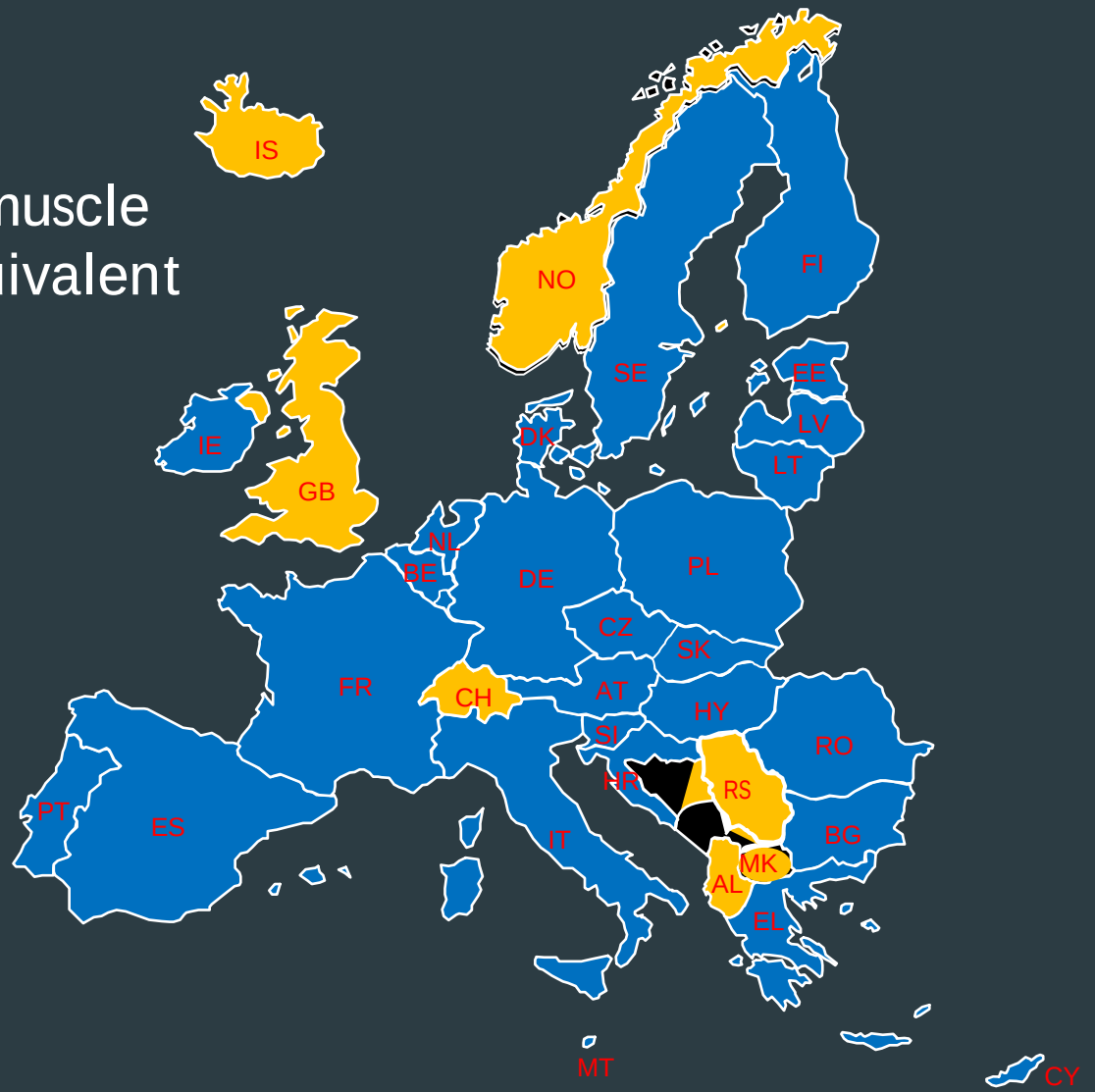
Proficiency testing on artificial digestion to detect *Trichinella* larvae in meat samples according to **ISO 18743:2015** or **Annex III Reg UE 2015/1375**

Purpose and participants

Purpose:

Test the capacity of NRLs to identify *Trichinella* muscle larvae in meat by applying ISO 18743:2015 or equivalent methods listed in reg. 2015/1375 (Annex III)

