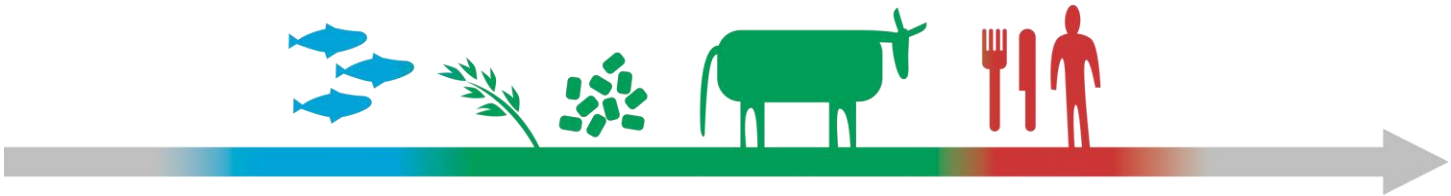
Veterinærinstituttet
Norwegian Veterinary Institute

One HEALTH EJP

AIR-SAMPLE

A low-cost screening tool in biosecured broiler production

Gro S. Johannessen/Food Safety and Animal Health Research/Norwegian Veterinary Institute



AIR SAMPLE

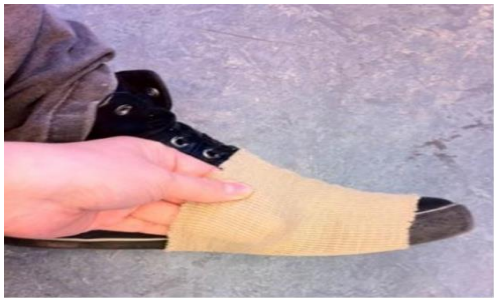
- Total funding: 620k Euro
- Period: 2 years (Jan 2018 - Dec. 2019), extended to 30.11.2020
- Coordinator: Jeffrey Hoorfar, DTU Food.

- Partners:

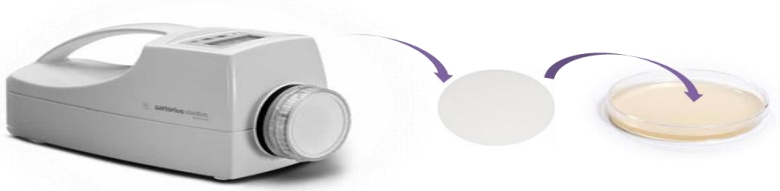
Julia Christensen & Jeffrey Hoorfar, DTU Food.	Denmark
Gro Johannessen, Mona Torp & Camilla Sekse, NVI.	Norway
Renáta Karpíšková & Ivana Koláčková, VRI.	Czech Republic
Kinga Wieczorek & Jacek Osek, NVRI.	Poland
Elisabetta Di Giannatale & Giuliano Garofolo, IZSAM.	Italy

Pre-slaughter sampling for *Campylobacter*

Current method: boot swabs or fecal droppings



Future method: air sampling



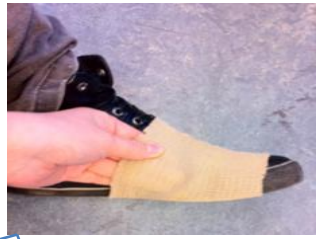
What have we done?

- Pilot study in 2018; testing the method and finalizing protocol, 5 countries
- Main study in 2019; multi-country study across Europe, 5 countries
- Metagenomics

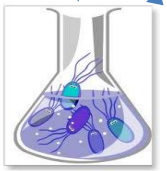
Pilot study 2018

Experimental set-up

Air flow rate 50 l/min,
15 min, total 750 l air



ISO 10272-1:2017,
Enrichment using
Bolton broth



Real-time PCR; enrichment/direct
(voluntary)

Selective plating

Confirmation of colonies with
PCR or Maldi-TOF

Results

Number of positive samples:

