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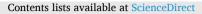
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# Food Control



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# A comparison of European surveillance programs for Campylobacter in broilers

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# ABSTRACT

Campylobacter is an important foodborne pathogen as it is associated with significant disease burden across Europe. Among various sources, Campylobacter infections in humans are often related to the consumption of undercooked poultry meat or improper handling of poultry meat. Many European countries have implemented measures to reduce human exposure to Campylobacter from broiler meat. In this paper, surveillance programs implemented in some European countries is summarized. Our findings reveal that many European countries test neck skin samples for Campylobacter as per the Process Hygiene Criterion (PHC) set by the European Regulation. Variations to the legal plan are seen in some countries, as in Norway and Iceland, where weekly sampling is performed during infection peak periods only, or in Iceland, where the Campylobacter limit is set at 500 CFU/g instead of 1000 CFU/g. Furthermore, northern European countries have implemented national Campylobacter surveillance plans. Denmark tests cloaca and leg skin samples at the slaughterhouses and meat samples at the retail, while Finland, Norway, and Sweden test caeca at slaughterhouses. In contrast, Iceland tests feces on farms. Iceland and Norway test flocks close to the slaughter date and when a farm tests positive, competent authority implement measures such as logistic slaughter, heat treatment or freeze the meat from these flocks. In Iceland, frozen meat is further processed prior to being put on the market. While the incidence of campylobacteriosis has declined in all European countries except France since the introduction of PHC in 2018, it is uncertain whether this decrease is due to prevalence reduction or underreporting during the COVID-19 pandemic. Future investigations with more comprehensive data, devoid of potential confounding factors, are necessary to validate this potential trend. However, it is evident that the implementation of national action plans can be successful in reducing the incidence of human campylobacteriosis, as demonstrated by Iceland.

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## 1. Introduction

*Campylobacter* (*C.*) is a Gram-negative bacterium known to cause campylobacteriosis, an acute diarrhoeal disease in humans, which has been the most frequently reported foodborne zoonosis in the European Union (EU)/European Economic Area (EEA) region since 2005 (EFSA & ECDC, 2022). Infections in humans can occur from a low infection dose where, besides diarrhoea, patients may also experience fever, headache and vomiting (ECDC, 2022; Teunis et al., 2018). The onset of symptoms occurs two to five days post-exposure and infected persons usually recover within a week. However, in children and individuals with a compromised immune system, the infection can be severe and develop into post-infectious sequelae such as gastrointestinal and joint disorders or immune-mediated neurological disorders, such as Miller-Fisher Syndrome and Guillen-Barré Syndrome (ECDC, 2022).

On average, 88% of the human Campylobacter infections in Europe are caused by C. jejuni and less frequently by other species such as C. coli (around 10%), C. fetus (0.2%), C. upsaliensis (0.1%) and C. lari (0.1%) (EFSA & ECDC, 2022). Campylobacter. Jejuni and C. coli are carried by livestock, such as poultry, cattle and pigs, even though poultry is recognised as the most common source of human infections (Mäesaar et al., 2020; Mota-Gutierrez et al., 2022). Poultry is identified as a natural amplifier for *Campylobacter*, because the birds have a higher metabolic temperature (42 °C) compared to other species, which promotes growth of these bacteria (Dedieu et al., 2002). A few of the Campylobacter species, such as C. jejuni, C. coli, C. upsaliensis and C. lari, have a relatively high optimum growth temperature and cannot grow below 30 °C, thus being called "thermotolerant". Interestingly, thermophilic Campylobacter cannot multiply outside the host due to the absence of micro-aerobic conditions but can survive when protected from dryness (Nicholson et al., 2005). Campylobacteriosis is primarily a sporadic disease with individual cases; however, outbreaks involving two or more individuals have been reported from across Europe. In 2021, a total of 249 Campylobacter outbreaks related to food were reported. These outbreaks resulted in 1,051 cases with 134 hospitalizations and six deaths. In seven out of the 20 outbreaks, trace back investigations identified broiler meat or broiler products as the source (EFSA & ECDC, 2022). The European Food Safety Authority (EFSA) regards Campylobacter as a high priority hazard in poultry due to the meat's high attribution in human campylobacteriosis cases, and due to the high prevalence of the organism in poultry carcasses (EFSA, 2012a). Infections in humans are often related to handling of raw contaminated poultry products or eating undercooked poultry meat (EFSA & ECDC, 2022).