Participants: **33 NRLs** (including 7 outside EU)



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Test material

Meat type: pork and horse meat

Samples weight: 35g and 100g

PT panel composition: 1 positive sample (5 *T. spiralis* larvae) and 2 negative samples

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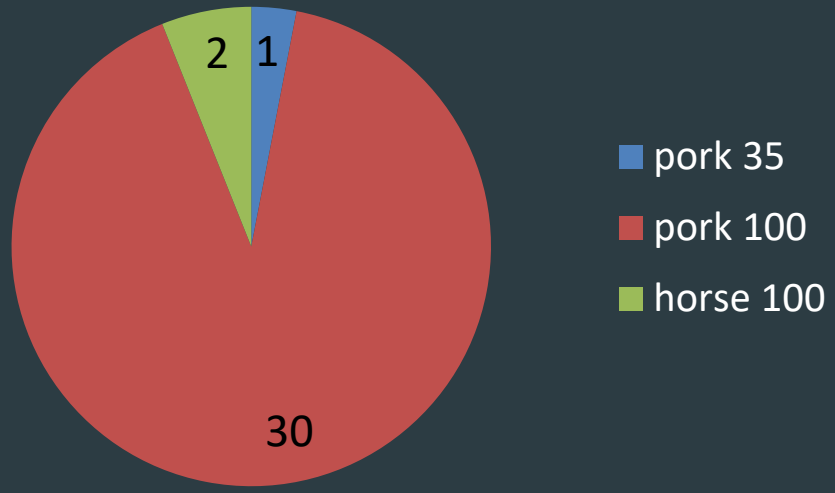
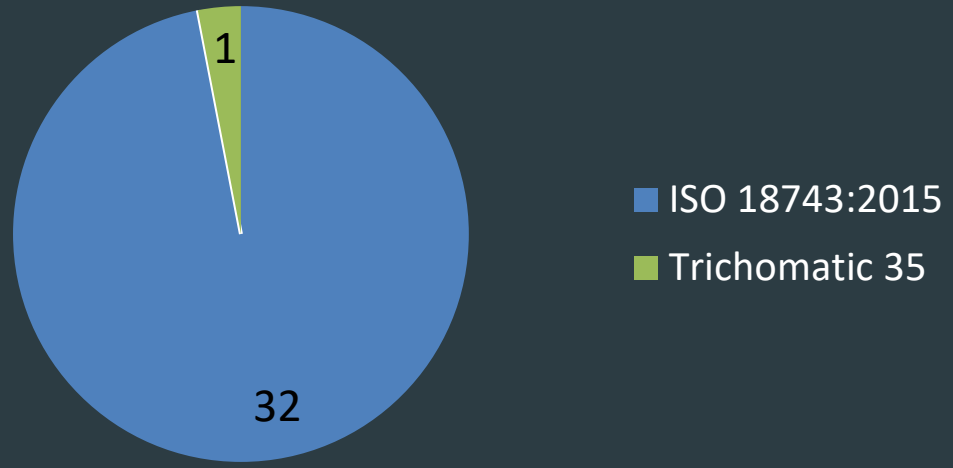
Samples and methods

Sample type



Method used



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Results

qualitative evaluation

Lab code	False negatives	False positives	PT final evaluation
NRL50	0	0	positive
NRL51	0	0	positive
NRL52	0	0	positive
NRL53	0	0	positive
NRL55	0	0	positive
NRL56	0	0	positive
NRL57	0	0	positive
NRL58	0	0	positive
NRL59	0	0	positive
NRL60	0	0	positive
NRL61	0	0	positive
NRL62	0	0	positive
NRL63	0	0	positive
NRL64	0	0	positive
NRL65	0	0	positive
NRL66	0	0	positive

Lab code	False negatives	False positives	PT final evaluation
NRL67	0	0	positive
NRL68	0	0	positive
NRL69	0	0	positive
NRL70	0	0	positive
NRL71	0	0	positive
NRL72	0	0	positive
NRL73	0	0	positive
NRL74	0	0	positive
NRL75	0	0	positive
NRL76	0	0	positive
NRL77	0	0	positive
NRL78	0	0	positive
NRL79	0	0	positive
NRL80	0	0	positive
NRL82	0	0	positive
NRL83	0	0	positive
TLE32	0	0	positive