Country	No. of flocks sampled	Cultivation method				PCR methods			
		Boot socks		Air samples		Boot socks		Air samples	
		Direct	Enrich.	Direct	Enrich.	Direct	Enrich.	Direct	Enrich.
Italy	10	**6/10**	0/10	0/10	**1/10**	**7/10**	**5/5**	**8/10**	**5/5**
Czech rep.	10	ND*	0/10	ND	0/10	ND	0/10	**2/9**	0/10
Norway	10	ND	**3/10**	ND	0/10	ND	ND	ND	ND
Poland	8	ND	**3/8**	ND	**1/8**	ND	ND	**3/8**	ND
Denmark	6	ND	**1/6**	ND	**1/6**	ND	ND	**1/6**	ND

*ND = Not done

Not all samples have been tested with all methods.

Results cont.

- Cultivation methods
  - 7 of 44 boot socks positive on enrichment, only two had corresponding positive air samples
  - One positive air sample was negative on corresponding boot sock
- PCR method - examples
  - Four of five partners reported PCR results
  - 14 of 33 air samples screened directly returned positive PCR
  - Five of the 14 were positive both directly and after enrichment of air samples, but negative on the corresponding cultivation of air samples

Published in: Johannessen *et al.* 2020. *Campylobacter* in chicken – critical parameters for international, multicentre evaluation of air sampling and detection methods, Food Microbiol, 90, doi: 10.1016/j.fm.2020.103455

Other observations

- Agreed on using ISO 10272-1, but still variations among labs in how the method was carried out
  - Volume of Bolton broth added to samples; fixed volume (90, 100 and 250 ml) or 1:10 weight volume dilution
- Partners doing PCR used primer-probe combination from Josefsen et al (2014), but used different DNA extraction methods and PCR platforms

Main study 2019

- Discussed experiences from the pilot study and finalised a protocol
  - Use ISO 10272-1:2017 and test both Bolton and Preston protocols
    - Use agar medium of own choice in Bolton protocol
  - Real-time PCR on DNA extracted directly from airfilters
    - Use the same DNA extraction protocol as agreed upon after meeting in Oslo
    - Use the same reagents and master mix distributed from the Danish partner and the PCR platforms as per partner

Published in: Hoorfar *et al*. 2020. Foodborne *Campylobacter*: A multi-center proposal for a fast screening tool in biosecured chicken flocks. Appl Environ Microbiol. AEM.01051-20.
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Experimental set-up

Air flow rate 50 l/min,
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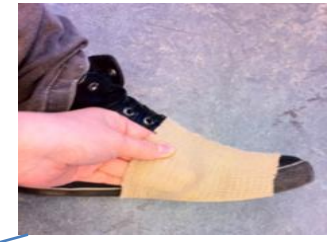
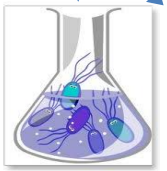


