104 (2025) 105465

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Full-Length Article

Assessment of process hygiene criteria in poultry slaughterhouses: A comparative analysis of own-checks and official controls in Northeast Italy (2021-2023)

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ARTICLE INFO

Keywords:
Process hygiene criteria
Moving window
Poultry
Campylobacter
Salmonella

ABSTRACT

Process hygiene criteria (PHC) for *Campylobacter* and *Salmonella* provide essential guidance on hygiene standards for poultry processing at slaughterhouses. Data collected by Member States (MSs) in Europe revealed inconsistencies between official verification and internal monitoring results, highlighting the need for a comprehensive investigation to uncover the reasons behind these discrepancies. This study investigated PHC in relation to operational factors at nine poultry slaughterhouses, integrating production characteristics and epidemiological surveillance data from a representative sample of supplying farms. The analysis is based on microbiological testing results collected between 2021 and 2023. PHC were monitored at regular intervals, creating a "moving window" of data to assess hygienic performance over time. The findings indicated that samples tested by competent authorities (CAs) showed higher prevalence rates of *Campylobacter* (27.6 %) and *Salmonella* (30.7 %) compared to those tested by food business operators (FBOs) (13.0 % and 8.7 %, respectively). However, statistically significant differences between paired observations ($P < 0.05$) were detected only in four high-throughput slaughterhouses. Six of the nine slaughterhouses face challenges in meeting the hygiene standards established by EC Regulation 2073/2005, with recurring non-compliance over three years, representing 17.0 % to 82.0 % of their total operational time. Serotyping of *Salmonella* isolates identified a high prevalence of *S.* Infantis (61.7 %), also prevalent in farm samples (72.0 %), frequently linked to human cases related to poultry meat in the region, underscoring its public health importance. The study highlights the importance of robust sampling protocols for comparative assessments by FBOs and CAs, alongside the need for continuous improvement in hygiene management. Discrepancies between FBO and CA sampling results, as reported by European Food Safety Authority (EFSA), in this study are largely attributed to the lower sampling frequency employed by CAs, which likely captures only a fraction of the microbial variability. Recurrent breaches of PHC underscore the ineffectiveness of current interventions in controlling microbial loads. These findings emphasize the need for comprehensive control measures throughout the production chain, including surveillance of emerging serovars and antimicrobial resistance monitoring.

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https://doi.org/10.1016/j.psj.2025.105465
Received 14 February 2025; Accepted 19 June 2025
Available online 20 June 2025

Introduction

In 2023, the main bacterial zoonotic diseases transmitted through food in Europe were caused by *Campylobacter* and *Salmonella*, with 148,181 and 77,486 reported cases, respectively (EFSA and ECDC, 2024). Poultry meat, especially broiler chicken, is a significant source of these infections (EFSA, 2010, 2012; Kaakoush et al., 2015; Pires et al., 2014). Both pathogens are often shed through the feces and contaminated feathers of clinically healthy animals (Newell and Fearnley, 2003; Shaji et al., 2023). Applying microbiological criteria on poultry carcasses at slaughterhouses can help assess and provide evidence of effective food safety risk management (Van Schothorst et al., 2009). In 2008, the European Food Safety Authority (EFSA, 2010) conducted surveys revealing a high prevalence of *Campylobacter* (76.0 %) in chicken carcasses across the European Union (EU), along with an average prevalence of *Salmonella* at 15.7 %, although this varied among Member States (MSs).

Modern poultry slaughterhouses differ in processing capacities, with some operating multiple slaughter lines simultaneously. Each facility has a unique design, and the equipment, especially in the evisceration room, can vary greatly (Rasschaert et al., 2020). The processing stages in modern poultry slaughterhouses are highly mechanized, needing minimal manual labor. Thus, maintaining batch dimensional uniformity is essential to ensure machines function correctly and minimize carcass contamination or recontamination (Althaus et al., 2017; Libera et al., 2023; Trevisani et al., 2024). The effect of processing between batches can be different, depending on *Campylobacter* and *Salmonella* flock prevalence, persistent shedding carriers and the effectiveness of hygiene management in slaughterhouses (Pacholewicz et al., 2015). The use of chemicals to decontaminate broiler carcasses is forbidden in the EU, leaving only the options of physical decontamination measures (Rasschaert et al., 2020), logistic slaughtering approach for *Salmonella* due to the presence of an ongoing surveillance system (Cota et al., 2024) and pre-harvest control measures for controlling *Campylobacter* and *Salmonella* in poultry farms (Koutsoumanis et al., 2020; Lu et al., 2020; Sibanda et al., 2018; Taha-Abdelaziz et al., 2023).

