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Use of harmonised epidemiological indicators (HEIs) for broilers in Europe

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ABSTRACT

In 2012, the European Food Safety Authority (EFSA) proposed harmonised epidemiological indicators (HEIs) for poultry at different production stages: *Salmonella*, *Campylobacter*, extended-spectrum β-lactamase/AmpC β-lactamase (ESBL/AmpC) producing *Enterobacteriaceae* and generic *Escherichia (E.) coli*. The HEIs are based on existing monitoring systems or the sampling strategies provided by EFSA. To realise the full benefit of HEIs, risk managers should use them for farm and abattoir categorisation and for adapting the existing meat inspection systems. As HEIs are not legal requirements within the European Union (EU), it is unclear which HEIs are used in which country, to date. Therefore, an online survey was conducted in Europe to gather knowledge about the implementation, application and consequences following on from the HEIs in existing official and private monitoring and surveillance systems (MOSS).

A total of 34 answer sets from participants working in the framework of official surveillance or as food business operators in broiler abattoirs were collected from eleven EU member states (EU-MS) and four non-EU countries.

While all participants stated that testing for *Salmonella* is performed, HEI 4-*Salmonella*, which corresponds to the process hygiene criterion (PHC) for *Salmonella* was applied by 62% of the participants. In total, 94% of the participants reported that they test for *Campylobacter*. Among them, 71% stated that testing is performed for HEI 5-*Campylobacter*, which corresponds to PHC for *Campylobacter*. Although testing neck skin samples for *Salmonella* and *Campylobacter* after chilling are official and mandatory MOSS in the EU, not all participants from EU-MS (*Salmonella*: 6/11 EU-MS; *Campylobacter*: 8/11 EU-MS) confirmed to comply with. Altogether, 56% of the participants (from 6 EU-MS and 2 non-EU countries) stated that they test for *E. coli*. Ten of them reported that the testing is performed at the abattoir after chilling according to the suggested HEI for generic *E. coli* as a hygiene indicator. Consequences that result from the existing MOSS for the three examined pathogens (*Salmonella*, *Campylobacter* and *E. coli*) were mainly rising awareness, farm risk categorisation and feedback to the farmer.

According to the answers from the participants, the HEIs suggested by EFSA for broilers are currently implemented in most EU-MS. One reason could be that some of the according MOSS are required by EU law. As intended by EFSA, the participants stated that they use HEIs for farm risk categorisation as one of the three top consequences following from MOSS for the three mentioned pathogens. For improving the knowledge and application of HEIs in the context of risk-based meat safety assurance systems, specific training could be helpful.

1. Introduction

1.1. Background

Poultry meat is the most widely consumed meat per capita worldwide, and the European Union (EU) ranked fourth in poultry production in 2022 (USDA, 2023). Every year, human cases of salmonellosis and campylobacteriosis as the main foodborne diseases are associated mainly with consumption of poultry meat and products thereof, with a total of 52,702 cases and 120,946 cases, respectively in the EU for 2020 (EFSA/ECDC, 2021). The chicken production system is strictly divided into egg (laying hens) and meat (broilers) production breeds (Flock &

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Preisinger, 2007; Preisinger, 2003; Tixier-Boichard et al., 2012). The organisation of broiler breeding programmes for meat is based on relatively few specialised grandparent flocks and parent *Gallus gallus* breeding flocks that produce eggs for hatcheries. They supply day-old-chicks to a large number of broiler fattening farms (Bessei, 1999; Laughlin, 2007). For commercial broiler meat production, this is organised in integrated systems (EFSA, 2012a), and in most European countries, broiler fattening farms deliver their birds to specialised slaughter companies. The European Food Safety Authority (EFSA) identified *Campylobacter*, *Salmonella* and extended-spectrum β-lactamase/AmpC β-lactamase (ESBL/AmpC) producing *Enterobacteriaceae* as the most relevant biological hazards in the context of poultry meat inspection (EFSA, 2012a). As poultry are carriers of these zoonotic agents and other pathogens (Blevins et al., 2018; Mead, 2004; Nørrung & Buncic, 2008), and foodborne disease is often connected to healthy animals not showing visible pathologic signs or lesions (Mead, 2004; Zweifel & Stephan, 2014), the hazards cannot be identified by ante- or post-mortem meat inspection carried out at the abattoir (EFSA, 2012a; Löhren, 2012). Different automated processing steps at poultry abattoirs reduce the bacterial loads on the carcass surfaces, but cannot completely exclude cross-contamination and carryover of these pathogens to the final product (Althaus et al., 2017; EFSA, 2012a; Mead, 2004).

1.2. Harmonised epidemiological indicators for broilers

It is important that control actions to minimise the incoming bacterial loads at the abattoir not only focus on the slaughter level but also include primary production, with its farm and feed levels (EFSA, 2012a; Klein et al., 2015; Maurischat et al., 2015; Vandeplas et al., 2008). To control these hazards, in 2012, EFSA suggested the use of so-called harmonised epidemiological indicators (HEIs) for poultry – namely for *Salmonella*, *Campylobacter*, ESBL/AmpC producing *Enterobacteriaceae* and generic *Escherichia* (*E.*) *coli*. A HEI is defined as "the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard that correlates to human health risk caused by the hazard" (EFSA, 2012b). HEIs should be used for risk analysis to decide whether meat inspection systems have to be adapted to control foodborne hazards in the broiler food chain (EFSA, 2012b). As each abattoir is unique, and different influencing factors on microbial loads exist (EFSA, 2012a; Habib et al., 2012; Pacholewicz et al., 2015), which HEI alone or in combination should be applied has to be decided on an individual basis (EFSA, 2012b). The official veterinarian (OV) takes a central role in this proposed risk-based meat safety assurance system (RB-MSAS) (Ferri et al., 2023). Additionally, EFSA suggested using HEIs for the categorisation of farms and abattoirs if current methods are not adequate (EFSA, 2012b). HEIs were only suggested by EFSA, but for some HEIs, their use is mandatorily laid down in EU regulations (i.e., *Salmonella* and *Campylobacter* at the abattoir), while for other HEIs, EFSA describes possible sampling schemes (i.e., generic *E. coli*) (EFSA, 2012b). In conclusion, some of the HEIs are implemented today as monitoring and surveillance systems (MOSS) or as official control programmes.

While monitoring can be defined as continuous data collection on a certain disease for reporting purposes, in surveillance, the gathered data are additionally used for specific actions to be taken. These actions will be taken when the MOSS provide results that are above a pre-defined threshold level (Christensen, 2001; Doherr & Audigé, 2001). The combination of MOSS with control and intervention strategies, and with the aim to reduce a specific disease over time, is defined as a (disease) control programme by Christensen (2001). The term MOSS and the related consequences are sometimes used collectively (Doherr & Audigé, 2001).