ISO 10272-1:2017,
Enrichment using
Bolton and Preston



Real-time PCR directly from air filters

Selective plating

Confirmation of colonies with
PCR or Maldi-TOF

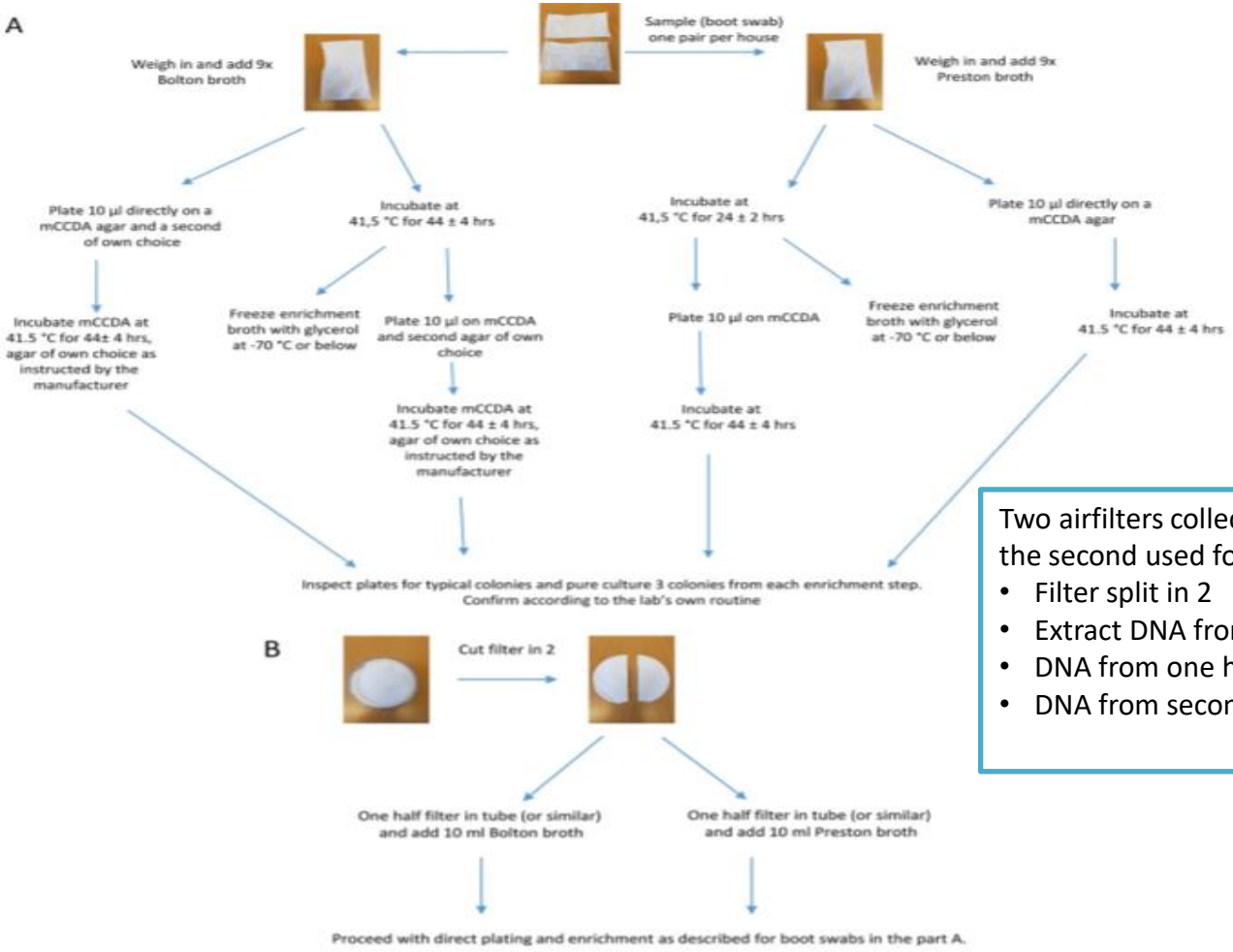


FIG 1 Flow diagram showing detection of *Campylobacter* spp. from boot swab samples (A) and air filters (B) using ISO 10272-1:2017.

Two airfilters collected per house,
the second used for molecular analyses:

- Filter split in 2
- Extract DNA from each half separately
- DNA from one half to shot-gun metagenomics
- DNA from second half to real time PCR

Results from boot swabs

		No. of positive boot swabs			
		Direct plating		Enrichment	
Country	No. of flocks	Preston	Bolton	Preston	Bolton
Czech Rep.	12	5	4	2	0
Denmark	18	0	0	6	0
Italy	10	10	10	10	10
Norway	10	0	0	0	0
Poland	12	6	6	5	0

Results from air filters

		No. of positive findings from air filters				
		Direct plating		Enrichment		Direct real-time PCR
Country	No. flocks	Preston	Bolton	Preston	Bolton	
Czech Rep.	12	0	0	1	2	5
Denmark	18	0	0	0	0	15
Italy	10	0	0	0	0	10
Norway	10	0	0	0	0	0
Poland	12	0	0	1	1	6