Many European countries have implemented a surveillance system for Campylobacter in the broiler meat chain, which aims to reduce broiler carcass contamination at the slaughterhouses. However, in a few countries, measures are also taken to reduce and control Campylobacter spread in broiler flocks. EFSA, however, is only responsible for monitoring Campylobacter in EU/EEA countries. Therefore, the responsibility for conducting surveillance Campylobacter lies with the individual EU/ EEA countries. The essential difference between monitoring and surveillance is that only in a surveillance system, besides the collection, analysis and interpretation of data, are control measures taken. These measures are often targeted interventions taken to mitigate the negative consequences of a pathogen in the food chain, which assist in controlling and preventing the transmission of pathogens (Christensen, 2001). The EFSA has been collecting and analyzing data on Campylobacter in broiler flocks and in broiler meat in EU and EEA countries (EC, 2003; 2005; 2017a) for many years. In 2018, Regulation (EC) No 2073/2005 introduced a process hygiene criterion (PHC) for Campylobacter in broiler carcasses. This was the first mandatory monitoring activity mandated by law in the EU (EC, 2005; 2017b). The implementation of PHC in the regulation was in response to the EFSA baseline survey carried out in 2008 that found approximately 76% of broiler carcasses are contaminated with Campylobacter in the EU (EFSA 2010a, b). This was also a part of the modernisation of the meat inspection process (EFSA, 2012a, EC,

2017a; 2019). Moreover, an EFSA scientific opinion also estimated that broiler meat alone accounted for 20-30% of human campylobacteriosis cases and found that these cases could be reduced by >50% or even >90%, if the microbiological criterion in all slaughter batches tested for neck and breast skin were set to a critical limit of 1000 or 500 CFU/g (EFSA, 2011). Besides surveillance at the slaughterhouse, EFSA recommends a range of additional interventions to control Campylobacter in primary production (EFSA BIOHAZ Panel, 2020). In addition to the application of the Regulation (EC) No 2073/2005 (EC, 2005), a few competent authorities (CAs) in the European countries have taken additional measures to improve food safety and have implemented national Campylobacter action plans. These plans include different activities such as short-term surveys of farms or poultry products, investigation of risk factors on farms, awareness campaigns for consumers on how to avoid Campylobacter infection and source attribution studies to identify the main sources of human infections. Additionally, many poultry companies and associations for primary producers have adopted their own self-inspection and control strategies for Campylobacter.

The national monitoring, surveillance and control measures implemented for *Campylobacter* in the broiler meat chain are not harmonised across EU/EEA countries. The aim of this work was to describe and compare the different surveillance programs for *Campylobacter* in broiler production across different European countries to identify the most promising practices to control *Campylobacter* along the broiler meat chain.

# 2. Materials and methods

Descriptive information on the monitoring and surveillance system for broilers in Denmark, Estonia, Finland, France, Germany, Iceland, Italy, Norway, Poland, Portugal, Serbia and Sweden was obtained from different experts within the European network of the COST Action 18,105 - Risk-Based Meat Inspection and Integrated Meat Safety Assurance (RIBMINS). The expert group included professionals from animal health, food safety and academia. Experts gathered information on the type of surveillance system in place in their respective countries in 2021 and provided information on the latest national action plans for controlling *Campylobacter* at farm and slaughterhouse level. All the information was collected and synthesised to look for similarities and differences in the approaches chosen to reduce *Campylobacter* prevalence in broilers and in broiler meat.

# 3. Results

# 3.1. Mandatory surveillance according to campylobacter process hygiene criterion (PHC)

All twelve participating countries have implemented the PHC defined by Regulation (EC) No 2073/2005 (EC, 2005) based on the quantification of *Campylobacter* on neck skin samples, whereby a limit on the acceptable threshold on the contamination of carcasses (<1,000 CFU/g) is set. These neck skin samplings are performed by the food business operators (FBOs), who are responsible for the slaughterhouses. In all countries except Finland and Norway, at least three or four chilled neck-skin random samples from broilers belonging to the same flock are collected at the slaughterhouses. In Finland and Norway, neck skin samples are collected prior to chilling.

