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# Microbiological sampling and analyses in the food business operators' HACCP-based self-control programmes

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A questionnaire was developed within the OH-HARMONY-CAP project providing an overview of current procedures for microbiological sampling and analyses in food business operators' HACCP-based self-control programmes in EU/EEA countries. It focused on six bacterial species: Salmonella spp., Listeria monocytogenes, Campylobacter spp., Shiga toxin-producing Escherichia coli, Shigella spp. and Yersinia spp.; and five parasites: Trichinella spp., Cryptosporidium spp., Echinococcus granulosus (Sensu lato), Echinococcus multilocularis and Toxoplasma gondii. Participating EU/EEA countries distributed the questionnaire to food business operators' laboratories within their countries and responses were received from nine countries. Feedback from 35 laboratories among 554 were considered for data analysis. Results showed that dairy products were analysed most frequently and the majority of laboratories analysed both ready-to-eat and non-ready-to-eat products. Laboratories analysing Salmonella spp. and Listeria monocytogenes processed the majority of samples. Accreditation for ISO-standards or an alternative method was in place in a considerable proportion of laboratories, but did not cover all pathogens investigated. Sending isolates for further confirmation to external laboratories was not common. In contrast, storing isolates was more frequently established. Around 60% of laboratories used more than one typing or characterisation method, predominantly MALDI-TOF, antimicrobial resistance typing and PCR, while 40% did not use any of these methods. Variability was observed as regards use of Whole Genome Sequencing; and participation in External Quality Assessment programmes. The study gathered insight into current practices of microbiological sampling and analyses performed in food business operators' HACCP-based self-control programmes, and showed that further efforts are needed for harmonisation of analytical protocols and characterisation of foodborne pathogens.

#### KEYWORDS

microbiological sampling, food business operator, HACCP, national reference laboratory, harmonisation

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# **1** Introduction

Laboratory analyses and detection protocols are essential for surveillance of zoonotic pathogens and outbreak investigations. The early detection of foodborne pathogens and comparable laboratory results across One Health sectors (public health, animal health and food safety) is crucial to reduce the burden caused by foodborne outbreaks (Jain et al., 2019). This implies the development and implementation of harmonised guidelines and sampling methods for the monitoring of zoonotic pathogens in the reservoir, in the food and feed chain, and in humans as well as similar capacities, capabilities and communication systems in place at a national level and if possible at EU level. However, large differences concerning the funding and resources for monitoring programmes, standardisation and harmonisation of surveillance systems, frequency and accessibility of data reports, consistency, and coordination for a rapid and efficient information sharing, are documented between EU/EEA countries (Todd, 2006; Mesa Varona et al., 2020). According to the Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, member states of the European Union are obliged to collect data on zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks (European Commission, 2003). Nevertheless, member states are not required to report the proportion of the animal population neither the products of animal origin tested by official routine control laboratories nor the sensitivity or specificity of the protocols. Additionally, mandatory notification of foodborne pathogens in food varies between member states, hampering reliable incidence and prevalence data and thus, comparable results. This represents a major challenge for a cross-sectoral consolidation of data, needed for a consistent interpretation of results (Uelze et al., 2021).

Therefore, within the framework of the One Health European Joint Programme (OHEJP) funded by the European Union's Horizon 2020 research and innovation programme, the OH-HARMONY-CAP project aims to collect information on current capabilities, capacities, adaptabilities and interoperability at both the National Reference Laboratory (NRL) and the official routine control laboratories. The project focuses on a set of microbial foodborne hazards to provide an in depth description of current procedures and sampling methods for the harmonisation of protocols to detect foodborne pathogens and antimicrobial resistance determinants across the One Health sectors at national and EU/EEA levels. To achieve this, the project was divided into five work packages, each one with its own overall objective, out of which work package 2 was the focus for the purpose of this study.