Results

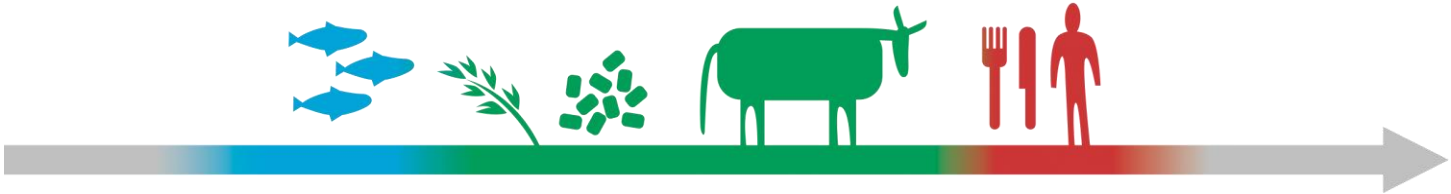
- Cultivation of boot swabs:
  - Highest number of positive samples from enrichment in Preston
- Air samples:
  - Higher frequency of positive samples from PCR (significantly different)
  - Cultivation gave very few positive samples

Concluding remarks

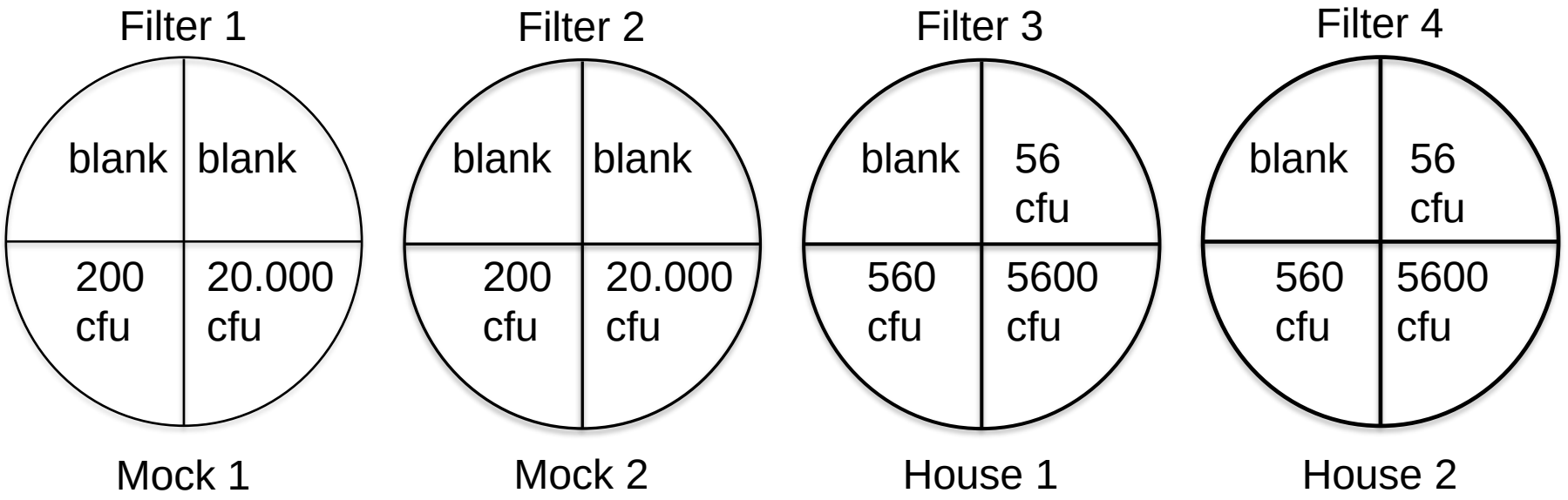
- Air sampling has been tested in areas with different *Campylobacter* pressure in the broiler population
- Air sampling combined with real-time PCR may be an alternative for screening for *Campylobacter*
- If isolates are desirable, an enrichment protocol should be preferred
- Air filters may be used for screening for multiple microbes, but needs further testing