In all countries, the samples for testing are generally taken from the slaughter batches collected mostly at the large slaughterhouses. In Estonia, which has only one major broiler slaughterhouse, sampling is done on randomly chosen monthly days and batches. In all countries, sampled carcasses are randomly selected from the slaughter batch on varying days, where the sampling frequency also depends on the slaughter plant capacity. For example, in Denmark, slaughterhouses processing  $\geq$ 10,000 to <1,000,000 broilers/year are sampled biweekly,

### Table 1

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National surveillance programs implemented in Denmark to reduce exposure to Campylobacter from broiler meat. -

Aim	Sample type <sup>a</sup>	Sampling point	Coverage	Responsible authority	Follow-up action
To obtain animal/farm-level prevalence to identify high-risk farms for potential future risk-based control and reduce the number of positive broiler flocks over a five- year period.	Cloaca – single pool of 12 cloacal swabs from 24 broilers (one swab per pair of broilers)	Slaughterhouse	Annual testing of 3,300–3,400 flocks. Only flocks with $\geq$ 500 broilers are sampled.	Danish Veterinary and Food Administration (DVFA) together with the industry (Danish Agriculture & Food Council) and the National Food Institute (DTU-Food)	None
To obtain post-harvest prevalence and obtain CFU/g of <i>Campylobacter</i>	Leg skin (single samples)	Slaughterhouse	Approximately one third of the flocks sampled for cloaca are tested from four large slaughterhouses.	DVFA	The prevalence and the CFU/g are fed into the risk assessment model that is used within the national action plan, to measure a consumer's risk of becoming ill with <i>Campylobacter</i> from eating Danish broiler meat. This model estimates the relative risk as it compares a consumer's current risk with the risk in previous years. The relative risk estimates are used to evaluate the effect of national action plans.
Test imported broiler meat to account for the source of infection and to detect any eventual outbreak due to imported meat	Frozen meat (single samples)	Retail (single samples in grams)	Proportionally stratified samples collected from six main supermarket chains with increased monthly sample sizes during summer-early autumn. Approx. 200 samples are tested, where isolates from 50 samples are used for whole genome sequencing.	DVFA	None
Test locally produced broiler meat to account for the source of infection and to detect any eventual outbreaks	Chilled meat (single samples)	Retail (single samples in grams)	The sampling scheme is the same as for the imported broiler meat at retail. Approx. 800 samples are tested, and isolates from 200 samples are used for whole genome sequencing.	DVFA	None
Apply whole genome sequencing on <i>Campylobacter</i> isolates from human and carcasses to identify different sources	Isolates from broiler carcasses, stool samples from humans	From contaminated samples, clinical human cases identified during outbreak investigations	The number of samples sequenced depends on the human cases identified.	DVFA, Statens Serum Institut, DTU- Food	None

<sup>a</sup> Cloaca samples are tested using PCR, whereas the thigh-skin and retail samples are tested according to NMKL 119, 2007 method. Source: Danish Campylobacter Action Plan 2022–2026 (Jensen, 2022) and Danish Annual Zoonoses Report

National surveillance activities implemented in Finland to reduce exposure to Campylobacter from broiler meat.

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$\operatorname{Aim}^{a}$	Sample type <sup>b</sup>	Sampling point	Coverage	Responsible authority	Follow-up action
During the high-risk season, between 1st June to 31st October, program aims to obtain the broiler-house-level prevalence of <i>C. jejuni</i> and <i>C. coli</i> ; during the rest of the year, the sampling plan is set to show prevalence of 1% with 1% accuracy and 95% confidence level	Caeca	Slaughterhouse	Mandatory for slaughterhouses slaughtering >150,000 broilers/year. From 1st June to 31st October, all slaughter batches are tested, and during the rest of the year, slaughter batches are sampled according to a sampling plan set by the FFA. Intact caeca from 10 birds/slaughter batch are collected and the sampling covers the whole batch. In the laboratory, the 10 caeca samples can be pooled together into one sample.	Finnish Food Authority (FFA)	The FFA notifies the laboratory that delivered the isolate, slaughterhouse, official veterinarian (OV) of the slaughterhouse, the farmer, regional state administrative agency and municipal veterinarian of the area where the farm is situated. If <i>Campylobacter</i> has been repeatedly found in slaughter batches of a holding, then the owner must evaluate the production hygiene and change the work and hygiene practices as necessary. The OV must check the changed procedures and give advice to rectify any shortcomings identified (MAF 10/EEO/2007; MAF 316/ 2021). If one or more slaughter batches have been positive on two consecutive slaughter ed at the end of the day. The procedure is continued until two consecutive slaughter batches test negative for <i>Campylobacter</i> . The broiler integrations also employ voluntary measures at holding after <i>Campylobacter</i> positive findings.