1.3. Monitoring and surveillance for Salmonella and Campylobacter

The occurrence of the zoonotic pathogens, *Salmonella* and *Campylobacter*, is reported at feed, farm and food levels (EU Commission, 2003a), based on sampling at the corresponding production stages along the food chain. Data are collected by competent authorities (CAs), and the EU member states (EU-MS) have to transmit their monitoring results to EFSA. Additionally, for *Salmonella*, Regulation (EC) No 2160/2003 (EU Commission, 2003b) lays down community targets for reducing the prevalence of *Salmonella* in general for poultry, followed by specifications in Regulation (EC) No 200/2012 (EU Commission, 2012) for broiler flocks. The food business operator (FBO) has to perform tests for *Salmonella* as a food safety criterion at retail or as a process hygiene criterion (PHC) in abattoirs (EU Commission, 2005). *Salmonella* must be absent a) in 25 g of poultry meat at retail level or b) in 25 g of pooled neck skin at slaughter level (EU Commission, 2005). In some of the Nordic countries, successful *Salmonella* intervention is based on intensive flock testing at farm level and *Salmonella*-free breeding flocks as a starting point (Blagojevic et al., 2021; Wegener et al., 2003). Following the results of the baseline survey for the prevalence of *Campylobacter* in broiler flocks and on carcasses (EFSA, 2010a,b), EFSA identified possible control actions in broiler production with the focus on reducing *Campylobacter* in primary production of broilers. Actions at this stage are expected to have the main impact on lowering the incidence of campylobacteriosis in humans (EFSA, 2011a; Nastasijevic et al., 2020; Nauta et al., 2009; Seliwiorstow et al., 2016). Since at farm level no control programme is yet implemented in the EU, EFSA recently updated possible suggestions for control options and proposed performing a new baseline study for *Campylobacter* (EFSA, 2020b). At slaughter level, the PHC for *Campylobacter* performed by FBOs was implemented in 2018, defining the threshold for control actions as 1,000 colony forming units/g in 15 pooled neck skin samples (10 samples from 2025 onwards) of 25 g (EU Commission, 2005). In setting these thresholds, it was estimated that the risk of human infection could be reduced by 50% if no batch of broilers exceeded these limits (EFSA, 2011a).

1.4. Monitoring and surveillance for ESBL/AmpC E. coli and generic E. coli

Poultry meat was identified as one of the main sources for ESBL/AmpC producing *Enterobacteriaceae* (EFSA, 2011b). Monitoring for antimicrobial resistance in general has to be performed according to Directive 2003/99/EC (EU Commission, 2003a), and annual reports are published (EFSA/ECDC, 2022). However, details on testing for ESBL/AmpC producing *Enterobacteriaceae* in broilers both at farm and slaughter levels are lacking in EU legislation. Even for *E. coli*, as one of the main representatives of this pathogen group, control actions for poultry have not yet been implemented. For other food producing animals, i.e., pigs, enumeration of *Enterobacteriaceae* on carcasses is required as a PHC at abattoirs (EU Commission, 2005). *Enterobacteriaceae* and *E. coli* can serve as indicators of faecal contamination of carcasses and, therefore, for assessing the process hygiene in abattoirs (Althaus et al., 2017; Hauge et al., 2022; Milios et al., 2014). Additionally for broilers, EFSA recommended that the HEI for generic *E. coli* can serve as a PHC in broiler abattoirs additionally to the PHC for *Salmonella* (EFSA, 2012a).

1.5. Objectives

Since the production stages, the samples to be taken and to be analysed as well as the application of the HEIs for poultry, proposed in 2012, are not regulated by law, the aim of this study was to investigate which of EFSA's HEIs for broilers are utilised within European countries and whether corresponding MOSS exist on an official or private basis. Additionally, resulting consequences of the existing MOSS and desired improvements were studied.

2. Materials and methods

2.1. Questionnaire development and structure

This study is part of a questionnaire-based study within the Cost Action CA 18105 - Risk-based meat inspection and integrated meat safety assurance ("RIBMINS"; www.ribmins.com) with the aim to collect information regarding the status quo and improvements of food chain information and HEIs as provided by EFSA for broilers, pigs and bovines. The questionnaire was drafted, and positive feedback and validation by two social scientists from the Agriculture Economics Research Institute (AGRERI) of ELGO-DIMITRA in Athens, Greece was given, after which the questionnaire was created using the cloud-based software and questionnaire tool, SurveyHero® (enuvoGmbH, Zurich, Switzerland). Ethical approval for the study was obtained from the Central Ethics Committee of Freie Universität Berlin, Germany under ZEA-Nr. 2022-008.

In the following, only the survey concerning broilers is described. The questionnaire for HEIs for broilers consisted of two parts, 'General information' and 'Harmonised Epidemiological Indicators (HEIs)' (see Supplementary material S1). The general information questions included the country in which the participant works, the participant's professional role and the slaughter capacity, i.e., the average number of animals slaughtered in the abattoir per hour. The HEI questions referred to the HEIs for *Salmonella*, *Campylobacter* and *E. coli* as proposed by EFSA (EFSA, 2012b). For each pathogen, questions were asked about the sampling point in the production process, the sample material and the methods used. At the end, the participants could provide suggestions for additional monitoring and testing. The following question types were used: single choice (only one answer could be chosen), multiple choice (single or multiple answers could be chosen) and open questions (text could be added). In total, the final questionnaire consisted of 47 questions, from which seven were mandatory questions and 40 were only displayed if the overriding question was answered with "yes" (see Supplementary material S1).

The term 'monitoring' instead of 'MOSS' was used in the detailed questions about each pathogen for the following reasons: i) parallel usage of the term MOSS for both monitoring and surveillance systems, as in German language, ii) the fact that some languages do not differentiate between both terms included in MOSS, iii) the assumption that the abbreviation MOSS was not known by all potential participants, and iv) the possibility of language limitations regarding the English terms.

2.2. Survey distribution and data collection

The final link to the online survey accompanied by application instructions were distributed by the RIBMINS Science Communication Manager to the 33 RIBMINS National Contact Points (NCPs) located in the EU-MS and non-EU countries. Each RIBMINS NCP was obliged to decide how many participants would be invited to answer the questionnaire to get an applicable picture according to the structure in their country. As a minimum, the NCPs were asked to ensure the participation of one OV/meat inspection officer (hereafter both are referred to as OV) and one FBO/quality assurance manager (hereafter both are referred to as FBO). Furthermore, the NCPs were free to send the questionnaire to other people who worked practically with HEIs in their country. The goal was to get a representative picture of the countries in Europe.

Data collection took place between 6th November and 16th December 2020. Anonymity was guaranteed for all participants according to Regulation (EU) 2016/679 (EU Commission, 2016).