Metagenomic sequencing of airfilters from Poultry farms

Thomas Haverkamp / Epidemiology

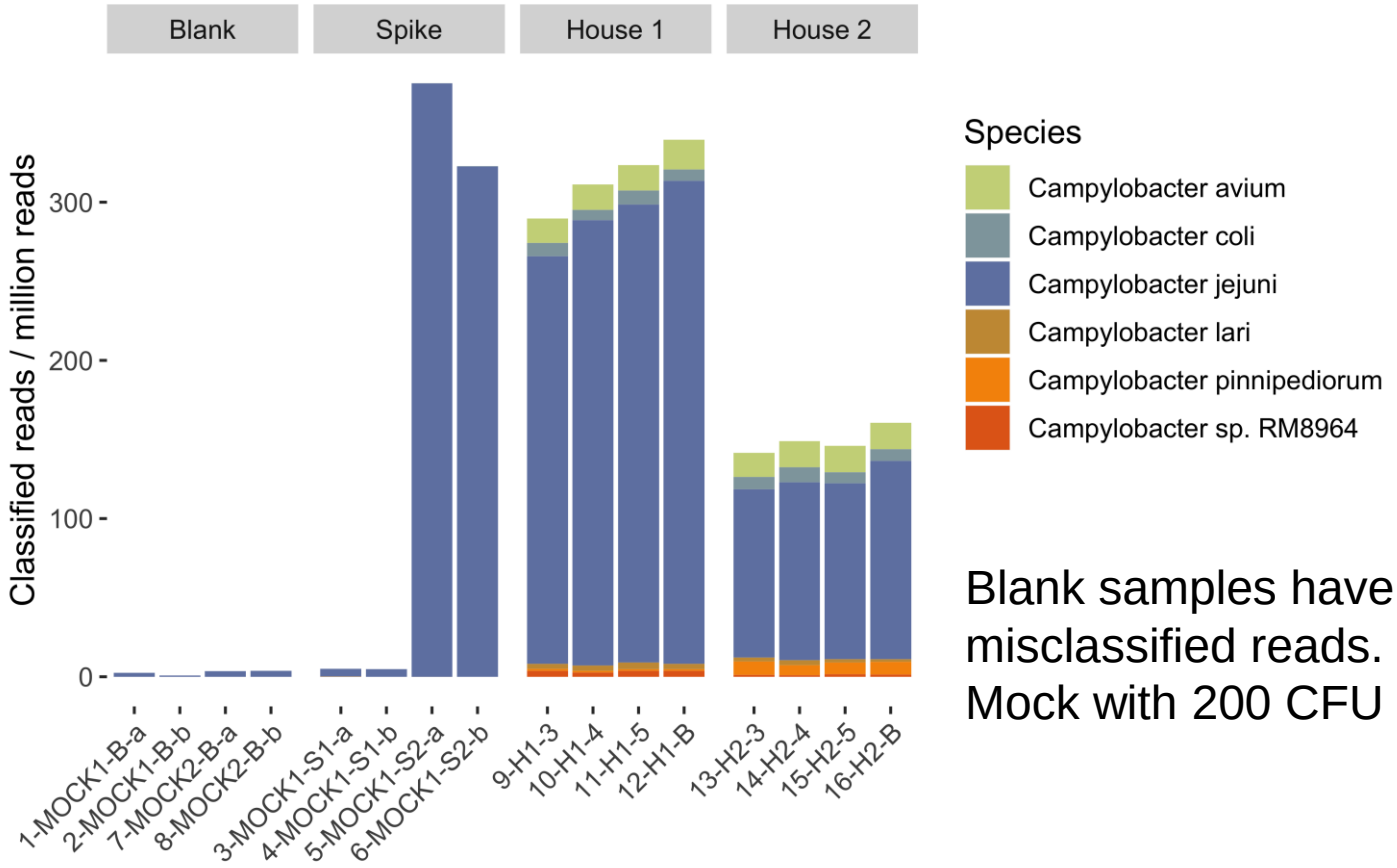


Pilot experiment



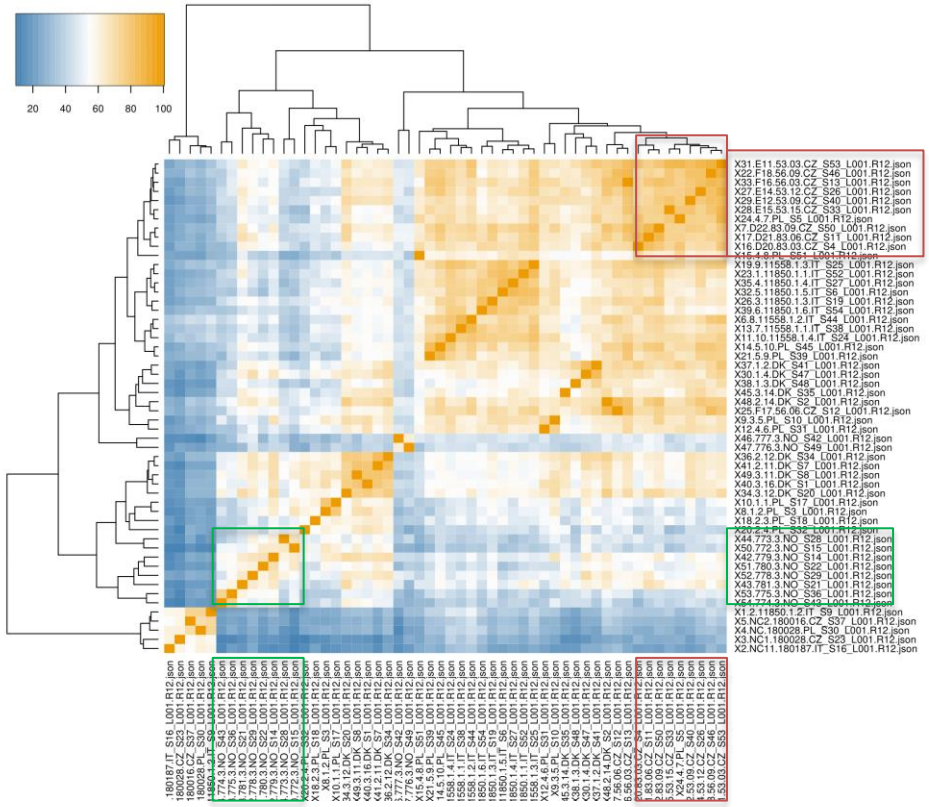
Spike (n) CFU *Campylobacter jejuni*

Pilot experiment



Blank samples have misclassified reads.
Mock with 200 CFU > 80 reads classified

Main experiment – preliminary results



Comparison of Poultry farms using Airfilter metagenomics.