At present, process hygiene criteria (PHC) for *Campylobacter* and *Salmonella* on poultry carcasses, as outlined in EC Regulation 2073/2005 and its 2017 amendment, provide guidance on the hygiene standards for poultry processing at slaughterhouses in Europe (EC, 2005, 2017). Monitoring data must be recorded over a 52-week period for *Campylobacter* and a 30-week period for *Salmonella* (EC, 2005). Acceptability criteria, established based on risk analyses (CAC, 1997; Comin et al., 2014; Rosenquist et al., 2003; Tromp et al., 2010) dictate that the hygiene of the slaughtering process is considered satisfactory if no more than 15 out of 50 samples (30 %) exceed 1,000 colony forming units (CFU)/g of neck skin for *Campylobacter* (EC, 2017; Gherman et al., 2023; Vose Consulting LLC, 2011) and no more than 5 out of 50 samples (10 %) test positive for *Salmonella* in 25 g of neck skin (Cegar et al., 2022; EC, 2005).

Monitoring sampling plans implemented by food business operators (FBOs) evaluate process performance over time, helping to identify trends, variations, and issues. This information guides decisions and corrective actions to maintain control within established limits. Regulations require interventions to effectively manage and reduce microbial loads on carcasses, ensuring food safety and regulatory compliance. Official controls ensure that FBOs meet regulatory standards and that their own-check processes align with official requirements and it builds trust among consumers and stakeholders by demonstrating that self-monitoring practices are reliable. Data collected by MSs in subsequent years showed discrepancies between official verification data and internal monitoring results, necessitating a more thorough investigation to understand the underlying reasons for these differences (EFSA and ECDC, 2024).

This study analyzed and compared own-check monitoring data from FBOs with official verification samples collected by CAs, focusing on potential biases in sampling procedures and analytical methods. It examined PHC in relation to slaughterhouse operational factors and epidemiological surveillance data from a representative sample of supplying farms. Additionally, the study assessed temporal trends and evaluated the effectiveness of corrective actions implemented by FBOs following non-compliant findings.

Materials and methods

Slaughterhouses and sampling of carcasses

The study, conducted from 2021 to 2023, involved all nine poultry slaughterhouses located in Emilia-Romagna. This region of Northeast Italy accounted for a significant portion of the Italian poultry meat production, contributing 20.6 % in 2024 (Vetinfo, 2025).

According to the Italian guidelines (Italian Republic, 2016) for implementation of EC Regulation 2073/2005 slaughterhouses are categorized based on their throughput (number of birds per year) in: high, over 15,000,000; middle, between 15,000,000 and 5,000,001; low, between 5,000,000 and 1,000,001. The characteristics of these slaughterhouses were collected through CAs, using a questionnaire specially designed for this purpose. The N data includes the questionnaire used for data collection at the slaughterhouses (section 2.4 and Appendix A). These slaughterhouses include four large and five small facilities. The birds they received originated from various farms, located in Emilia-Romagna, and from the nearby regions of Lombardy, Piemonte, and Veneto.