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Overtime comparison

Last five years

Laboratory code		50	51	52	53	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	82	83	TLE32
Year	2019	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P	-
	2020	P	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	-	-
	2021	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	F	P	-
	2022	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NA	P	P	P	P	P	P	-
	2023	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

P = passed F = failed

Sporadic failures involving no more than one participant

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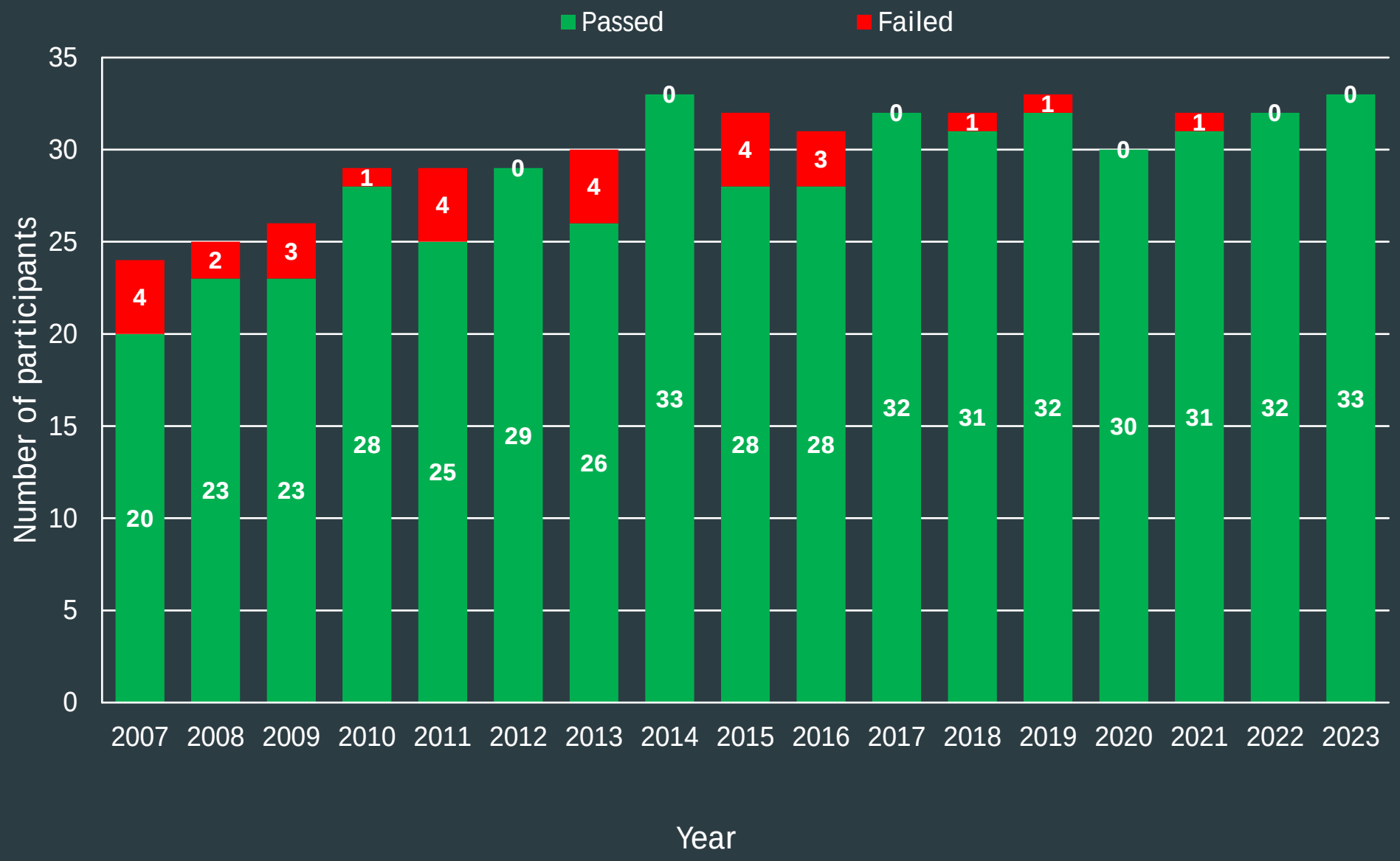
Overtime comparison