Work package (WP) 1 was responsible for the project coordination and ensured progress, outreach and engagement with EU stakeholders, including ECDC and EFSA, and other OHEJP projects. WP2 addressed the development of "OHLabCap", a monitoring tool to assess One Health laboratory capability, capacity, interoperability and performance across EU/ EEA countries. The aim of WP3 was to review scientific and grey literature regarding laboratory interoperability to identifying current best practices for food sampling and testing, characterisation of isolates, and data management and harmonised reporting in One Health sectors. WP4 designed harmonised protocols for detection and typing of Shiga toxin-producing *Escherichia coli* (STEC), Enterotoxigenic *E. coli* (ETEC), *Cryptosporidium* spp. and

antimicrobial resistance in *Salmonella* spp. and *Campylobacter* spp.; and WP5 focused on developing e-learning training and practical workshops for detection, typing and characterisation of STEC, ETEC and *Cryptosporidium* spp.

The aim of WP2 was to establish a generic benchmarking instrument "OHLabCap", adjustable and sustainable at both the EU/ EEA and national level, that would support harmonised data collection and validation, encourage cross-sectoral communication, and assist competent authorities in establishing and strengthening national networks. This required identifying and characterising One Health laboratory interoperability, capacity and performance in each step within the system (e.g., coordination, data collection, analysis, interpretation, and dissemination of data), which was achieved by conducting surveys to approach the NRL and both official control and private laboratories. For the purpose of this study, food business operators' laboratories were considered within the group of private laboratories. Therefore, a survey was designed and conducted for the first time in EU/EEA countries, providing an overview of the current procedures for microbiological sampling and analyses in food business operators' HACCP-based self-control programmes. Self-control programme of food companies is mandatory and it is required, by Regulation 852/2004 on the hygiene of foodstuffs, to comply with the HACCP principles (European Commission, 2004a). Each food company designs a specific self-control programme depending on the food product that is handled or processed in the establishment. The purpose of a HACCP-based self-control programme in food companies is to obtain safe food for the consumer.

Current investigations conducted in the framework of national monitoring programmes or as part of official investigations that include food sampling and laboratory testing by competent authorities, differ largely and cannot be compared with the ones performed by the food business operators' self-control programmes (van Asselt et al., 2021). Therefore, the rationale behind this study was to collect information on how and which sampling and analytical methods do food business operators (FBO) carry out; to support more comparable microbiological sampling and testing programmes in the future. This insight would evidence the gaps, necessities and differences between FBO HACCP-based self-control programmes and official monitoring programmes within and between countries, needed as a baseline for further harmonisation and standardisation of sampling and analytical protocols of foodborne pathogens, not only at a national level but in a European context.

# 2 Methods

An online questionnaire was developed within the OH-HARMONY-CAP project, using the EU Survey tool, an open source application available in 23 EU languages and secured through the European Commission authentication system (European Commission, 2012). It focused on regulated and nonregulated pathogens, namely, six bacterial species: Salmonella spp., Listeria monocytogenes, Campylobacter spp., Shiga toxin-producing E. coli, Shigella spp. and Yersinia spp.; and five parasites: Trichinella spp., Cryptosporidium spp., Echinococcus granulosus (Sensu lato), Echinococcus multilocularis and Toxoplasma gondii. Participating EU/EEA countries contacted their respective associations of

EU/EEA countries	Number of laboratories			
	Contacted	Respondent	Response rate (%)	Considered for data analysis
Sweden	56	11	19.6	3
Netherlands	27	8	29.6	8
Portugal	51	8	15.7	8
Germany	116	5	4.3	4
Finland	37	4	10.8	4
Norway	26	3	11.5	3
Denmark	9	2	22.2	2
France	200	2	1.0	2
Latvia	32	1	3.1	1
Total	554	44	13.1	35

#### TABLE 1 Survey participants.

independent testing laboratories, which provided a list of associate private laboratories that tested, analysed, and measured safety and quality of food products. The list included laboratories analysing food hazards for FBO with HACCP-based self-control programmes, thereafter called FBO laboratories. Only FBO laboratories that analysed microbiological hazards were selected for the study and FBO laboratories analysing chemical and physical hazards were discarded. The selected FBO laboratories received a letter of invitation for participation in the survey, translated into the official language of each country, attached with the online questionnaire.