Carcass sampling plans: own-checks and official controls

According to EC Regulation 2073/2005, neck skin samples must be randomly collected from at least 15 poultry carcasses after chilling, with samples from at least three carcasses combined into a single 26 g sample. Each sampling session consists of five samples, which are properly identified, labeled, and maintained at $3 \pm 2°C$ during transport to the laboratory. FBO must sample at different intervals, according to the throughput of the slaughterhouses: weekly for high, bi-weekly for middle, and monthly for low. The frequency can be reduced if the hygienic performance related to *Campylobacter* is good for 52 weeks and those related to *Salmonella* are good for 30 weeks (EC, 2005; Italian Republic, 2016). Slaughterhouses must monitor their performance using a moving window of 50 samples (i.e. 10 sampling sessions) and a two-class sampling plan, classifying samples as acceptable or unacceptable based on wheter they are below/equal to or above the limit "M". Unacceptable values (M) are defined as the presence of *Salmonella* in 25 g and *Campylobacter* counts exceeding 1,000 CFU/g in aggregated neck skin samples. The allowed tolerance is set at 10 % ($c = 5/50$) for *Salmonella* and 30 % ($c = 15/50$) for *Campylobacter*. If these moving window criteria are breached, slaughterhouses are required to implement corrective actions.

To ensure compliance with the Italian guidelines (Italian Republic, 2020) the CAs must collect a minimum of 49, 15, and 5 samples per year in the slaughterhouses with high, middle, and low -throughput, respectively. This means a minimum of 10, 3 and 1 sampling sessions, contextual to FBOs monitoring. While the guidelines outline the frequency of sampling and the number of samples to be collected from each batch or production cycle, the infrequent sampling conducted by CAs fails to adequately address potential significant fluctuations in hygiene conditions over time or across different product batches. FBOs are required to submit their scheduled sampling events in advance to the CAs, enabling the CAs to collect samples from the same batches on the same day. This coordination ensures that paired samples are temporally aligned.

Microbiological analysis

Microbiological analyses were performed either at internal laboratories within high-throughput slaughterhouses (limited to own-checks) or at external laboratories, including the official regional laboratory, the Istituto Zooprofilattico Sperimentale of Lombardy and Emilia-Romagna (IZSLER). All laboratories are accredited by the national System for the Accreditation of Laboratories in accordance with UNI CEI EN ISO/IEC 17025 standards. For enumeration of thermophilic *Campylobacter* and detection of *Salmonella* spp. from neck skin samples, a 26 g test portion was combined with 234 mL of buffered peptone water at room temperature (EC, 2005). The mixture was homogenized, and 10 mL (~1 g of sample) was used for *Campylobacter* enumeration according to ISO 10272-2:2017 or an equivalent validated method (AFNOR BRD). At IZSLER suspect colonies of thermofilic *Campylobacter* were also further caracterized using MALDI-TOF. The remaining 250 mL (~25 g of sample) were used for *Salmonella* detection following the ISO 6579-1:2017 method or an equivalent validated method. *Salmonella* serotyping at IZSLER laboratory was performed following the full Kauffman-White-Le Minor method (ISO/TR, 6579-3:2014), while laboratories internal of high-throughput slaughterhouses 1 and 2 identified the serogroup (B, C1, C2, D, E and G) through a real time-PCR, to exclude the *Salmonella* serotypes relevant to the plan. Molecular serotyping was conducted using a bead-based suspension array system with Luminex xMAP technology, adhering to protocols by Fitzgerald et al. (2007) for somatic (O) antigens and McQuiston et al. (2011) for flagellar (H) antigens. This method involves PCR amplification of specific gene targets, hybridization with oligonucleotide probes on microspheres, and fluorescence detection via streptavidin-phycoerythrin labeling. This alternative method is validated by the national reference laboratory for *Salmonella* and meet the necessary standards for accuracy and reliability (IZSVE, 2020).

Comprehensive questionnaire for the collection of production data, slaughtering procedures and hygiene improvement strategies

A questionnaire was developed to gather production data, report slaughtering procedures, and investigate preventive and corrective actions to improve slaughter hygiene. The respondents were CAs controlling the slaughterhouses under study. The questionnaire included 30 open-ended questions for detailed responses. The questionnaire consisted of two parts: the first part collected general information about the establishment, while the second part focused on the sampling plan and results. Data was collected via email between September-November 2024 and compiled into an Excel database. The questionnaire is provided in the supplementary data (Appendix A).