<sup>a</sup> Sampling scheme set by Finnish Food Authority (MAF 10/EEO/2007; MAF 316/2021). <sup>b</sup> Ginne 2001 off the correction set food Authority (MAF 10/2022) 1 of set of an it doed a correction

Since 2021, all the samples are tested using EN ISO 10272-1 or a method validated according to the latest version of EN ISO 16140-2 against EN ISO 10272-1, and that includes isolation of the Campylobacter strain from the positive sample (MAF 316/2021). Prior to this (2004–2019), the NMKL No 119 method with modifications (no enrichment) was used for detection (Finnish National Zoonoses Monitoring Report 2004, 2007;

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and those processing >1,000,000 broilers yearly are sampled weekly. Making changes to the sampling protocol is allowed only if approved by the competent authority (EC, 2005). For example, when the prevalence of Campylobacter is low in a country, then the country could be exempted from monthly sampling. Hence, in Norway and Iceland, weekly samples are collected only during peak infection periods (June to October in Norway, and May 15 to October 15 in Iceland since 2022). In Sweden, slaughterhouses slaughtering >100, 000 broilers yearly must be sampled at least once a week between June and September. In Iceland and Finland, slaughterhouses are allowed to reduce sampling to every two weeks if the PHC has been met in the previous year. Additionally, in Finland, the frequency of sampling can be further reduced during some specific periods of the year, since during winter and spring, Campylobacter prevalence is lower than during other periods. Thus, between November and the following May in Finland, the sampling for Campylobacter PHC can be limited to once per month. In some countries, such as Denmark, Norway, Sweden and Finland, broilers from certain flocks are exempt from neck-skin sampling for PHC, e.g., those sent to smaller slaughterhouses (processing <10,000 broilers per year in Denmark, <100,000 in Sweden and <150,000 broilers per years in Finland), and those that are a part of the national surveillance program (50 days old at slaughter) in Norway. In all countries samples are tested as per the regulation, and the PHC limit is set to 1,000 CFU/g in 15 out of 50 samples. In Iceland, however, the CA has set the PHC limit to 500 CFU/g in a maximum of 10 out of 50 samples, within their national action plan. Furthermore, it is important to note that from the January 1, 2025 the PHC limit will be set to 1000 CFU/g in 10 out of 50 in all the member states (EC, 2017b).

In all countries, when the FBOs fail to comply with the limit, they are required to implement corrective actions based on hazard analysis and critical control points (HACCP) principles and good manufacturing practices (GMP) and additional measures described in Regulation (EC) No 2073/2005 (EC, 2005). Furthermore, according to the Regulation (EU) 2019/627 (EC, 2019), FBOs' compliance is further verified by the CAs choosing the following approaches: implementing ad hoc official control on the reported carcasses or collecting all the available information from the samples collected by the FBOs to verify the compliance with the PHC.

# 3.2. Other national surveillance programs

In addition to the PHC for *Campylobacter* in Denmark, Finland, Iceland, Norway and Sweden, other surveillance programs are run at national level (Tables 1–5). The objectives of *Campylobacter* sampling and testing within these national action plans differ among the five Nordic countries. Although differences exist, the central aim is always the same, which is focused on diminishing the potential for human exposure to *Campylobacter*.

In Denmark and Finland, samples are gathered regularly, with increased sampling during summer and autumn months (high-risk period), while in Norway, sampling occurs exclusively during the highrisk period. Elsewhere, in Sweden and Iceland sampling is conducted regularly the whole year. The test samples include cloaca and leg skin samples (Denmark), ceca (Finland, Norway, Sweden), feces (Iceland), and meat samples (Denmark). The cloacal, leg skin, caecal and feces samples are collected either on farms (Iceland, Norway), or at the slaughterhouse (Denmark, Finland, Sweden), whereas the meat samples are routinely taken at the retail outlets. Follow-up actions by local authorities also differ, with only Finland, Iceland and Norway involving CAs in the direct control measure implementation when flocks test positive. The follow-up actions include improving process hygiene and farm biosecurity, implementing logistic slaughter (processing infected flocks last) and applying heat-treatment or freezing for meat from positive flocks. However, heat treatment or freezing are not required in Finland. In Iceland, frozen meat from positive flocks is further processed before it is made available on shelves. More permanent interventions,