2.3. Data analysis

Responses from the United Kingdom (UK) were included in the answers of EU-MS, as the survey was conducted at the end of 2020, when the Brexit transition phase was still ongoing, and the UK continued to be subject to EU rules at that time. To guarantee anonymity of the participants, answers from Norway and Iceland were included in non-EU countries although they belong to the European Economic Area together with Liechtenstein and all 27 EU-MS.

Free text answers from the category 'other' were placed in one of the given categories if they matched the content. In cases where the answer from the category 'other' showed that the participant had not understood the question correctly or that the given answer did not fit the context, the authors decided to not take these answers into account. Those answers were counted as if the question was not answered.

Only completely answered questionnaire sets were included in the final analysis.

MS Excel® 2016 (Microsoft, Redmond, WA, USA) and IBM SPSS version 28 (SPSS for Windows (IBM®, Armonk, NY, USA) were used to analyse the data in a descriptive way and to create figures.

3. Results and discussion

3.1. General information concerning the participants

In total, 48 persons participated in the survey, of which 34 completely replied to the HEI questions and were finally included in the analysis. The answers were generated from 15 countries, which were eleven EU-MS (73%), i.e., Croatia, Denmark, France, Germany, Ireland, Latvia, Poland, Portugal, Romania, Sweden and the UK, as well as four non-EU countries (27%), i.e., Albania, Iceland, Norway and Serbia. Significantly more participants worked in two of the EU-MS, with nine and six participants, followed by one country with three participants and four countries with two participants each. For the remaining eight countries, only one participant per country answered the questionnaire (Table 1).

Even though it was our aim to get answers from at least one OV and one FBO per country, we also wanted to get an overview of the situation in Europe, which is why we wanted to include as many countries as possible. Thus, we included in the analysis all responses, also those from countries where only one response was available, as all participants were experts, and the responses could, therefore, be considered of high quality and informative value. By analysing all fully answered questions, it was possible to include responses from four of the five countries with the highest number of broilers slaughtered per year, but also one country with one of the lowest number of broilers slaughtered in 2021 (Eurostat, 2023). Most answers were received from OVs (21/34; 62%): 16 from EU-MS (76%) and five from non-EU countries (24%). In total, ten FBOs (10/34, 29%), all from EU-MS, answered the questionnaire. Three participants could not be connected to one of the pre-defined categories and were classified as "others" (3/34, 9%): two from EU-MS (one university professor and a member of a governmental organisation) and one from a non-EU country (a member of a governmental organisation) (Table 1).

The participants assigned themselves to an abattoir size according to the number of slaughtered animals per hour in the pre-defined abattoir size categories. Most answers were collected from medium-sized abattoirs with slaughter capacities of 3,001 to 10,000 broilers per hour (17/34). From small abattoirs with slaughter capacities of less than 3,000 broilers per hour and large abattoirs with slaughter capacities over 10,000 birds per hours, the number of responses was almost the same with eight and nine answers, respectively. Answers from OVs and FBOs were collected from each pre-defined category (Table 1). Therefore, we assume that our results reflect a representative picture in terms of the EU's structural aspects of broiler abattoirs.

3.2. Testing for Salmonella

All participants from the 15 participating European countries answered that they test for *Salmonella* (100%; 34/34). The participants reported that the performed MOSS is official in 97% (33/34) of the cases or private in 74% (25/34) of the cases. In all included countries (11 EU-

Table 1
Overview of the answers received per country in an according to country status and the number of answers per participant role and per abattoir size category to which the participant assigned themselves (anonymous presentation).

Country-ID	Country status	Answers received						
		Total	Role of participant			Abattoir size category (birds slaughtered/h)		
			OV	FBO	Other	<3,000 (small)	3,001–10,000 (medium)	>10,000 (large)
1	EU-MS	2	1	1		1	1	
2	EU-MS	1		1			1	
3	EU-MS	9	5	4			6	3
4	EU-MS	1	1					1
5	EU-MS	1	1				1	
6	EU-MS	1			1		1	
7	EU-MS	1	1			1		
8	non-EU	2	2			2		
9	EU-MS	3	2		1	2	1	
10	EU-MS	3	2	1			3	
11	non-EU	2	1		1		1	1
12	EU-MS	5	2	3			1	4
13	non-EU	1	1			1		
14	EU-MS	1	1				1	
15	non-EU	1	1			1		
Total		34	21	10	3	8	17	9

OV: official veterinarian or meat inspection officer.
FBO: food business operator or quality assurance manager.
other: professor or member of a governmental organisation.
EU-MS: EU member state.
Non-EU: countries from European Economic Area or other European countries not being an EU member state.

MS, 4 non-EU countries), official MOSS for *Salmonella* was conducted (Table 2; Supplementary material S2 & S3). Interestingly, one participant from an EU-MS, and working in a large abattoir as an OV, answered that only a private MOSS for *Salmonella* is performed. As the participant was an OV, and the *Salmonella* monitoring at abattoirs is performed by FBOs (when testing their process hygiene using the PHC), the participant was probably unaware that the PHC for *Salmonella* that is tested by the FBO is an official MOSS (since the OV only checks the results of these self-checks) (EU Commission, 2005). Additionally, private MOSS for *Salmonella* was performed in ten countries (7 EU-MS; 3 non-EU countries). In conclusion, *Salmonella* testing was performed in all included countries, which is an encouragingly positive result, as monitoring of *Salmonella* is mandatory following the EU regulations (EU Commission, 2003a,b, 2005, 2012).

Most participants (32/34, 94%) reported that testing for *Salmonella* is mainly conducted at farm level. Testing at slaughter level before chilling (14/34, 41%) and after chilling (21/34, 62%) were mentioned as the second and third most frequent sampling points. Testing the transport vehicles for *Salmonella* is performed according to seven answers from two EU-MS. In the category 'other', two participants from the same EU-MS mentioned that they test fresh meat or products in the abattoirs as additional sample material.

Not all participants who stated that they test for *Salmonella* at a specific sampling point answered the questions regarding the sampled material and the methods used. For each sampling point, different sample materials and methods were mentioned. Multiple answers were possible for these sub-questions, which did not allow a direct connection between the sample materials taken and the methods performed.

At the hatchery and/or the beginning of a new production cycle, 75% (9/12) of the participants, and at the farm prior to slaughter, all participants (30/30) stated that boot swabs or pooled faeces are taken. In total, six participants (6/34, 18%) stated transport vehicles are sampled using swab samples. At the abattoir before chilling, caecal content and tissue samples (the latter specified as skin samples), were mentioned by an equal number of participants (34%; 4/11). At the abattoir after chilling (21/34; 62%) tissue samples, specified as skin samples, were taken in all seven countries (6 EU-MS, 1 non-EU-country) in which testing was performed at this sampling point. All sample materials were analysed (20/20, 100%) with microbiological methods. Depending on the sampling point, PCR was mentioned as the only or as a second examination technique (Fig. 1).