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Conclusions

All participanting laboratories successfully passed the PT demonstrating their competence in detecting Trichinella larvae in meat samples

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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: **25 NRLs** (including 5 outside EU)

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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. murrelli	10	
T. nelsoni	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

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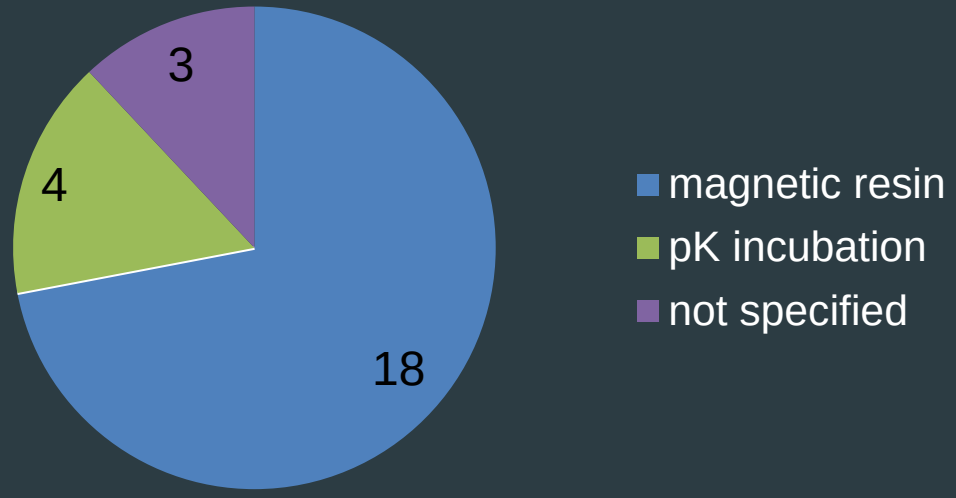
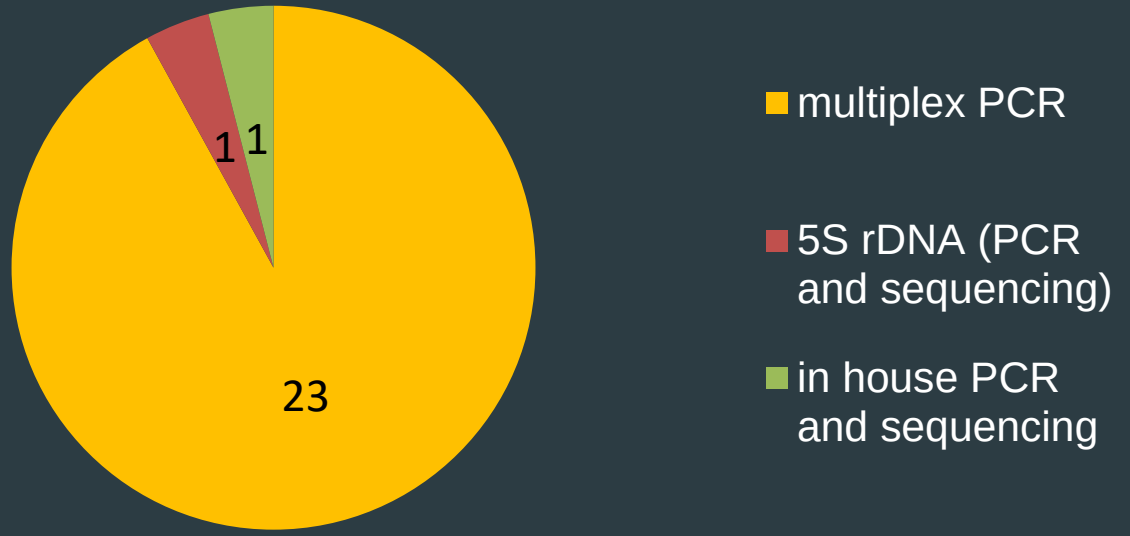
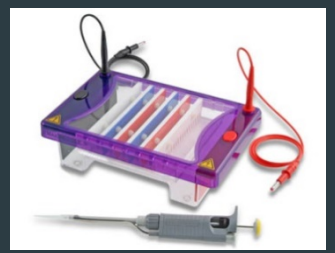
Samples and methods