Data collection

The data from microbiological analyses conducted on samples collected by the CA were extracted from the laboratory information system (IZSLER). From the national information system (www.vetinfo.it), the following were downloaded: i) the own-check data for PHC at the slaughterhouse (National Information System of Zoonoses, SINZOO) conducted on samples collected by FBO and, ii) the own-check data collected by breeders in broiler farms within three weeks prior to sending to slaughterhouse (National Plan for the Control of Salmonellosis) in the Emilia-Romagna region (Salmonellosis Information System, SISalm). All data was downloaded for the three-year period 2021-2023.

Statistic analysis

Data from samples collected by FBOs and CAs in slaughterhouses were summarized using absolute and relative frequencies, along with the 95 % confidence interval (CI) for the positivity rate in the analyzed samples. Fisher's exact test was used to compare the positivity rates. Data concerning FBO monitoring samples and contextual CA verification samples were analyzed with the Wilcoxon Mann-Whitney test for paired data using R software (version 4.3.0). Test results, including effect size as an evaluation of the results' importance, were computed with the rstatix package (Kassambara, 2023) and are reported accordingly.

Results

Slaughterhouses characteristics

Table 1 provides information on the technical characteristics of the examined slaughterhouses, including uncaging and stunning methods, scalding water temperature, evisceration method, carcass chilling time, and temperature. Four slaughterhouses have a high-throughput of over 15,000,000 birds per year, and three of them have a throughput (average daily slaughter, ADS) exceeding 100,000, with a line speed between 6,900 and 12,000 birds per hour (bph). One slaughterhouse has an ADS of 45,000 and a line speed of 4,600 to 6,600 bph. All these facilities are equipped with a fully automated evisceration system. In contrast, the other five slaughterhouses have a throughput of less than 5,000,000 heads per year, with ADS ranging from 2,400 to 12,600 and line speeds between 600 and 1,720 bph. These facilities utilise a mechanical eversion system for the intestinal package, which is removed manually. In slaughterhouses 5, 6, 7, 8, and 9, where other poultry species (e.g., hens or guinea fowl) are also processed, these are slaughtered only after all broiler batches have been completed. Broiler processing always occurs at the start of the production day. Thorough cleaning and disinfection are carried out between broiler and other species processing, in accordance with standard hygiene protocols to prevent cross-contamination.

Prevalence of Campylobacter and Salmonella

From 2021 to 2023, a total of 3,766 neck skin samples were tested for *Campylobacter* by the FBOs, and 797 samples were tested by the CAs (Table 2). Notably, Fisher's exact test indicated that CAs more frequently identified a higher count of positive samples: overall, 489 (13.0 %) of the samples collected by the FBOs tested positive for *Campylobacter*, while the CAs detected 220 (27.6 %) positive samples. This higher frequency at the CA level was observed for slaughterhouses 1, 3, 4, 6, and 7.

In the same period, 3,930 samples were tested for *Salmonella* by the FBOs, and 797 samples by the CAs (Table 3). Similar to the *Campylobacter* results, a Fisher's exact test also showed a higher positive rate in the CA data (251 positive samples, 31.5 %) compared to the FBOs' data (328 positive samples, 8.3 %). This higher frequency at the CA level was observed for slaughterhouses 1, 2, 3, 4, 7, and 8.

Focusing on batches, 182 out of 754 batches (24.1 %) sampled by the FBOs and 71 out of 177 batches (40.1 %) sampled by the CAs tested positive for *Campylobacter* (Table S1). Moreover, 193 out of 786 batches (24.6 %) sampled by the FBOs and 84 out of 177 batches (47.5 %) sampled by the CAs tested positive for the presence of *Salmonella* (Table S2). This variability is clearly illustrated in Fig. S1 which show how many out of five samples per sampling event tested positive for FBOs and CAs. The graphs related to *Salmonella* and *Campylobacter* indicate that the samples collected by CAs did not adequately capture the substantial variability present across batches.