EFSA proposed four HEIs for *Salmonella* (EFSA, 2012b), hereafter referred to as HEI 1-*Salmonella* to HEI 4-*Salmonella*. HEI 1-*Salmonella* and HEI 2-*Salmonella* focus on the farm on flocks of breeding parents and poultry prior to slaughter, respectively. We focussed especially on broilers, so we can only evaluate the realisation of the latter.

In total, 91% (31/34) of the participants reported sampling flocks on the farm for *Salmonella* testing. Out of them 30 participants reported that they use pooled faeces or boot swabs which is described for HEI 2-*Salmonella* (using pooled faeces (i.e., boot swabs) and microbiological analysis for detection and serotyping). In total, 87% (27/31) use classical microbiological methods, while 26% (8/31) reported that PCR is performed, in four cases additionally to classical microbiology and in four cases solely. The participants sampling HEI 2-*Salmonella* originated from ten EU-MS and from three non-EU countries. EFSA suggested that the monitoring data from the national control programmes is used for this HEI 2 (EFSA, 2012b). According to the answers, this is done in nine of the eleven participating EU-MS, meaning that they comply with the legal requirement for *Salmonella* control in broiler flocks (EU Commission, 2012). One OV from an EU-MS stated that testing at the farm for HEI 2-*Salmonella* is conducted, but did not specify the material sampled or the method used. Thus, we assume that the participant was aware that official monitoring took place at farm level, but did not perform these tests personally and could not, therefore, specify more in detail what was done.

HEI 3-*Salmonella* (farm audits for controlled housing conditions) was reported as being performed by two participants (OV and FBO), both from the same EU-MS. EFSA proposed requirements for controlled housing conditions in its report (EFSA, 2012b), but as we did not ask in this survey for the detailed measures applied, we cannot interpret these answers further. We assume that it would be much easier to comply with these requirements in integrated production systems than in small holdings, and that FBOs and OVs will have access to management and biosecurity data from farms in integrated systems, and which can be gathered by one-off or frequent audits.

Sampling at the position of HEI 4-*Salmonella* (testing at the abattoir after chilling using neck skin or breast skin for microbiological detection and serotyping) was stated as applied by 62% (21/34) of the

Table 2
Overview of the received answers by participant, their background and information on the monitoring systems in place for *Salmonella*, *Campylobacter* and *E. coli* (more than one answer allowed per participant) (anonymous presentation).

	ID	Role	CID	Abattoir size	*Salmonella*							*Campylobacter*							*E. coli*						
					Test	OM	OS	O-MOSS	PM	PS	P-MOSS	Test	OM	OS	O-MOSS	PM	PS	P-MOSS	Test	OM	OS	O-MOSS	PM	PS	P-MOSS
EU-MS	1	FBO	1	S	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y
	2	OV	1	M	Y	Y		Y	Y		Y	Y	Y		Y				Y	Y		Y	Y		Y
	3	FBO	2	M	Y		Y	Y		Y	Y	Y	Y		Y										
	4	FBO	3	M	Y	Y	Y	Y	Y		Y	Y				Y		Y	Y				Y		Y
	5	OV	4	L	Y		Y	Y				Y	Y		Y				Y	Y		Y			
	6	OV	5	M	Y	Y	Y	Y				Y	Y		Y										
	7	Other	6	M	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y							
	8	OV	7	S	Y	Y		Y				Y	Y		Y										
	9	FBO	3	M	Y	Y		Y				Y	Y		Y				Y	Y		Y			
	10	FBO	3	L	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y	Y				Y		Y
	12	OV	9	M	Y		Y	Y				Y	Y		Y	Y		Y	Y	Y		Y			
	13	FBO	10	M	Y	Y		Y	Y		Y	Y				Y		Y	Y	Y		Y	Y		Y
	14	Other	9	S	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y	Y	Y		Y			
	15	OV	9	S	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y	Y		Y			
	16	OV	10	M	Y		Y	Y		Y	Y	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y
	18	OV	12	L	Y					Y	Y	Y					Y	Y	Y		Y	Y		Y	Y
	19	FBO	12	L	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y
	20	OV	12	L	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y							
	22	OV	3	M	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y		Y	Y							
	23	OV	14	M	Y	Y		Y				Y				Y		Y							
	25	OV	10	M	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y							
	26	OV	3	L	Y		Y	Y		Y	Y	Y	Y		Y				Y	Y		Y			
	27	OV	3	L	Y		Y	Y		Y	Y	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y
	29	FBO	12	L	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y					Y	Y
	30	OV	3	M	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y										
	31	OV	3	M	Y	Y		Y				Y	Y		Y										
	33	FBO	12	M	Y	Y		Y	Y		Y	Y	Y		Y										
	34	FBO	3	M	Y	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y	Y		Y			
Total EU-MS					28	17	15	27	12	12	21	28	20	6	24	12	7	18	17	12	2	14	7	3	10
Non-EU	11	OV	8	S	Y		Y	Y		Y	Y	Y					Y	Y							
	17	OV	11	L	Y		Y	Y				Y		Y	Y										
	21	OV	13	S	Y		Y	Y		Y	Y	Y					Y	Y	Y		Y	Y			
	24	OV	8	S	Y	Y		Y	Y		Y														
	28	Other	11	M	Y		Y	Y		Y	Y	Y		Y	Y		Y	Y							
	32	OV	15	S	Y	Y	Y	Y											Y	Y	Y	Y			
Total non-EU					6	2	5	6	1	3	4	4	0	2	2	0	3	3	2	1	2	2	0	0	0
Total					34	19	20	33	13	15	25	32	20	8	26	12	10	21	19	13	4	16	7	3	10
%					100	56	59	97	38	44	74	94	63	25	81	38	31	66	56	68	21	84	37	16	53

ID – Participant; CID – ID of the country; OM – Official monitoring system in place; OS – Official surveillance system in place; O-MOSS – Official monitoring and surveillance system in place (answer generated from participants' answers counted together when OM, OS or both were selected); PM – Private monitoring system in place; PS – Private surveillance system in place; P-MOSS – Private monitoring and surveillance system in place (answer generated from participants' answers counted together when PM, PS or both were selected); EU-MS – Member state of the European Union; non-EU – countries from European Economic Area or other European countries not being an EU member state; FBO – Food business operator/Quality assurance manager; OV – Official veterinarian/Meat inspector; Other – professor or member of a governmental organisation; S – small abattoir with slaughter capacity of <3,000 broilers/h; M – Medium-sized abattoir with slaughter capacity of 3,001–10,000 broilers/h; L – Large-scale abattoir with slaughter capacity of >10,000 broilers/h; Y – Yes; % – Percentage based on numbers of participants testing for the respective pathogen.