Statens Serum Institut (SSI), the French Agency for Food, Environmental and Occupational Health and Safety (ANSES), the Finnish Food Authority, the German Federal Institute for Risk Assessment (BfR), the Institute for Food Safety, Animal Health and Environment (BIOR), the Norwegian Veterinary Institute, the National Institute of Health Doutor Ricardo Jorge (INSA), the Swedish Food Agency and the National Institute for Public Health and the Environment (RIVM) were the institutions that distributed the letter of invitation for participation in the survey within each country. The survey was anonymous and conducted during a period of 6 months from February to July 2021. Information was collected on: food categories or the food areas that were examined, number of analysed samples, implementation of ISO-standards and laboratory accreditation, whether FBO laboratories sent their isolates for external analysis or not, length of storage of isolates, typing or characterisation methods used for each microorganism, and participation in External Quality Assessments or Proficiency Testing programmes. The link to the online questionnaire is provided for more details of the study: https://ec.europa.eu/eusurvey/runner/Foodsector\_Survey.

# 3 Results and discussion

Responses were received from FBO laboratories in Denmark, Finland, France, Germany, Latvia, Norway, Portugal, Sweden and Netherlands. The response rate between countries was not homogeneous, varying from 1% in France to 29.6% in Netherlands. Overall, 554 FBO received the letter of invitation for participation in the survey, and feedback from 35 FBO laboratories working with one or more of the six priority bacteria and five priority parasites were considered for data analysis (Table 1).

The information provided by the FBO laboratories should be interpreted cautiously as the relatively small turnout might be subject to at least three limitations. First, the survey was sent out and circulated in the EU/EEA countries during summer season, when the staff member availability is the lowest during the year. Second, the majority of FBO laboratories contacted for the study were private; therefore, the willingness of staff members to answering surveys is not a priority nor an obligation and it varies over time. Third, the impact of the COVID-19 pandemic and the lockdown measures adopted during 2020 still with repercussions during 2021. These factors might have resulted in missing data and bias (Ray et al., 2021).

### 3.1 Food categories and food areas

The food categories considered in the survey included the food groups most commonly consumed by the population. The food categories and food areas analysed by the FBO laboratories and considered in the survey were the following: dairy products, fish and seafood, meat and products thereof, vegetables and fruits and products thereof, bakery products, egg products, beverages, ready-to-eat mixed meals, and food processing areas and environmental surfaces. Food processing areas referred to any place where foodstuffs are prepared or processed for human consumption, such as rooms for cutting, grinding, cooking or packing food products; and environmental surfaces included surfaces of equipment, floors, walls, ceilings, drains, among others within a food processing plant.

Each food category or food area was analysed by a minimum of 14 and a maximum of 29 FBO laboratories. Beverages was the food

category analysed by the lowest proportion (40%) of FBO laboratories participating in the survey, whereas dairy products was analysed by the highest proportion (82.9%) of laboratories. The global trend of dairy consumption has been stable over the time compared to meat and eggs consumption. Currently, Europe has the highest level of dairy consumption with 443.5 kg/capita per year, with the projection to increase (Henchion et al., 2021). Hence, food safety concerns on dairy products, consisting of milk, butter and ghee, and cream could explain the highest proportion of FBO laboratories analysing them. A large proportion (65.7%) of FBO answering the questionnaire analysed five or more food products in their laboratories. Additionally, the majority (62.9%) of FBO laboratories analysed both ready-to-eat and non-ready-to-eat products, probably because nearly all food categories, except beverages, include products in both presentations for human consumption.

