Detection of Anisakidae L3 larvae in fish fillets

PT Results of the German NRL for Anisakis

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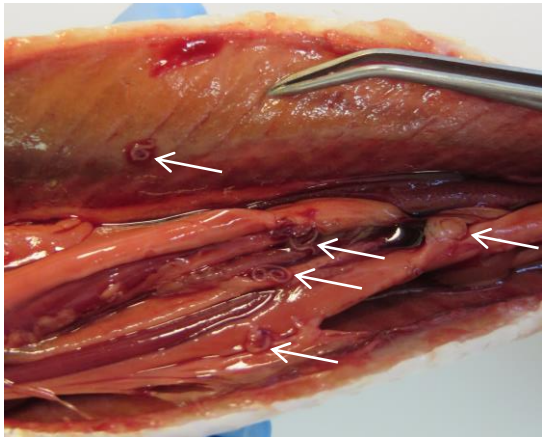
Preparation of the proficiency samples

Isolation of Anisakidae L3 larvae

Living Anisakis larvae were isolated from the intestines of fresh North Sea herring (*Clupea harengus*) and Pseudoterranova from fresh smelt (*Osmerus eperlanus*).

The nematodes are killed by freezing for easier spiking.



Spiking fish samples

- Dead larvae were added to the skinned fillets of farmed fresh fish by scoring the musculature with a scalpel and placing the larvae in the resulting pockets.
- Spiked fillets were individually packed in PE bags.
- The samples were stored at -24° C until shipping.

7th Proficiency testing (2020)

Participants

- Candling & digestion method: 14 resp.18 labs from surveillance, trade and industry

Each participant got a description of the methods. Any deviation from the described method should be reported in a form.

Samples

- Candling & digestion method

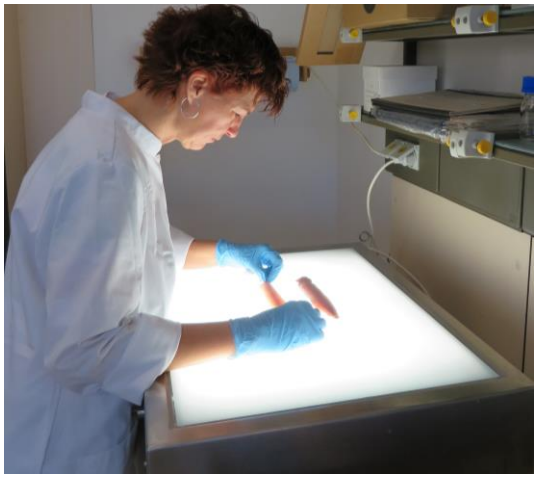
6 x ~100 g skinned fillets of farmed trouts (*Salmo trutta*)

Number of samples	1	1	1	1	1	1
Number of Anisakidae larvae	0	1 Pseudoterranova	1 Anisakis	3	5	10

The samples must be stored deep-frozen until the test is performed.

Participants are requested to examine the samples within two months.

Results evaluation



- **Candling**

  Evaluation of candling results was only qualitative.

- **Digestion and UV-press method**

➢ Sample without larvae

Results evaluation was qualitatively and reported as correct or false-positive.

➢ Low spiked samples (n = 1 to 4 nematodes)

Results evaluation was qualitatively and reported as correct, false-negativ, sub- or excess-finding.

Tolerance range (setpoint 3 larvae): 1-4; Tolerance range (setpoint 4 larvae): 2-6

➢ High spiked samples (n ≥ 5 nematodes)

A quantitative evaluation was made based on the calculation of the z-score.

7th Proficiency testing (2020)

Results

- Candling (14 participants)

Sample	0 NL	1 Pseudo.	1 Anis.	3 Anis.	5 Anis.	10 Anis.
	Number of participants					
correct	13	9	2	2	1	1
false-negative		5	11	8	7	5
false-positive	1					
sub-finding				4	6	8
excess-finding			1			

- Pseudoterranova was clearly better detected than Anisakis.
- From a total of 280 added nematodes, only 76 were recovered (Recovery 27%).
- No nematodes were observed in 51% of all spiked samples.