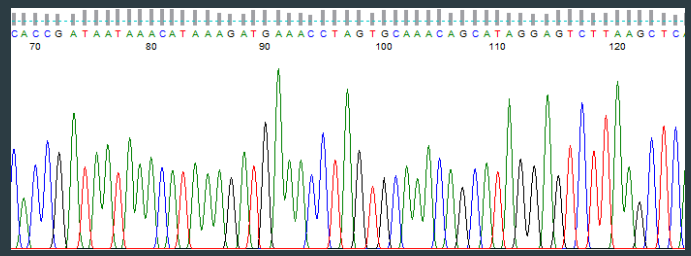
DNA purification methods



Detection methods used for larvae identification at species level







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Results

Laboratory code	Right identification	Wrong identification	Missed identification	Final evaluation
NRL50	4	0	0	positive
NRL51	4	0	0	positive
NRL52	2	2	0	negative
NRL55	4	0	0	positive
NRL56	4	0	0	positive
NRL57	4	0	0	positive
NRL59	4	0	0	positive
NRL61	4	0	0	positive
NRL62	4	0	0	positive
NRL63	4	0	0	positive
NRL65	4	0	0	positive
NRL66	4	0	0	positive
NRL70	4	0	0	positive
NRL71	4	0	0	positive
NRL72	4	0	0	positive
NRL73	4	0	0	positive
NRL74	4	0	0	positive
NRL76	4	0	0	positive
NRL77	3	1	0	negative
NRL78	4	0	0	positive
NRL79	4	0	0	positive
NRL80	4	0	0	positive
NRL82	4	0	0	positive
NRL83	4	0	0	positive
NRL84	4	0	0	positive

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Incorrect identifications

Lab	Reported species	Correct species	Causes
NRL77	*T. nativa*	*T. murrelli*	Missed amplification of the *T. murrelli* specific band because of suboptimal PCR mastermix
NRL52	*T. nativa*	*T. murrelli*	???
	T. spiralis	*T. nelsoni*	???

	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		

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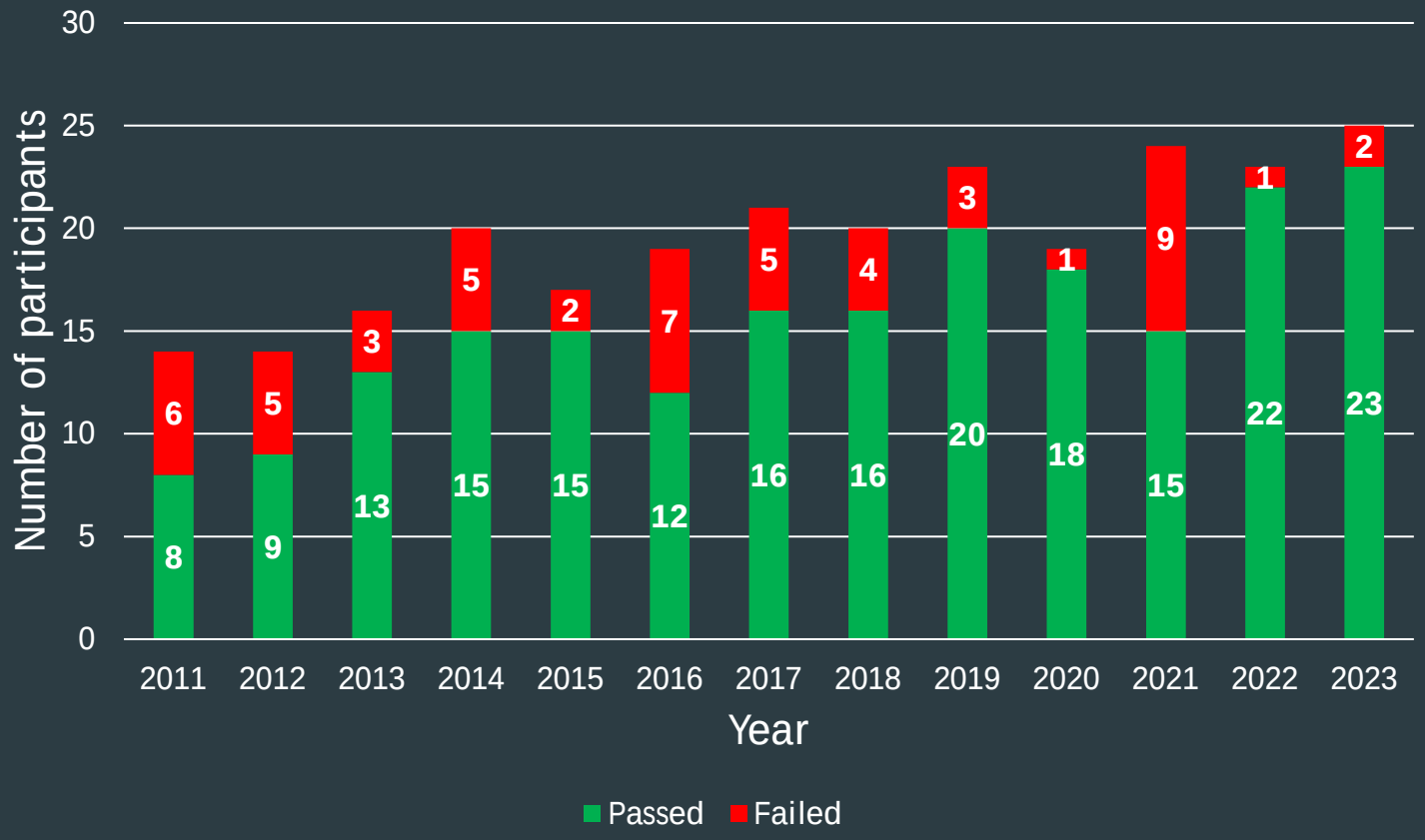