Finland's Annual Zoonoses Reports

Aim Sample Sam type <sup>a</sup>	Sampling point G	Coverage	Responsible authority	Follow-up action
To detect <i>Campylobacter</i> Feces Farm before distribution to reduce consumers exposure		All poultry flocks where meat is intended to be distributed fresh (non-heat treated and unfrozen); 10 fecal samples pooled into one sample. The sample must be taken within 5 days before slaughter.	Icelandic Food and Veterinary Authority	Meat from positive rearing flocks cannot be distributed as fresh meat. Alternatively, if sample during rearing has not been taken or is invalid, a negative sample taken during slaughter is sufficient for distributing meat & fresh.
Keep contamination during Neck skin Slaug poultry slaughter within set samples limits	Slaughterhouse A. 56 Fi Fi W	As per PHC according to reg. (EU) mr. 1495/2017, but the limit is set to 500 CFU/g in a maximum of 10 out of 50 samples. From 2022 onwards, sampling is reduced to seasonal sampling between May 15 to October 15. Furthermore, the abattoirs are allowed to reduce sampling to every two weeks if the PHC has been met in the previous year.	Icelandic Food and Veterinary Authority	The actions for non-compliance are in line with reg (EU) nr. 1495/ 2017. Improvements in slaughter hygiene, review of process controls of animals' origin and of the biosecurity measures in the farms of origin.

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Table 4   National surveillance activities implemented in Norway to reduce exposure t	vities imple	mented in Nor	rway to reduce exposure to <i>Campylobacter</i> from broiler meat.		
Aim	Sample type <sup>a</sup>	Sample Sampling type <sup>a</sup> point	Coverage	Responsible authority	Follow-up action
To reduce exposure in the Caeca human population to <i>Campylobacter</i>	Саеса	Farm	All flocks with broilers that are a maximum of 50 days old at time of collaboration between Norwegian slaughter are sampled between May and October. The sample is Food Safety Authority, industry, an taken up to six days before slaughter, so the <i>Campylobacter</i> status is administrative support institutions known before the time of slaughter. From each slaughter batch, intact caeca are collected from 10 birds and covers the whole slaughter batch. At the laboratory, the 10 caeca samples are pooled together into one sample.	Collaboration between Norwegian Food Safety Authority, industry, and administrative support institutions	The slaughterhouse must heat treat or freeze ( $-18$ °C or colder for at least three weeks) all approved slatherings from a positive herd. If the slaughterhouses choose to slaughter positive flocks before negative flocks, they must have implemented commensurate measures with the risk involved. If the slaughter batches of a holding test positive repeatedly, then the owner must evaluate the production hygiene and change the management and hygiene practices as necessary.
<sup>a</sup> Samples are tested using PCR.	ng PCR.				

#### Table 5

National surveillance activities implemented in Sweden to reduce exposure to Campylobacter from broiler meat.

Aim	Sample type <sup>a</sup>	Sampling point	Coverage	Responsible authority	Follow-up action
To achieve an annual prevalence of <10% in slaughter batches of broiler and reduce exposure in humans	Caeca	Slaughterhouse	The program covers over 99% of the broilers slaughtered in Sweden. From each slaughter batch, intact caeca are collected from 10 birds. If a flock is slaughtered at multiple time points with an interval exceeding 4 days, samples are collected from both batches; otherwise, samples are taken from only one of the batches. At the laboratory, the 10 caeca samples are pooled together into one sample.	Managed by Svensk Fågel (Swedish Bird, primary producers' association) under the supervision by the Swedish Board of Agriculture, Swedish Food Agency, National Veterinary Institute and Public Health Agency of Sweden.	If farms test positive for <i>Campylobacter</i> for more than one-year rearing batch per year, then they are considered to have a high prevalence. For these farms, a specific action plan is drawn, and following investigations take place: In-depth analysis of the actual production units – investigation and measures in the facilities where elevated presence of <i>Campylobacter</i> is recorded. Risk assessment to identify conditions and/or behaviors that contribute to the increased risk of introduction and spread of <i>Campylobacter</i> .

<sup>a</sup> EN ISO 10271-1. Starting in 2017, *Campylobacter* isolates gathered during two 2.5-week periods, commencing at week 8 and week 31, undergo whole genome sequence testing. These periods were chosen to align with the collection of human domestic isolates.

such as covering the broiler house with flynets, are also used in Iceland. Moreover, in Denmark, CAs uses the outcome of the test results to set targets for *Campylobacter* prevalence on positive farms at farm level and for proportion of carcasses testing positive for *Campylobacter* (>10 CFU/g) at slaughterhouses and in retail sample testing in the national action plans (Danish Annual Zoonoses Report, 2008–2021). Furthermore, in Iceland, public education about the dangers of foodborne bacteria is extensively conducted by using various media platforms and informational brochures.

# 4. Discussion

In this study, we have gathered up-to-date information on the different types of surveillance programs for *Campylobacter* in various European countries. This paper describes the ongoing initiatives implemented according to the current EU legislation (*Campylobacter* PHC at the slaughterhouse) as well as national initiatives.