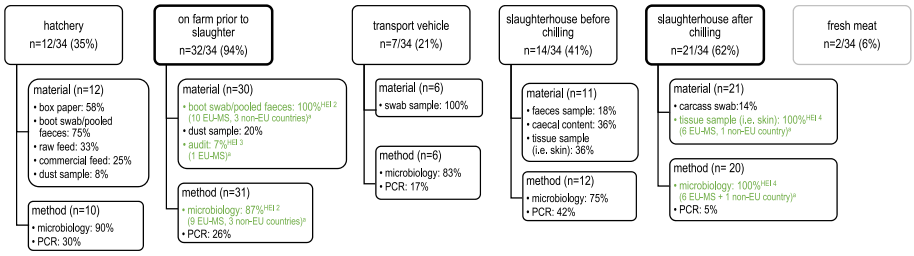

Fig. 1. Flowchart of participants' answers for implemented monitoring and surveillance systems for *Salmonella* in broilers (N = 34; multiple answers allowed).
black box – pre-defined answers options; light grey box – answers given as "other" but no further questions were linked for material used and methods performed if "other" was selected; bold box – HEI 2, HEI 3 and HEI 4 for *Salmonella;* green letters – sampling material and method for HEIs
[a] number of countries in which participants worked (multiple participants per country possible, but each country is only counted once in the figure).

participants. Using neck skin samples for classical microbiology at this position methodologically satisfies the PHC for *Salmonella* (EU Commission, 2005). In total, 20 participants stated that tissue samples, which were specified as skin samples and in one case as an organ sample, are used for the microbiological analysis. Three participants from three EU-MS reported that carcass swabs are used. One reported that the swabs are used instead of neck skin samples, whereas the other two stated swabs are used additionally to neck skin. This is not in accordance with the defined PHC for *Salmonella* or with HEI 4-*Salmonella*, and as the participant worked as an OV in a large abattoir, it is inexplicable why this answer was given. Our analysis was additionally hampered because other participants from the same EU-MS stated that neck skin is used as the sample material. In conclusion, all 20 participants from six of the ten participating EU-MS and the one participant from a non-EU country, all of whom use neck skin samples after chilling, complied methodologically with the PHC for *Salmonella*, and so also cover HEI 4-*Salmonella*. However, we cannot explain the fact that some of our survey participants, who were from EU-MS, did not state that neck skin samples are used as an official MOSS in their country.

Action plans against *Salmonella* infections include a combination of farm and slaughter level MOSS in almost all countries, as laid down in EU regulations (EU Commission, 2003a,b, 2005, 2012). Despite reports of practical and efficient interventions for *Salmonella* being rarely implemented at farm level at national or regional levels, the Nordic countries, Finland, Norway and Sweden, have demonstrated that successful interventions for *Salmonella* in cattle, pigs and poultry at herd/flock level have been in place since the 1980s. Later on in 2018, Denmark was accepted by the EU Commission to have the same state as the Nordic countries mentioned above regarding *Salmonella* in broilers. The focus of intervention strategies in these countries is based on testing regimes at farm level and integrated controls at farm and slaughter levels (Forshell & Wierup, 2006; Heier et al., 2022; Maijala et al., 2005; Wegener et al., 2003).

3.3. Testing for Campylobacter

In total, 94% (32/34; from 11 EU-MS and 3 non-EU countries) of the participants reported that *Campylobacter* testing is conducted in their country. Testing for *Campylobacter* is performed mainly as official MOSS (81%, 26/32) and less so as private MOSS (66%, 21/32) (Table 2; Supplementary material S2 & S3). The two participants who stated that *Campylobacter* is not tested for worked in two non-EU countries. Deviating responses were received from another country, as one participant stated testing is not conducted, while another participant stated that private monitoring is implemented. Both worked as OVs, and in this non-EU country, testing for *Campylobacter* is officially regulated and is mandatory as a PHC. Again, the OVs could have misunderstood and misinterpreted the question, as they likely just check the self-check PHC that is performed by FBOs (see Section 3.2).

The 32 participants testing for *Campylobacter* stated they test mainly at the abattoir after chilling (23/32, 72%), while fewer test before chilling (12/32, 38%). According to the answers received, testing on farm, at hatchery or taking other samples like fresh meat were performed rarely, <10% for each sampling point (3/32; 3/32; 1/32, respectively).

As previously reported for *Salmonella*, not all participants who stated that they test for *Campylobacter* provided answers on the sample material for each sampling point. Data on sample material at the abattoir before chilling was provided by 11 of 12 participants. According to those participants' answers, caecal content is used in 73% (8/11), tissue samples, i.e., skin samples in 64% (7/11) and cloacal swabs in 9% (1/11) of cases. All samples were analysed using classical microbiological methods (82%, 9/11) or PCR (27%, 3/11). For the sampling point at the abattoir after chilling, 22 participants (22/32, 69%) answered how testing is performed, and all stated that tissue samples, specified as skin samples, are used. Additionally, one participant reported that carcass swabs are taken at that position. Analyses were performed by classical microbiological methods in 91% (20/22) and by PCR in 9% (2/22) of the cases (Fig. 2).

For *Campylobacter*, five HEIs (hereafter referred to as HEI 1-*Campylobacter* to HEI 5-*Campylobacter*) were proposed by EFSA (EFSA, 2012b).

HEI 1-*Campylobacter* (testing flocks on the farm with caecal droppings by real-time PCR) was applied by three participants, all from one non-EU country.

HEI 2-*Campylobacter* (farm audits for controlled housing conditions) was not applied by any of the participants.

As HEI 3-*Campylobacter* (partial depopulation of the flock analysed using FCI) was not included as a specific question in our survey, and as no one provided information about this HEI as an additional answer, we cannot report if this HEI is used or not.

HEI 4-*Campylobacter* (testing at evisceration stage using caecal content for microbiological enumeration) was reported to be applied by 25% (8/32) of the participants. The answers originated from participants in five EU-MS.