## 3.2 Analysed samples

FBO laboratories were requested to provide the average number of analysed samples for each of the priority pathogens investigated in the period of 2018-2020. Regarding the six bacterial species, FBO laboratories analysing Campylobacter spp. processed 6,007 samples out of which 636 were positive, FBO laboratories analysing Listeria monocytogenes processed 168,522 samples out of which 62,698 were positive, FBO laboratories analysing Salmonella spp. processed 203,078 samples out of which 61,107 were positive, FBO laboratories analysing Shiga toxin-producing E. coli processed 18,307 samples out of which 128 were positive, FBO laboratories analysing Shigella spp. processed 286 samples out of which 1 was positive, and FBO laboratories analysing Yersinia spp. processed 704 samples out of which 14 were positive. Regarding the five parasites, FBO laboratories analysing Trichinella spp. processed 9,000 samples out of which 1 was positive, FBO laboratories analysing Cryptosporidium spp. processed 800 samples out of which 300 were positive, FBO laboratories analysing Echinococcus granulosus (Sensu lato) and Echinococcus multilocularis processed 500 and 300 samples, respectively, which were all negative, and FBO laboratories analysing Toxoplasma gondii did not process any sample in this period.

Samples of Campylobacter spp., Listeria monocytogenes, Salmonella spp. and Shiga toxin-producing E. coli accounted for 97.2% of the samples analysed by the FBO laboratories during the period of 2018-2020. The four pathogens are regulated by Directive 2003/99/EC, EU member states are obliged to include them in their zoonoses and zoonotic agents monitoring programmes, and moreover, are associated with the highest risk of bacteria-related foodborne diseases in the EU (European Commission, 2003; Lee & Yoon, 2021). FBO laboratories analysing Salmonella spp. processed 49.9% of the samples, followed by FBO laboratories analysing Listeria monocytogenes which processed 41.4% of the samples; probably as a result of the fact that 31 and 28 FBO laboratories considered for this study analysed Salmonella spp. and Listeria monocytogenes, respectively. Regarding parasite-related foodborne diseases, FBO laboratories analysing Trichinella spp. received the majority of samples (9,000), most likely due to Trichinella spp. being the only of the investigated parasites for which there is compulsorytargeted surveillance in slaughterhouses and meat-producing animals placed on the EU market (European Commission, 2015; van der Giessen et al., 2021).

# 3.3 Implementation of ISO-standards or an alternative method

Respectively, 77.1% and 74.3% of FBO laboratories had ISOstandards or an alternative method implemented. Alternative methods included national standards, such as the DIN standards for Germany, and validated alternative methods certified by organizations such as AFNOR, MicroVal, NordVal or others. Accreditation for ISO-standards or an alternative method was reported in 68.6% and 62.9% of FBO laboratories, respectively.

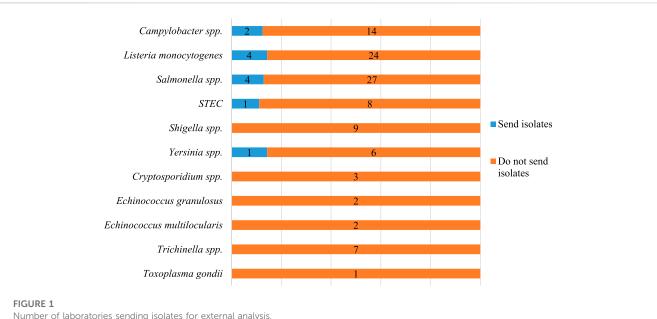
Implementation and accreditation for the ISO-standards or an alternative method was in place in a considerable proportion of FBO laboratories, but did not cover all the pathogens investigated. Official food controls and veterinary laboratories in the European Union, are required by Regulation (EC) No. 882/2004 to implementing a quality management system based on international laboratory management standards, such as EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories' (European Commission, 2004b; Wilson et al., 2015). The aforementioned aims the improvement of products and customer satisfaction, and to add quality and reliability to the services provided by these laboratories (Neves et al., 2017). Therefore, the respective 77.1% and 74.3% of FBO laboratories with ISO-standards or an alternative method implemented found in the study could be explained.