Paired comparison: CAs vs FBOs sampling

We analyzed contextual sampling data to determine if analytical results varied between samples collected on the same days and from the same product batches. The number of paired batches analyzed ranged from 30 (slaughterhouse 1) to 6 (slaughterhouse 7) (Table 4). We used the Wilcoxon-Mann-Whitney test for paired data to analyze differences in positive units of *Campylobacter* and *Salmonella* in neck skin samples

Table 1
Technical information about the slaughterhouses.

SH[a]/ T[b]	ADS[c] (birds/ day)	MLS[d] (bird/hour)	Categories or species	Uncaging method	Scalding temperature (°C)	Evisceration method	Chilling time (minutes or hours) and temperature
1/H	~125,000	~12,000	Broilers	Automatic	47-54	Automatic	3 h at 0°C
2/H	~105,000	6,000-9,000	Broilers	Automatic	52-53	Automatic	3 h / 0-1°C
3/H	~100,000	6,900-7,800	Broilers	Automatic	51-54	Automatic	2.5-3 h / 0-1°C
4/H	~45,000	4,800-6,600	Broilers, hens, guinea fowl	Manual	49.9-53.3	Automatic	1.5-2 h / 6 and −1°C
5/L	~12,600	~1,700	Broilers, hens, guinea fowl	Manual	51-52	Mechanical eversion of the intestinal package completed manually	15 min / 0-1°C
6/L	~8,000	1,500 broilers, hens, guinea fowl; 800 capons	Broilers, hens, guinea fowl, capons	Manual	51-52	Mechanical eversion of the intestinal package completed manually	2-3 h / 0-5°C
7/L	~5,400	1,000-1,400	Broilers, hens, guinea fowl	Manual	52-53	Mechanical eversion of the intestinal package completed manually	1.5 h / 2°C
8/L	~4,200	1,000 hens; 600-700 guinea fowl, capons, broilers; 150 ducks	Broilers, hens, guinea fowl, capons, ducks	Manual	50-52	Mechanical eversion of the intestinal package completed manually	3 h / 0-1°C
9/L	~2,400	600 broilers; 200 turkeys	Broilers, turkeys	Manual	54	Mechanical eversion of the intestinal package completed manually	6 h / 1°C

[a] Slaughterhouse.
[b] Throughput: high (H), processing over 15,000,000 broilers per year (b/yr); low (L), processing 5,000,000 - 1,000,001 b/yr
[c] Average daily slaughter: value obtained from the extraction of data regarding 90 days of slaughter (https://www.vetinfo.it/j6_bdn/avimacellazione/avimac/).
[d] Maximum line speed.

Table 2
Campylobacter data collected by food business operators (FBOs) and the competent authorities (CAs) during the period 2021-2023.

SH[a]/ T[b]	FBO						CA						$P < 0.05$[c]
	No samples tested				No positive samples	% (95 % CI)	No samples tested				No positive samples	% (95 % CI)	
	2021	2022	2023	Total			2021	2022	2023	Total			
1/H	260	260	260	780	183	23.5 (20.5-26.6)	50	50	50	150	58	38.7 (30.8-47.0)	CA>FBO
2/H	280	305	280	865	91	10.5 (8.6-12.8)	50	50	50	150	26	17.3 (11.6-24.4)	-
3/H	165[d]	130[d]	130[d]	425	92	21.6 (17.8-25.9)	50	50	50	150	43	28.7 (21.6-36.6)	CA>FBO
4/H	265	270	265	800	59	7.4 (5.7-9.4)	25	15	15	55[e]	29	52.7 (38.8-66.3)	CA>FBO
5/L	51	60	65	176	10	5.7 (2.8-10.2)	25	30	20	75	9	12.0 (5.6-21.6)	-
6/L	50	65	50	165	46	27.9 (21.2-35.4)	25	45	25	95	42	44.2 (34.0-54.8)	CA>FBO
7/L	125	125	125	375	0	0.0 (0.0-1.0)	13	25	25	63	11	17.5 (9.1-29.1)	CA>FBO
8/L	40[d]	25[d]	25[d]	90 (85[f])	0	0.0 (0.0-4.0)	8	5	5	18 (17[f])	1	5.6 (0.1-27.3)	-
9/L	30[d]	30[d]	30[d]	90	8	8.9 (3.9-16.8)	11	15	15	41	1	2.4 (0.1-12.9)	-
Total	1,266	1,270	1,230	3,766	489	13.0 (11.9-14.1)	257	285	255	797	220	27.6 (24.5-30.8)	CA>FBO

[a] Slaughterhouse.
[b] Throughput: high (H), low (L).
[c] P: *p*-value.
[d] Reduced frequency.
[e] Sampling plan not meeting law requirements.
[f] Samples carried out on capons.

from paired controls between FBOs and CAs illustrated in Fig. 1.