Even though the monitoring of PHC at the slaughterhouses became mandatory in 2018, the first reports of the data were provided only by a few countries from the RIBMINS consortium (i.e., Denmark, Estonia, Germany and Sweden) (EFSA & ECDC, 2021). In this study, only the PHC data collected by FBOs from countries reporting data to EFSA for the two consecutive years 2020 and 2021 were evaluated (Supplementary Table S1). The data on CFU limit (>1,000/g) in neck skin samples show that less than 2% of the samples tested in Estonia, Finland, Norway, and Sweden exceeded the set microbiological limit. Moreover, when looking at the human incidence of campylobacteriosis for the same period in these countries, Estonia had a lower incidence (14-20 per 100,000) compared to Finland (33-39 per 100,000) and Sweden (33-38 per 100,000) (Supplementary Table S2). In a recent study, broiler meat samples tested from Estonian retail stores had a lower prevalence of Campylobacter in domestic products (1.8%) than in products that were of Latvian (36.8%) and Lithuanian (66.9%) origin (Tedersoo et al., 2022). Also, Campylobacter counts in imported products were significantly higher compared to products originating from Estonia. Moreover, for C. jejuni, the same genotype was found in both broiler meat and human samples, both of which were related to imported products. Hence, imported fresh broiler meat could potentially be the main cause of human campylobacteriosis in Estonia (Tedersoo, et al., 2022). However, further research involving the use of whole genome sequencing techniques and source attribution studies are needed to substantiate these findings. In Finland and Sweden, however, the occurrence of human campylobacteriosis has been associated with broiler meat. A recent study from Finland used whole genome sequencing techniques and successfully

traced back 18.4% of the domestically acquired human *C. jejuni* infections (n=50) to chicken meat. Additionally, the study found that 59.2% of the human samples of *Campylobacter* shared the genetic sequence type with those found in a batch of chickens slaughtered prior to the onset of the illness in humans, suggesting a possible link or source of infection (Llarena & Kivistö, 2020). In Sweden, according to Lindqvist et al. (2022), broiler prevalence with a 2-week lag period can partly explain the human cases. However, additional factors including consumer practices must be evaluated in order to understand the transmission routes and epidemiology of campylobacteriosis.

In general, since the introduction of Campylobacter PHC in 2018, the incidence rate of Campylobacter infections in humans decreased in all the countries except France (Supplementary Table S2). In Denmark, the observed trend in campylobacteriosis incidence appears to be characterised by some fluctuations, which makes it difficult to draw any definitive conclusions at this time (Supplementary Table S2). Anyway, due to varying surveillance and reporting systems among EU/EEA countries, which can also result in underreporting, these results should be interpreted cautiously. As we are comparing the data reported from the selected countries in the two-year period 2020-2021, the underreporting effect of the COVID-19 pandemic on the reduced incidence of several zoonoses, including campylobacteriosis, should also be carefully considered (EFSA & ECDC, 2022). Thus, it remains uncertain if the tightening of hygiene measures in slaughterhouse has had an impact on the reduction of human incidence rates from the outset, despite the simultaneous decrease in positive neck skin samples and disease observed in Finland, Germany and Sweden in particular, or whether the general underreporting trend from 2020 to 2021 has affected the official incidence of the human disease (Supplementary Tables S1, S2).

Among the different countries within our consortium, only the Nordic countries have implemented national actional plans for *Campylobacter*, where Norway and Iceland collect caecal and faecal samples on farms. Whereas, in Denmark, cloacal samples, and in Finland and Sweden caecal samples are taken at the slaughterhouses. The *Campylobacter* prevalence in broilers, representing farm-level prevalence, for the countries are published in national reports or on the CA's website (Supplementary Table S3). These countries use the prevalence results from broiler flocks on farms to implement on-farm measures, such as improved biosecurity and hygiene, or at the slaughterhouse to plan for a logistic slaughter. When *Campylobacter* is introduced into a flock, nearly all the birds are colonised rapidly, whereby they shed up to 10<sup>8</sup> *Campylobacter* per gram of caecal content (Wagenaar et al., 2013). Given this, it becomes challenging to prevent cross-contamination at the slaughterhouse or in the poultry products from positive flocks, as the

high bacterial counts persist until slaughter age, which typically is around 35–42 days of age in conventional production systems. For the logistic slaughter to be effective, broiler flocks should be tested closer to the slaughter date, and this method is only effective when slaughterhouses take additional hygiene measures to reduce the bacterial contamination on the carcasses e.g., freezing (Havelaar et al., 2005). Therefore, broilers in Iceland and Norway are tested close to the slaughter age. Thus, sampling before slaughter enables the planning of preventive measures for the upcoming slaughter of *Campylobacter*-positive broiler flocks, whereas sampling at slaughter provides only retrospective information. In addition, sampling before slaughter can be used for categorisation of farms, as suggested by EFSA with the proposed harmonised epidemiological indicators (HEIs) for *Campylobacter* in poultry (Cameron, 2012; EFSA 2012b).