HEI 5-*Campylobacter* (testing neck skin or breast skin after chilling for microbiological enumeration) was applied by 72% (23/32) of the participants, but only 22 of them gave information regarding the sample material. They all reported that neck skin is used as the sample material, while one mentioned carcass swabs additionally. The participants who answered that this sampling is conducted in their country worked in eight EU-MS and in two non-EU countries. Using neck skin for classical microbiological analysis after chilling methodologically satisfies the PHC for *Campylobacter* at the abattoir (EU Commission, 2005), so it was expected that all EU-MS test at this sampling point. Interestingly, participants from only eight of the eleven included EU-MS answered that this test is performed at the abattoir. From one EU-MS, both participants (OV and FBO) answered that official monitoring in the abattoir for *Campylobacter* exists. However, they reported that the sampling position is before chilling and that caecal content is used – which complies with HEI 4-*Campylobacter* – along with neck skin as sample materials. This methodology does not satisfy the mandatory PHC for *Campylobacter*. The other missing answers from EU-MS could be because the participants understood the question referred to testing that was conducted by

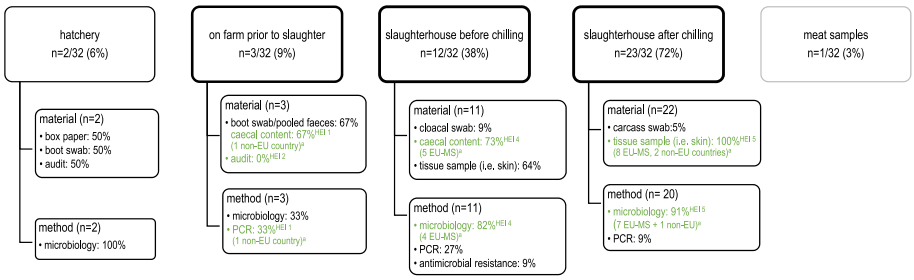

Fig. 2. Flowchart of participants' answers for implemented monitoring or surveillance system for *Campylobacter* in broilers. (N = 32; multiple answers allowed).
black box – pre-defined answers options; light grey box – answers given as "other" but no further questions were linked for material used and methods performed if "other" was selected; bold box – HEI 1, HEI 2, HEI 4, and HEI 5 for *Campylobacter*; green letters – sampling material and method for HEIs
[a] number of countries in which participants worked (multiple participants per country possible, but each country is only counted once in the figure).

themselves personally, which was not our intention.

Two studies from Denmark and Sweden – performed before the PHC for *Campylobacter* was set – concluded that higher levels of *Campylobacter* in caecal contents at the farm resulted in higher loads on neck skin samples at slaughter taken after defeathering or before chilling (Hansson et al., 2007; Rosenquist et al., 2006). In contrast, Reich et al. (2018) concluded that no prediction on *Campylobacter* counts of neck skin is possible because they did not find correlations between caecal samples and neck skin samples from the same batch. Additionally, Rosenquist et al. (2006) could not recover *Campylobacter* from all tested intestine samples in their study, even though the batch was *Campylobacter* positive before slaughter. These contradictory results show that at the moment, no simple or stand-alone sampling strategy with the intention of eliminating *Campylobacter* in the broiler food chain exists, as also reported by Nauta (2016). Additionally, Nauta et al. (2016) assumed that no general prediction for *Campylobacter* counts on meat using pre-slaughter samples would be possible. Thus, testing only caecal content prior to slaughter (HEI 1-*Campylobacter*) will not help to predict *Campylobacter* loads at the end of the slaughter process, but can indicate the possible incoming risk and, therefore, will help to risk categorise flocks and to adjust the meat inspection system at the abattoir as intended by EFSA (2012b). Reduction strategies for *Campylobacter* should be used in an integrated approach and should be cost effective and applicable (Vandeplas et al., 2008) for the FBOs at farm and slaughter levels, with the main focus on reducing positive flocks at the abattoir (EFSA, 2011a, 2020b; Gölz et al., 2014; Hansson et al., 2007; Rosenquist et al., 2006). In this framework, using the suggested HEIs 1, 2, 4 and 5 for *Campylobacter* could help FBOs and OVs as key actors in the RB-MSAS, by enabling categorisation of farms prior to slaughter and then adaption of processing and meat inspection at abattoirs as suggested for improving food safety (EFSA, 2012b), and by enabling work with the farmer on individual intervention strategies for *Campylobacter*. One approach can be seen in Norway, where an action plan against thermophilic *Campylobacter* based on flock categorisation and testing prior to slaughter was established in 2001. Carcasses from positive flocks have to be heated or frozen prior to sale in order to reduce the potential for human exposure to this pathogen (Hofshagen & Kruse, 2005).

3.4. Testing for E. coli

In total, 56% (19/34; 6 EU-MS, 2 non-EU countries) of the participants stated that they test for *E. coli*. The 15 participants not testing for *E. coli* came from ten countries (7 EU-MS with 10 answers; 3 non-EU countries with 5 answers). According to the answers, testing for *E. coli* is mainly done using an official MOSS (84%, 16/19), but 53% of participants (10/19) stated that private MOSS is implemented in their country (Table 2; Supplementary material S2 & S3). Once more, for three EU-MS, deviating answers from the same country were received. Interestingly, in these responses from two EU-MS, even those who indicated testing for *E. coli* is conducted contradicted each other, as official MOSS, private MOSS, and both MOSS were used. EU-wide monitoring for *E. coli* as a PHC is mandatory only for minced meat, mechanically separated meat and meat preparations (EU Commission, 2005), but not specifically for poultry meat. For one EU-MS, from which all participants reported official MOSS for *E. coli* is performed, two participants mentioned sampling meat or the final product as additional samples. For the participants from the other EU-MS, there was no specification in this regard. As our participants worked at slaughter level, we assume that they were aware of the existence of official monitoring for *E. coli*. However, as relatively few answers were received for *E. coli* MOSS, and the participants did not provide this MOSS as additional information or for other processing stages, we hypothesise that *E. coli* MOSS only exists in these few cases which were mentioned.

The 19 participants who answered that testing for *E. coli* is performed reported that this is done mainly at slaughter level, with 47% (9/19) testing before chilling and 53% (10/19) testing after chilling. Testing of fresh meat or products was mentioned in two cases (16%; 3/19) as another sampling position, while another participant (5%, 1/19) answered that testing is conducted at the hatchery or on the farm prior to slaughter.

Reported samples at the abattoir before chilling were caecal content (78%, 7/9), tissue samples (33%, 3/9) and faeces (11%, 1/9). According to the participants, all sample materials were used for microbiological analysis. At the abattoir after chilling, all participants (n = 10) using this sampling point took tissue samples, i.e., skin, and 20% (2/10) took additional swab samples. All stated that the sample materials are analysed using classical microbiological methods (Fig. 3).

HEI 1-*E. coli*, the only HEI for generic *E. coli* (testing neck skin or breast skin at the abattoir after chilling for microbiological enumeration), was declared to be conducted by 29% (10/34) of the participants, who work in four EU-MS.

