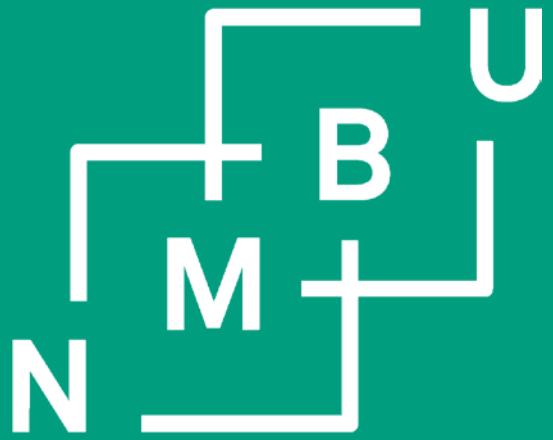
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Parasite contamination of leafy greens and berries in Norway

Norwegian University of Life Sciences

Faculty of Veterinary Medicine, Parasitology Unit

Ingrid Olstad, November 2024

Who are we?

Photo: NMBU

- Research, teaching veterinary students/nurses and diagnostics
- NRL: fresh produce, drinking water and honeybee health regarding parasites

Contamination of leafy greens and berries - what are the big questions?

- What parasites are present on leafy greens and berries in Norway?
- Pathogen surveillance

Photo: Shutterstock

Leafy geens and berries as vehicles for parasites

- «Sticky» parasite transmission stages
- Berry surfaces
- Environmental sources of contaminants

Photo: Shutterstock

Which parasites to focus on?

- The Norwegian Scientific Committee for Food and Environment (VKM): risk ranking of foodborne pathogens with highest public health impact (2021)
  - 1st place: *Toxoplasma* gondii
  - (2nd place: *Campylobacter* spp.)
  - 3rd place: *Echinococcus multilocularis*
  - (…)
  - 9th place: *Cryptosporidium* spp.

  - *Cyclospora cayetanensis*

Implementation of parasite screening of leafy greens and berries

- The PARA-BERRY project as a part of a PhD project by Tamirat Tefera Temesgen: *Detection and viability assessment of foodborne parasites of public health importance on berries* (2018-2021)
- IMPACT project (consortium including ISS and NMBU): *Molecular detection of Cryptosporidium oocysts in leafy greens by real-time PCR*
- Training at ISS
- Norwegian distributor of leafy greens and berries send samples for diagnostic screening (2022, 2023, 2024)

Method – berry samples

Tamirat washing berries. Photo: NMBU

30 g of berries

↓

Wash berries with 200 ml
0.1 % Alconox solution

↓

Shake for 10 minutes

↓

Concentration by centrifugation

↓

DNA isolation from pellet, using
DNeasy PowerSoil Kit

↓

Multiplex qPCR: *Toxoplasma gondii*,
Echinococcus multilocularis, *Cyclospora cayetanensis*
Simplex qPCR: *Cryptosporidium* spp.

Method – salad samples

Photo: Shutterstock

30 g of salad

↓

Add 200 ml Glycine buffer
(1 M, pH = 5.5) into stomacher bag

↓

Vigorous washing using a
paddle beater

↓

Concentration by centrifugation

↓

DNA isolation from pellet, using
DNeasy PowerSoil Kit

↓

Multiplex qPCR: *Toxoplasma gondii*, *Echinococcus multilocularis*, *Cyclospora cayetanensis*
Simplex qPCR: *Cryptosporidium* spp.

What do we find? – PARA-BERRY project

- Samples collected 2019/2020
- 820 samples of berries analysed (blueberries/raspberries/strawberries)
- Overall occurrence was:
  - 2.9% for *T. gondii*
  - 6.6% for *C. cayetanensis*
  - 8.3% for *Cryptosporidium* spp.
  - *E. multilocularis* was not detected

Berry type	No. analysed (% of total)	Parasites detected; number positive (%)			
		Echinococcus multilocularis	*Toxoplasma gondii*	*Cyclospora cayetanensis*	*Cryptosporidium* spp.
Blueberries	274 (33.4)	0	4 (1.5)	15 (5.5)	9 (3.3)
Raspberries	276 (33.7)	0	10 (3.6)	24 (8.7)	26 (9.4)
Strawberries	270 (32.9)	0	8 (2.9)	13 (4.8)	33 (12.2)
Total	820	0	24 (2.9)	52 (6.6)	68 (8.3)

Temesgen TT, Stigum VM, Robertson LJ. (2022). Surveillance of berries sold on the Norwegian market for parasite contamination using molecular methods. *Food Microbiol.* **104**:103980

What do we find? – PARA-BERRY project

Country	Blueberries		Raspberries		Strawberries	
Belgium					32	1 Toxo; 2 Crypto
Chile	28	1 Toxo; 1 Cyclo; 1 Crypto				
Egypt					14	3 Crypto
Morocco	26	1 Toxo	82	4 Cyclo; 18 Crypto	2	2 Crypto
Netherlands	6	3 Cyclo	2	1 Toxo	108	2 Toxo; 4 Crypto
Norway			20	1 Toxo; 1 Crypto	66	4 Toxo; 1 Cyclo; 19 Crypto
Peru	116	1 Toxo; 8 Cyclo; 3 Crypto				
Poland	46	1 Cyclo			10	1 Toxo; 2 Crypto
Portugal	6		122	8 Toxo; 9 Cyclo; 6 Crypto		
South Africa	34	2 Cyclo; 5 Crypto				
Spain			50	11 Cyclo; 1 Crypto	38	1 Crypto
Sweden	2					
Zimbabwe	10	1 Toxo				

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What do we find? – Routine diagnostics

Samples from 2022 (berries):

- 7 samples of berries analysed
- All samples were negative

Strawberries for sale. Photo: Shutterstock

What do we find? – Routine diagnostics

Samples from 2023 (berries):

- 15 samples of berries
- Two raspberry samples (Spain + Portugal) positive for *T. gondii*

Samples from 2024 (berries + salad):

- 7 samples of berries and 8 samples of salad
- One raspberry sample (Morocco) was positive for *T. gondii* and *Cryptosporidium* spp.
- One iceberg lettuce sample (Spain) was positive for *Cryptosporidium* spp.

Iceberg lettuce. Photo: Shutterstock

What does the results mean?

- Positive qPCR results = DNA detected
- Contamination?!
- DNA from parasites are not uncommon on berries, regardless of origin

Strawberry field in Norway. Photo: Shutterstock

The way forward

- The industry decides what to do
- Knowledge is key –
  diagnostics is a part of the puzzle to secure food safety
- Awereness on foodborne parasites

Photo: Shutterstock

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