Farm-level prevention measures are efficient tools in preventing Campylobacter contamination in further production steps. Therefore, in Finland, the broiler industry insisted on keeping the farm-level sampling as a part of the Campylobacter control program together with neck skin sampling at the slaughterhouse. Similarly, other countries, such as Sweden, have implemented on-farm measures based on the test sample results obtained at the slaughterhouse (Table 5). Farm-level information allows broiler farmers to implement hygiene measures on farms with a higher probability of Campylobacter-positive broiler flocks. In the context of high contamination at the farm-level, the slaughterhouse does not have a central role in carcass contamination, since the highest relevance is the high bacterial loads of infected batches entering the slaughterhouse. Conversely, when the epidemiological situation changes, in the context of low Campylobacter contamination on farm, the neck skin sampling at the slaughterhouse can be more informative to identify contaminated carcasses as result of both contaminated flocks and cross-contamination due to transport and slaughter phases (Marotta et al., 2015). Recently, EFSA estimated, for on-farm measures, the relative risk reduction in EU human campylobacteriosis linked to broiler meat consumption (EFSA, 2020). Several potential on-farm control interventions that could help reduce Campylobacter flock prevalence were evaluated, focusing on the reduction of caecal concentration of the pathogen. The selected interventions included vaccination, feed and water additives, discontinued thinning, employing few and well-trained staff, avoiding drinkers that allow standing water, the addition of disinfectants to drinking water, hygienic anterooms, and designated tools per broiler house. Overall, the most effective interventions seemed to be vaccination (27%; 90% probability interval (PI) = 4-74%), followed by feed and water additives (24%; 90% PI = 4-60%). Although large variations in PIs attributable to the selected control options were observed, a 3-log<sub>10</sub> reduction in broiler caecal concentrations was estimated to reduce the relative EU risk of human campylobacteriosis attributable to broiler meat by 58% (EFSA Panel on Biological Hazards, 2020). With regards to vaccination as an effective strategy, it is important to note that while there has been promising progress in the development of candidate vaccines, they are not yet commercially available.

Among the countries that have implemented a national action plan, Iceland has the lowest reported incidence of campylobacteriosis in humans (Supplementary Table S2). At present, the Campylobacter-positive samples from poultry flocks are very low in Iceland, but between June 1998 and March 2000, the number of human cases reached epidemic proportions. The infections were mostly related to the consumption of fresh broiler meat from the domestic market. Therefore, the authorities, in the beginning of 2000, established an extensive surveillance program for Campylobacter in poultry (Reiersen et al., 2002). Hence, improved on-farm biosecurity measures can contribute to reducing consumer exposure, with measures including the installation of flynets on the windows, emphasis on cleaning and disinfection of broiler houses between flocks, effective cleaning, and the disinfection of crates for transporting live birds to reduce cross-contamination (Newell et al., 2011). To enhance public health protection in Iceland, all poultry flocks were tested for Campylobacter no later than five days before being sent to

the slaughterhouse, and meat from positive flocks was frozen before being placed on the market (Stern et al., 2003), with frozen poultry generally further processed before being placed on the market. An important factor in campylobacteriosis reduction in Iceland was the education of consumers on food hazards carried out by specialists in generally available media and broad distribution of a pamphlet on foodborne bacteria in society (Reiersen et al., 2002). The actions taken in Iceland have thus contributed to the reduction of flock prevalence of *Campylobacter* from over 20%–2.1% in the period from 2001 to 2018 (Seman et al., 2020). The incidence rate has decreased from 42.0 cases per 100,000 population in 2017 to 15.7 cases in 2021 (Supplementary Table S2).