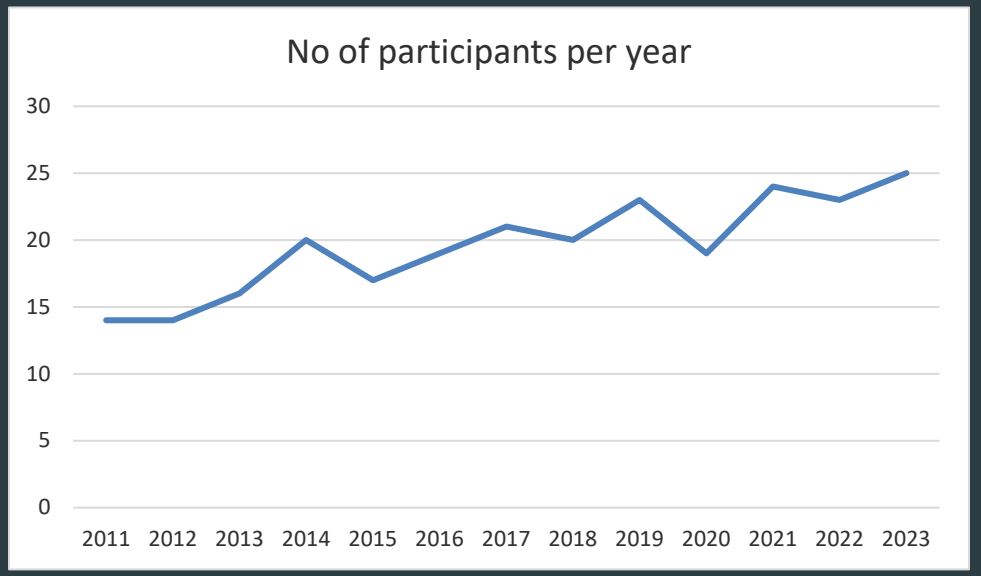

Increased number of participants linked to augmented number of NRLs that validated molecular method for *Trichinella* species identification

Failure reasons:

- Mistakes in recognizing the specific band pattern of non-European *Trichinella* species
- Use of suboptimal reagents

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Conclusions

- DNA purification was done mainly by commercial kits based on magnetic beads and the multiplex PCR was the most used method to identify larvae at species level

- The laboratories that failed the PT misidentified *T. murrelli* and *T. nelsoni* the two non-European species present in the PT panel most probably because of the use of suboptimal reagents and lack of experience in recognizing the specific band pattern

Thanks for your attention