It could be seen as one limitation of this study that we did not ask about EFSA's proposed HEI 1-6-ESBL/AmpC producing *E. coli* in our survey. It was decided to ask only about *E. coli* instead of about ESBL/AmpC producing *E. coli*, even though EFSA proposed separate HEIs for ESBL/AmpC producing *E. coli* and for generic *E. coli*. Our decision was based on the assumption that if testing for *E. coli* is not performed, then testing for ESBL producing *E. coli* is not conducted either. Differing from EFSA, we not only asked for information on HEI 1-*E. coli*, but we also asked about monitoring at the same sampling points as are required for *Salmonella* and *Campylobacter*. Some of these sampling points would satisfy the sampling point requirements in the HEIs for ESBL/AmpC producing *E. coli*, and if answers had been generated regarding such sampling points, the information would have been relevant for ESBL/AmpC producing *E. coli*. However, the questions asking about MOSS on *E. coli* led to only two statements regarding ESBL producing *E. coli* and antimicrobial resistance; these answers discussed MOSS for ESBL producing *E. coli* as an additional examination and as proposed additional MOSS (see Section 3.5). Additionally, using generic *E. coli* or *Enterobacteriaceae* as general indicators of faecal contamination and in process hygiene control was suggested by different studies (Althaus et al., 2017; Buess et al., 2019; Hauge et al., 2022; Milios et al., 2014). EFSA also proposed the use of HEI 1-*E. coli* for this purpose and for other animal

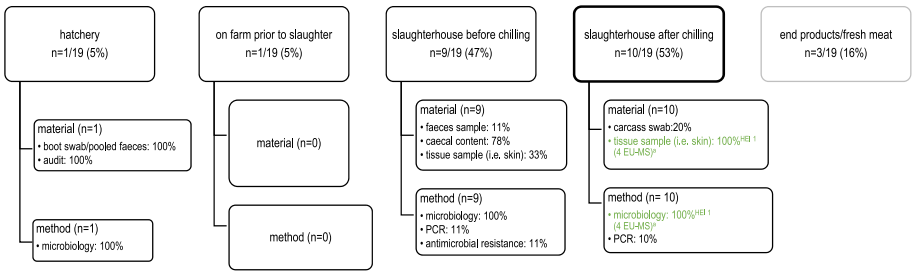

Fig. 3. Flowchart of participants' answers for implemented monitoring and surveillance systems for *E. coli* in broilers. (N = 19; multiple answers allowed).
black box – pre-defined answers options; light grey box – answers given as "other" but no further questions were linked for material used and methods performed if "other" was selected; bold box – HEI 1 for generic *E. coli;* green letters – sampling material and method for HEI
[a] number of countries in which participants worked (multiple participants per country possible, but each country is only counted once in the figure).

species, so today, examination of pig and bovine carcasses for *Enterobacteriaceae* is required (EU Commission, 2005). We concluded, and stand by our decision, that asking about the MOSS for *E. coli* was sufficient to give an overview of existing MOSS and of the proposed HEIs regarding ESBL/AmpC producing *E. coli* and generic *E. coli.*

3.5. Additional monitoring and other information

In total, 79% (27/34) of the participants indicated that no additional monitoring system apart from the three pathogens examined in the survey is needed. The other 21% (7/34), all from EU-MS, stated that additional monitoring systems are desired. In total, four participants proposed monitoring systems for other specific pathogens, which were *Listeria* (2/4), ESBL producing bacteria and methicillin-resistant *Staphylococcus aureus* (MRSA) (1/4), as well as SARS-CoV-2 (1/4). The other two participants (2/7) did not provide a specific pathogen on which an additional monitoring system should focus. Answers from one participant could not be evaluated as they did not fit in the context of the question.

One participant specified the sampling points and the consequences that will follow the monitoring for the three given pathogens, *Salmonella*, *Campylobacter* and *E. coli*. The proposed sampling points and consequences were listed in our questionnaire for each of the pathogens. We assume that this participant is not testing in this way and wanted to inform us that these points and consequences would be helpful for the three pathogens.

The answer regarding SARS-CoV-2 must be considered with caution. The survey was conducted at the height of the SARS-CoV-2 pandemic, including existing lockdowns of the social life in many countries at the end of 2020. At that time, many studies dealt with SARS-CoV-2, as not much was known. Possibly, therefore, monitoring was desired, but it was shown that chicken are not susceptible to SARS-CoV-2 (Schlottau

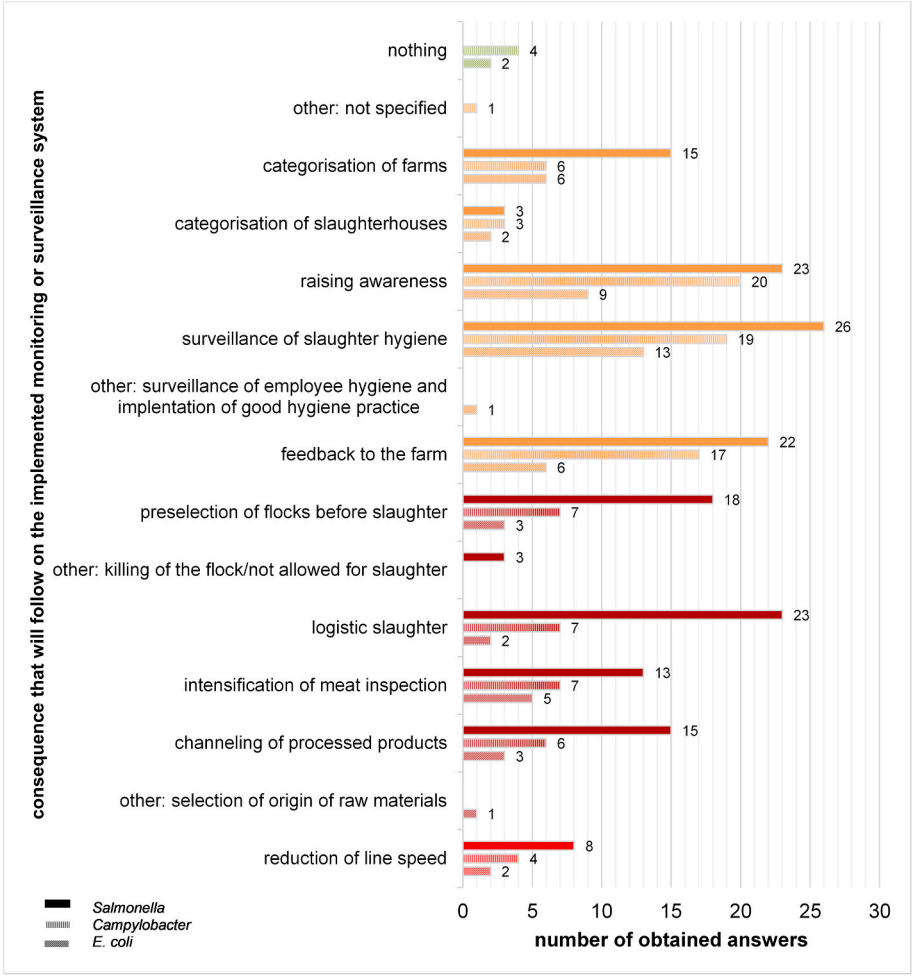

Fig. 4. Overview of participants' answers regarding the consequences that follow on from the implemented monitoring or surveillance systems for *Salmonella* (n = 34), *Campylobacter* (n = 32) and *E. coli* (n = 19) in their country.
The answers of selected pre-defined categories and the individual answers given as "other" have been combined in this figure. The consequences are classified according to their expected impact (monetary or on the slaughter process, from low at the top (green) to high at the bottom (light red)). Multiple answers were allowed in the survey.

et al., 2020; Shi et al., 2020). Even though food can be cross-contaminated with SARS-CoV-2, and the virus was found on frozen foods and their packaging materials (Han et al., 2021), food is not considered as a vehicle of transmission at the moment (BfR, 2022; EFSA, 2020a; FASANZ, 2021; WHO, 2020). Overall, we do not believe that monitoring of SARS-CoV-2 in broilers would be useful.