Additionally, accreditation for ISO-standards or an alternative method was reported in 68.6% and 62.9% of FBO laboratories, respectively. This majority of accredited FBO laboratories could be due to the fact that laboratory accreditation provides a level of standardisation and ensures integrity and accuracy of current food testing results required by national regulatory institutions and other end users to accept laboratory data in support of an integrated food safety system (Wangsness et al., 2017).

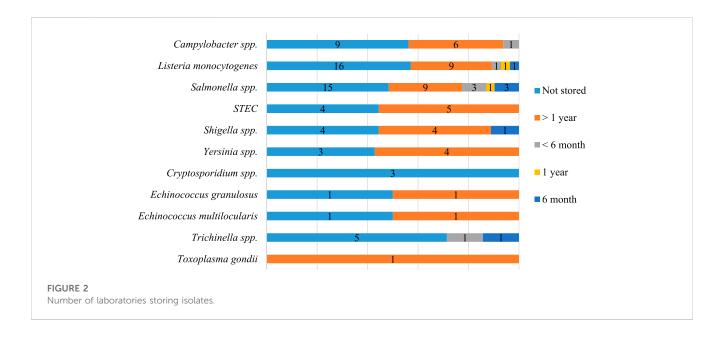
### 3.4 Sending of isolates for external analysis

10.4% of FBO laboratories sent their isolates for further confirmation to external laboratories (Figure 1).

Sending isolates for further confirmation to external laboratories was not performed by FBO laboratories analysing parasites neither was common for FBO laboratories analysing bacteria. The issue behind this could be that the majority (89.6%) of FBO laboratories have the capacity to process the samples and subtype the isolates received from self-control programmes and consequently do not send their isolates to external laboratories. Moreover, national and international regulatory frameworks support early identification of foodborne outbreaks, and thus the sharing of information and prompt communication, comparability of data, efficient laboratory subtyping and intersectoral collaboration. Nevertheless, this remains occasional and mainly occurring when laboratories are required to a further confirmation of their results by sending their isolates to National Reference Laboratories (Mylius et al., 2018; O'Brien et al., 2020).





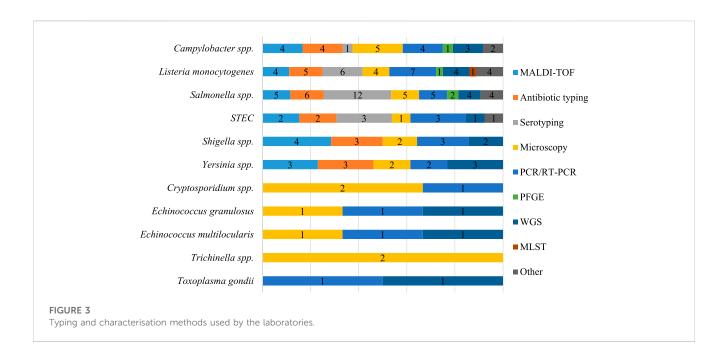


# 3.5 Storing of isolates

Storing isolates was more frequently established but not for all the pathogens investigated. Therefore, 51.1% and 45.8% of FBO laboratories analysing bacteria and parasites, respectively, stored their isolates. FBO laboratories storing their isolates, stored them typically for longer than 1 year (Figure 2).

In order to send isolates for further analyses to National Reference Laboratories or for characterisation and typing of isolates for research, source attribution, collection or conservation purposes at a later stage, laboratories need to improve their preservation procedures and storage capacity. An average of 48.5% of FBO laboratories storing their isolates

may reflect lack of awareness of the relevance of storing isolates, insufficient storage capacity and limited budget allocation for the costly use of a cold chain for preservation of isolates. Additionally, the viability of isolates could be reduced after a long-term storage (more than 1 year), hence the laboratories might not invest for this purpose. For instance, isolates of Salmonella spp., E. coli and Shigella spp., preserved by using Tryptic Soy Broth with 15% glycerol, may remain 100% viable even after 5 years of storage under deep freezing (-70 to -80 °C); however, Campylobacter spp. isolates may be more sensitive to temperature changes and preservation techniques, maintaining a lower viability (66.7%) under the same conditions (Sunarno et al., 2021).