The data from Denmark, Finland and Sweden demonstrate that the incidence of campylobacteriosis in humans cannot be linked only to the presence of the pathogen in chicken. In the case of Denmark, the national program has resulted in the reduction of the occurrence of Campylobacter in broiler flocks and meat, but only a small decrease in the number of human cases of Campylobacter infections have been reported (Boysen et al., 2014). Therefore, the effect of the implemented measures may have been offset by other factors, such as other sources of infection and the importation of infected poultry from other countries (Boysen et al., 2014). More recently, a source attribution tool has been included in the latest Danish national action plan (2022-2026) (Jensen, 2022), with the aim that this tool could help identify the sources of infection to help implement source-specific interventions. In recent years, decreasing costs and improved use of whole genome sequencing (WGS) have significantly increased the accuracy of Campylobacter source attribution studies. In France, for example, WGS assigned 31-63% of the clinical isolates to broiler meat consumption, especially undercooked broiler meat, but 22-55% of the human clinical cases were attributed to undercooked beef meat, tripe, liver or raw milk, and consumption of water contaminated by bovine manure. Companion animals, such as dogs and cats, were associated with 4%-12% of the human cases (Thépault et al., 2018). In Denmark, with the use of WGS data and advanced network analysis, over 50% of the human Campylobacter isolates were attributed to broilers, while ducks were not associated with human infections (Wainaina et al., 2022). Recently, Mäesaar et al. (2020) conducted population genetic analyses to attribute clinical C. jejuni isolates originating from Estonia, Latvia and Lithuania to their most likely sources. The studies from these Baltic countries demonstrated that poultry is the main source (88.3%) of C. jejuni human infections, followed by cattle (9.4%) and wild birds (2.3%) (Aksomaitiene et al., 2019; Mäesaar et al., 2020; Meistere et al., 2019).

Campylobacter can be carried by different animal species, and therefore, information should also be collected on different animal sources and the environment. Since Campylobacter can survive better in warm climates, a climate change might further pose a challenge in the prevention and control of Campylobacter. In fact, it is predicted that the number of cases of campylobacteriosis could increase by 25% by the end of the 2040s and 196% by the end of the 2080s (Kuhn et al., 2020a). Higher temperatures and heavy rainfall in many European countries could create favourable conditions for the survival and growth of Campylobacter in the environment. High incidences of human campylobacteriosis occur in Norway and Sweden, where there is a high degree of water coverage, as direct water, wet sand and mud contact increase the risk of infection (Kuhn et al., 2020b). One of the sources of infection in broiler houses is insects, which can be vectors for microorganisms transmitted from the contaminated environment. A simulation model showed that the effective protection of farms from insects had the strongest impact of all tested biosecurity factors on Campylobacter contamination in broiler houses and slaughterhouses in the Netherlands by reducing the peak percentage of contaminated broilers from 51% to 26% and the neck samples of broiler carcasses from 13% to 8% (Horvat et al., 2022).

### 5. Conclusions

In conclusion, managing Campylobacter in broiler flocks or broiler meat is challenging. Several efforts are being made by some EU countries to implement national surveillance activities in broilers both on farms and at slaughterhouses, each of them trying to find the best practices to protect human health. However, high efficiency and significant reductions of contaminated broiler and broiler meat at the same time can only be achieved by a multi-factorial approach both on farms and in slaughterhouses, including transportation, farm hygiene and visitor control, like in a risk-based meat safety assurance system. More efforts should be promoted in the future, since campylobacteriosis is still the most commonly reported zoonosis in Europe, while also addressing the interventions in animal species other than poultry, and keeping the consumers informed about the risks of foodborne diseases related to some domestic practices. This is demanding, especially for the numerous countries for which the surveillance of Campylobacter continues to remain focused on the PHC defined by the current EU legislation.

# CRediT authorship contribution statement

Abbey Olsen: Conceptualization, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision. Silvia Bonardi: Investigation, Writing - original draft, Writing - review & editing. Lisa Barco: Investigation, Writing - original draft, Writing review & editing. Marianne Sandberg: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. Nina Langkabel: Investigation, Writing - original draft, Writing - review & editing. Mati Roasto: Investigation, Writing - original draft, Writing review & editing. Michał Majewski: Investigation, Writing - original draft, Writing - review & editing. Brigitte Brugger: Investigation, Writing - original draft, Writing - review & editing. Arja H. Kautto: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. Bojan Blagojevic: Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition. Joao B. Cota: Conceptualization, Investigation, Writing - review & editing. Gunvor Elise Nagel-Alne: Investigation, Writing - original draft, Writing - review & editing. Adeline Huneau: Investigation, Writing - review & editing. Riikka Laukkanen-Ninios: Conceptualization, Investigation, Writing - review & editing. Sophie Lebouquin-Leneveu: Investigation, Writing - review & editing. Ole Alvseike: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. Maria Fredriksson-Ahomaa: Conceptualization, Investigation, Writing - review & editing. Madalena Vieira-Pinto: Investigation, Writing review & editing. Eija Kaukonen: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

# Data availability

No data was used for the research described in the article.

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# Appendix A. Supplementary data

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