5 Countries:

Czech Republic

Denmark

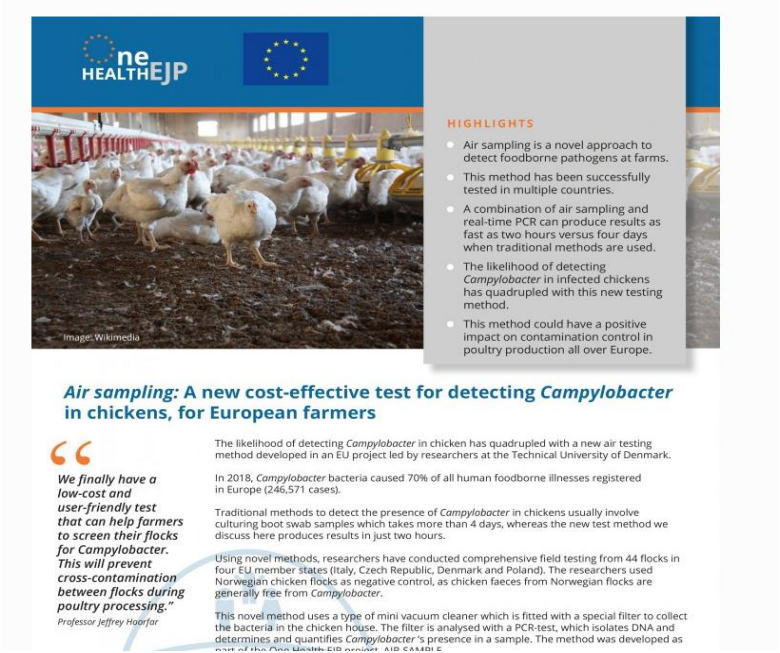
Italy

Norway

Poland

Same country farms form clusters

What we have delivered



HIGHLIGHTS

- Air sampling is a novel approach to detect foodborne pathogens at farms.
- This method has been successfully tested in multiple countries.
- A combination of air sampling and real-time PCR can produce results as fast as two hours versus four days when traditional methods are used.
- The likelihood of detecting *Campylobacter* in infected chickens has quadrupled with this new testing method.
- This method could have a positive impact on contamination control in poultry production all over Europe.

Image: Wikimedia

***Air sampling:* A new cost-effective test for detecting *Campylobacter* in chickens, for European farmers**

We finally have a low-cost and user-friendly test that can help farmers to screen their flocks for Campylobacter. This will prevent cross-contamination between flocks during poultry processing."

Professor Jeffrey Hoorfar

The likelihood of detecting *Campylobacter* in chicken has quadrupled with a new air testing method developed in an EU project led by researchers at the Technical University of Denmark.

In 2018, *Campylobacter* bacteria caused 70% of all human foodborne illnesses registered in Europe (246,571 cases).

Traditional methods to detect the presence of *Campylobacter* in chickens usually involve culturing boot swab samples which takes more than 4 days, whereas the new test method we discuss here produces results in just two hours.

Using novel methods, researchers have conducted comprehensive field testing from 44 flocks in four EU member states (Italy, Czech Republic, Denmark and Poland). The researchers used Norwegian chicken flocks as negative control, as chicken faeces from Norwegian flocks are generally free from *Campylobacter*.

This novel method uses a type of mini vacuum cleaner which is fitted with a special filter to collect the bacteria in the chicken house. The filter is analysed with a PCR-test, which isolates DNA and determines and quantifies *Campylobacter's* presence in a sample. The method was developed as

› Appl Environ Microbiol. 2020 Aug 7;AEM.01051-20. doi: 10.1128/AEM.01051-20. Online ahead of print.

Foodborne *Campylobacter*: A multi-center proposal for a fast screening tool in biosecured chicken flocks

Jeffrey Hoorfar [1], Ivana Koláčková [2], Gro S Johannessen [3], Giuliano Garofolo [4], Francesca Marotta [4], Kinga Wieczorek [5], Jacek Osek [5], Mona Torp [3], Bjørn Spilsberg [3], Camilla Sekse [3], Natasia Rebekka Thornval [6], Renáta Karpíšková [2]

Affiliations + expand

PMID: 32769183 DOI: 10.1128/AEM.01051-20

› Food Microbiol. 2020 Sep;90:103455. doi: 10.1016/j.fm.2020.103455. Epub 2020 Feb 8.

Campylobacter in chicken – Critical parameters for international, multicentre evaluation of air sampling and detection methods

Gro S Johannessen [1], Giuliano Garofolo [2], Gabriella Di Serafino [2], Ivana Koláčková [3], Renáta Karpíšková [3], Kinga Wieczorek [4], Jacek Osek [4], Julia Christensen [5], Mona Torp [1], Jeffrey Hoorfar [6]

Affiliations + expand

PMID: 32336358 DOI: 10.1016/j.fm.2020.103455

Other publications.

Video: https://www.youtube.com/watch?v=S9mapXSM8tw&feature=youtu.be

Aknowledgements

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- Sartorius for providing the Air samplers and air filters
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