## 3.6 Typing and characterisation methods

Regarding characterisation and typing methods, around 60% of FBO laboratories participating in the study used more than one typing or characterisation method, predominantly MALDI-TOF, antimicrobial resistance typing and PCR, while 40% of FBO laboratories did not type nor characterise the investigated pathogens. Although characterisation methods provide various advantages, like being more sensitive, reliable, time-efficient and less laborious than conventional culture-based methods to detect viable pathogens in foods (Zhao et al., 2014; Foddai & Grant, 2020), they generally demand higher costs, require trained personnel and specialised instruments (Law et al., 2015), which FBO laboratories might not be willing to pay as it is no legal requirement for selfcontrol programmes.

FBO laboratories analysing bacteria used a wider variety of typing and characterisation methods than FBO laboratories analysing parasites. Variability between FBO laboratories was also observed as regards use of Whole Genome Sequencing (WGS) (Figure 3). Despite providing substantial support for surveillance programmes and foodborne outbreak investigations, source attribution studies, and genomic information for antimicrobial resistance, pathogenicity and virulence, only 17.1% of FBO laboratories reported using it (Uelze et al., 2020). Culture-based methods are still considered the gold standard for microbiological analysis of food. These methods represent the first alternative for FBO to comply with Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs, because they need easy to use, sensitive and inexpensive tests to monitor foods for the presence of particular pathogens in certain categories of food products (European Commission, 2005; Foddai & Grant, 2020), for either a prompt communication with the competent authorities or to take the correspondent actions in case of unsatisfactory results based on the aforementioned legislation.

In addition to conventional methods, WGS is employed to subtype bacteria by having the ability to look at the entire genome and therefore identifying genes that would determine its survival (Franklin et al., 2021). This, although advantageous for food monitoring and assessments, is not a compulsory requirement for FBO self-control programmes to implementing corrective actions when the microbiological criterion is not met. Moreover, introduction and application of WGS in routine monitoring programmes remain challenging as long as national authorities allocate insufficient resources, like budget and expertise, for WGS capacity building at public health and food safety/veterinary laboratories (García Fierro et al., 2018). Furthermore, the lack of harmonised data, concerns on the legal aspects associated with the collection of genomic data, and in some countries the limited number of collected isolates, generate a false perception that investment in WGS implementation is unnecessary (Nouws et al., 2020).

# 3.7 Participation in External Quality Assessments

The majority (76.5%) of FBO laboratories participated in External Quality Assessment (EQA) programmes or proficiency testing programmes, probably because it is considered key component to monitor laboratory performance through reverification of samples, inter-laboratory comparisons and onsite evaluations; and pivotal in laboratory quality management systems to detect and control infections in humans, animals and food (James et al., 2014; Carter, 2017). Additionally, participation in EQA programmes is a requirement for international laboratory accreditation (Manjengwa et al., 2021). Nevertheless, participation in EQA programmes demands costs and resources for capacity-building programmes to support continuous training, which might explain the 23.5% of FBO laboratories that did not participate (Pedersen et al., 2018; Mogeni et al., 2021).

Five countries accounted for the 23.5% of FBO laboratories that did not participate in EQAs: France, Portugal, Netherlands, Finland and Sweden. The two respondent FBO laboratories in France, the eight respondent FBO laboratories in Portugal and the eight respondent FBO laboratories in Netherlands did not participate in EQAs for the analysis of seven (*Listeria monocytogenes*, Shiga toxin-producing *E. coli*, *Shigella* spp., *Yersinia* spp., *Cryptosporidium* spp., *Echinococcus granulosus* (*Sensu lato*), and *Echinococcus multilocularis*), six (*Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes*, *Shigella* spp., *Yersinia* spp., and *Trichinella* spp.) and four (*Campylobacter* spp., Shiga toxinproducing *E. coli*, *Shigella* spp., and *Yersinia* spp.) of the pathogens investigated, respectively. *Shigella* spp., and *Yersinia* spp. were not analysed by any of these countries.