As relatively few participants stated that additional monitoring or surveillance are necessary, and the given answers were in an individual context for the already existing MOSS, we conclude that the current MOSS of suggested HEIs for broilers are appropriate in Europe at the moment.

3.6. Consequences resulting from the monitoring or surveillance system in place

All participants stating that the respective MOSS for *Salmonella*, *Campylobacter* or *E. coli* are performed gave information on consequences that result from the MOSS for each pathogen (Fig. 4). Multiple answers regarding the consequences could be given.

For all three pathogens, surveillance of slaughter hygiene (*Salmonella*: 77%, 26/34; *Campylobacter*: 59%, 19/32; *E. coli*: 68%, 13/19) and raising awareness (*Salmonella*: 68%, 23/34; *Campylobacter*: 63%, 20/32; *E. coli*: 47%, 9/19) were the most or second most mentioned consequences, followed by feedback to the farm (*Salmonella*: 65%, 22/34; *Campylobacter*: 53%, 17/32; *E. coli*: 32%, 6/19). Categorisation of abattoirs, one use for the HEIs as proposed by EFSA, was the least mentioned consequence that would follow a MOSS for *Salmonella*, *Campylobacter* or *E. coli*. As additional consequences to the pre-defined categories for *Salmonella*, three participants, working in one EU-MS and one non-EU country, reported killing of positive flocks at the farm. This consequence is allowed to be a defined measure in national *Salmonella* control programmes according to EU regulations (EU Commission, 2003b, 2012). Additionally, one participant from one EU-MS answered that a close look at the Hazard Analysis and Critical Control Point (HACCP) system at each processing step is a consequence resulting from *Salmonella* monitoring. This consequence, along with the consequence on HACCP, can be classified as a consequence of raising awareness, since neither of these consequences specify in detail the action to be taken. For *Campylobacter*, no additional consequences to the pre-defined categories were given. The selection of raw materials, as well as surveilling employee hygiene plus implementing good hygiene practice (GHP) were mentioned by two participants, both from EU-MS, as two additional consequences following on from *E. coli* monitoring. These consequences can be seen in the framework of process hygiene, for which EFSA intended HEI-*E. coli* to be used (EFSA, 2012b).

We used the term 'monitoring' in the sub-questions for each pathogen in the questionnaire instead of a differentiation of MOSS (see Section 2). In this aspect, inaccuracies in answers to the question about consequences could have occurred. This was imprecise wording, as consequences after data analysis are applied in surveillance systems. As mentioned above, the term 'monitoring and surveillance' is confusingly used to describe both monitoring and surveillance in some countries (see Sections 1.2 and 2.1). However, since the question on consequences was phrased in terms of reactions/consequences on the monitoring (see Supplementary material S1), we conclude that participants correctly understood the questions and, thus, correctly named those consequences.

3.7. Limitations of the study

This study has some limitations. Firstly, we did not ask whether and to what extent the term 'HEI' was known to the participants. This would have led to clearer statements regarding the use of HEIs in the different countries. Additionally, it would have been interesting to determine if the term HEI was known, since this data is lacking in the literature. Still, meaningful and insightful data on the use of HEIs in the individual countries was provided by the participants.

In some questions, our intention that the participants should answer for the entire broiler food chain if MOSS is performed was likely not clear. It seems that some participants only answered for tests they themselves were performing. This was not our intention, because the participants were selected as experts in the field of meat inspection, and as having in-depth knowledge of all MOSS performed in that framework in the country in which they work.

Another limitation is that we asked separately for each sampling material and method performed, but did not ask for the sampling point when consequence(s) will follow. Therefore, we have provided an overview of all methods performed and all possible consequences, but cannot connect them to specific sampling points or sample materials. As the questions on sample materials and sample methods used were multiple answer questions, a direct match could be made only if just one material and method was used. For the consequences, the sampling point at which the consequence(s) will follow can be assumed for some of the consequences that are at the beginning or the end of the broiler chain. However, the most frequent consequence, i.e., raising awareness, applies at all stages of the broiler chain. Therefore, in future research that will focus on consequences, this point should be considered.

4. Conclusion

Overall, the survey provided a good overview of existing testing strategies for MOSS in broilers in Europe. In contrast to the situation in 2012, today, some HEIs in the broiler chain are implemented in monitoring and control programmes, but they are not used all over Europe. Unfortunately, follow-on consequences resulting from HEIs are rather unspecific and only partly result in the categorisation of farms or abattoirs, which is one of the main reasons for using HEIs as proposed by EFSA. However, it is clear that if appropriate information on pathogens is available, the consequence of raising awareness is applied today to adapt the existing meat inspection system, just as EFSA proposed in the report on HEIs for broilers in 2012. In RB-MSAS, HEIs and other data from the farm, including food chain information, should be combined to decide on adaptions of the meat inspection system.

The HEIs for broilers are still considered as appropriate because the incidences of salmonellosis and campylobacteriosis in humans remain high in most European countries, and the pathogens cannot be detected by visual meat inspection. Communicative feedback to the farmer from the FBO or OV, and equally importantly, communication from the farmer and veterinarian responsible for the farm to the FBO or OV, is essential to improve animal health at the farm and consumer safety. Overall, we conclude that the awareness of HEIs and the possibilities of using the data collected in the framework of already existing MOSS need to be further improved. Appropriate information, e.g., in training opportunities, must be provided for FBOs, OVs and CAs.

CRediT authorship contribution statement

Nina Langkabel: Investigation, Data curation, Visualization, Writing – original draft. **Diana Meemken:** Conceptualization, Methodology, Supervision, Writing – review & editing, Project administration. **Ting-Ting Li:** Data curation, Writing – review & editing. **Smaragda Sotiraki:** Methodology, Validation, Writing – review & editing. **Sofia Anastasiadou:** Validation, Writing – review & editing. **Truls Nesbakken:** Conceptualization, Methodology, Writing – review & editing. **Susann Langforth:** Investigation, Data curation, Writing – review & editing.

Declaration of competing interest

All authors declare no conflict of interest.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2023.110020.

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