7th Proficiency testing (2020)

- Digestion (18 participants)

Sample	0 NL	1 Pseudo.	1 Anis.	3 Anis.	5 Anis.	10 Anis.
	Number of participants					
correct	15	16	15	12	11	9
false-negative			3			
false-positive	3					
sub-finding				6	7	9
excess-finding		2				
					1 border. 1 outside	

With two exceptions, the results for samples with 3 to 10 NL were within the tolerance range.

One result was in the borderline range and one was outside the acceptable limits.

- 2 labs correctly identified all samples
- 7 labs correctly identified 5 samples
- 6 labs correctly identified 4 samples
- 1 lab correctly identified 3 samples
- 2 labs correctly identified 2 samples

8th Proficiency testing (2022)

Participants

- Candling & digestion method: 14 resp. 18 German labs from surveillance, trade & industry
  + 1 Polish lab (industry)
- UV-press method; 3 German labs (surveillance)

Samples

- Candling & digestion method: 6 x ~100 g skinned trout fillets (*Salmo trutta*)

ISO 23036-2: 2021 Microbiology of the food chain – methods for the detection of Anisakidae L3 larvae in fish and fishery products - Part 2: Artificial digestion method

Number of samples	1	1	2	1	1
Number of Anisakidae larvae	0	1	3	4	11 (10 Anis. + 1 Pseudo.)

- UV-press method: 4 x ~80 g skinned gilthead seabream fillets (*Sparus aurata*)

ISO 23036-1: 2021 Microbiology of the food chain – methods for the detection of Anisakidae L3 larvae in fish and fishery products – Part 1: UV-press method

Number of samples	1	1	1	1
Number of Anisakidae larvae	0	1	2 (1 Anis. + 1 Pseudo.)	5

8th Proficiency testing (2022)

Results

- Candling (14 participants)

Sample	0 NL	1 Anis.	3 Anis.	3 Anis.	4 Anis.	11 (10 Anis. + 1 Pseudo.)
	Number of participants					
correct	14	1	1			
false-negative		12	8	9	12	6
false-positive						
sub-finding			5	5	2	8
excess-finding		1				

- In no case nematodes were assumed in the control sample (0 NL).
- Only 53 of the 308 added nematodes were found (Recovery 17%).
- No nematodes were observed in 67% of all spiked samples.

8th Proficiency testing (2022)

- Digestion (19 participants)

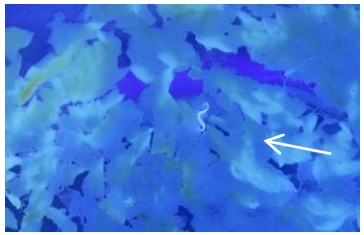
Sample	0 NL	1 Anis.	3 Anis.	3 Anis.	4 Anis.	11 (10 Anis. + 1 Pseudo.)
	Number of participants					
correct	18	13	6	12	5	5
false-negative		1	2	1	1	
false-positive	1					
sub-finding			9	5	9	12
excess-finding		5	2	1	4	2
			2 outside	2 outside	4 outside	5 borderline 2 outside

- DIN method: 9 labs without any changes, 3 labs extended the digestion time to 60 min or 120 min.
- 7 labs worked according to their former method.
- The most correct results were given by the labs, that did not work according to the DIN procedure, but according to their old familiar method.
- Only one lab correctly identified all samples.

8th Proficiency testing (2022)

- UV-Press method (3 participants)



Sample	0 NL	1 Anis.	2 (1 Anis. + 1 Pseudo.)	5 Anis.
	Number of participants			
correct	3	2	3	3
excess-finding		1		

Overview of the correct results (2022)

No. correct samples - Digestion	No. of labs	No. correct samples – UV-press	No. of labs
6	1 (5.3%)	4	2 (67%)
5	1 (5.3%)	3	1 (33%)
4	5 (26.3%)	2	0
3	6 (31.6%)	1	0
2	4 (21.1%)	0	0
1	2 (10.5%)		
0	0		

Discussion

There are still problems with the digestion method.

Critical points

- Performance of the digestion
  - temperature
  - stirring speed
  - digestion time
  - ratio of fish to digestion solution
  - pepsin activity
- Complete transfer of the nematode larvae from the sieve to the counting basin
- Interpretation of fragments
- Verification by microscopic examination
  - differentiation between head, tail and middle pieces
  - knowledge of the appearance of nematodes
  - differentiation between bones and nematodes
- Training of staff