Seven out of nine FBO laboratories analysing *Shigella* spp., the only non-regulated pathogen among the investigated foodborne hazards, did not participate in EQAs. All participating EU/EEA countries use a passive surveillance system for *Shigella* spp. France, for instance, has a voluntary notification system for this pathogen and therefore laboratories are not required to participate in EQAs for further accreditation (ECDC, 2022a). Since according to Directive 2003/99/EC it is not compulsory to include *Shigella* spp. in the monitoring of zoonoses and zoonotic agents in EU member states (European Commission, 2003), laboratories do not have to undergo proficiency testing or EQAs to evaluate and compare their detection protocols for this pathogen with other internal and external laboratories.

Four out of seven FBO laboratories analysing *Yersinia* spp. did not participate in EQAs. Although *Yersinia* spp. is a regulated pathogen, its monitoring is subject to the epidemiological situation in each EU/EEA country (European Commission, 2003). For instance, there is neither active monitoring of *Yersinia* spp. in foods nor national coverage for yersiniosis infections in France. Detection of *Yersinia* spp. in France follows ISO 10273 standard, based solely on conventional microbiology, followed by isolation of the strain and PCR only in case of positive findings. In Netherlands, *Yersinia* spp. surveillance system does not exist (ANSES, 2017; ECDC, 2022b). This absence of mandatory surveillance and national coverage in some EU/EEA member states may contribute to lower participation of FBO laboratories in EQAs as accreditation of detection methods for this pathogen may not be relevant.

Four out of nine FBO laboratories analysing Shiga toxinproducing *E. coli* (STEC) in food did not participate in EQAs. The four FBO laboratories were from France and Netherlands. STEC is notifiable at a national level in all participating EU/EEA countries (European Commission, 2003), except for France where reporting is instead voluntary and based on paediatric haemolyticuremic syndrome (HUS) surveillance (Jones et al., 2019; ECDC, 2022c); therefore, EQAs for detection methods might be unnecessary for FBO laboratories (EFSA & ECDC, 2021). Furthermore, as stated in the tenth EQA scheme for typing of Shiga toxin-producing *E. coli* in Europe, further reasons for nonparticipation in EQAs could be: lack of laboratory capacity, lack of financial means, characterisation or typing method was not relevant to the laboratory, among others (ECDC, 2021).

Harmonisation of sampling and analytical protocols between official laboratories and FBO laboratories could strengthen

relationships and build trust between the public and private sectors. Multiple investigations for reconfirmation of results would not be necessary and therefore interventions would become more cost-effective for both sectors. FBO could obtain better trading opportunities by following official control analytical protocols. For instance, the compliance of official control analytical protocols could be used as recognition by the FBO to allocate their products in the market with, perhaps, an increased consumer acceptance. In a hypothetical scenario, national stakeholders and the EU commission could promote the use of official analytical protocols such as the total aerobic viable count (TVC), ELISA, chromatographic tests (e.g., HPLC), the use of ISOstandards as analytical reference methods (European Commission, 2005), and in the same way consider the potential of rapid on-site testing of microbial contamination in food products (Visciano & Schirone, 2020; Santovito et al., 2022), through regulations that reward the FBO that apply these control protocols and consequently, encourage the rest of FBO to perform accordingly.

# 4 Conclusion

The study gathered insight into current practices of microbiological sampling and analyses performed in FBO HACCP-based self-control programmes in EU/EEA countries, considering the response rate of the survey.

Further efforts are needed for harmonisation and standardisation of analytical protocols and further characterisation of foodborne pathogens. A need for regular participation in EQAs and the implementation of rapid on-site testing for assessing food safety might be considered in the future.

# Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

# Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

# Author contributions

CF, NB, and FS were responsible for the conceptualisation of the study. CF was responsible for the data collection. MA was responsible for the data curation, interpretation of results and writing. AK was responsible for reviewing and editing the manuscript. All authors contributed to the article, read and approved the submitted version. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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