Significant discrepancies ($P < 0.05$) were found between FBO and CA results for *Campylobacter* in slaughterhouses 1 and 4, with moderate and large effect sizes, respectively (Table 4). For *Salmonella*, significant discrepancies ($P < 0.05$) were observed in slaughterhouses 1 (large effect size), 2, and 3 (moderate effect size). Notably, all slaughterhouses with significant differences exhibited high throughput (ADS >100,000; slaughter line speeds 6,000-12,000 bph). Slaughterhouse 8 was excluded from this analysis due to inconsistent sample collection by the CA, precluding direct comparisons.

Fig. S2 shows the counts of thermophilic *Campylobacter* in neck skin samples taken at slaughterhouse 2. Quantitative data were not made available by the laboratory of the other slaughterhouse, which only reported numbers in categories between zero and 1,000 CFU/g or above. It is useful to note that many of the data points were close to the limit of acceptability. Therefore, small differences in the counts of *Campylobacter* spp. and the calculated proportions of thermophilic isolates, estimated based on identification tests, could have affected the number of violative sample units reported by FBOs and CAs.

Table 3
Salmonella spp. data collected by food business operators (FBOs) and the competent authorities (CAs) during the period 2021-2023.

SH[a]/T[b]	FBO						CA						$P < 0.05$[c]
	No samples tested				No samples positive	% (95 % CI)	No samples tested				No positive samples	% (95 % CI)	
	2021	2022	2023	Total			2021	2022	2023	Total			
1/H	260	260	260	780	103	13.2 (10.9-15.8)	50	50	50	150	69	46.0 (37.8-54.3)	CA>FBO
2/H	280	305	280	865	90	10.4 (8.4-12.6)	50	50	50	150	56	37.3 (29.6-45.6)	CA>FBO
3/H	260	260	260	780	74	9.5 (7.5-11.8)	50	50	50	150	58	38.7 (30.8-47.0)	CA>FBO
4/H	265	270	265	800	10	1.3 (0.6-2.3)	25	15	15	55[d]	13	23.6 (13.2-37.0)	CA>FBO
5/L	55	65	65	185	8	4.3 (1.9-8.3)	25	30	20	75	8	10.7 (4.7-19.9)	-
6/L	50	65	50	165	26	15.8 (10.6-22.2)	25	45	25	95	13	13.7 (7.5-22.3)	-
7/L	55	60	60	175	17	9.7 (5.8-15.1)	13	25	25	63	31	49.2 (36.4-62.1)	CA>FBO
8/L	40[e]	25[e]	25[e]	90 (85[f])	0	0.0 (0.0-4.0)	7	6	5	18 (17[f])	3	16.7 (3.6-41.4)	CA>FBO
9/L	30[e]	30[e]	30[e]	90	0	0.0 (0.0-4.0)	11	15	15	41	0	0.0 (0.0-8.6)	-
Total	1,295	1,340	1,295	3,930	328	8.3 (7.5-9.3)	256	286	255	797	251	31.5 (28.3-34.8)	CA>FBO

[a] Slaughterhouse.
[b] Throughput: high (H), low (L).
[c] P: *p*-value.
[d] Sampling plan not meeting law requirements.
[e] Reduced frequency.
[f] Samples carried out on capons.

Table 4
Comparison of *Campylobacter* and *Salmonella* results obtained by food business operators (FBOs) and competent authorities (CAs) in samples collected from the same batches.

SH[a]/T[b]	Contextual sampling for *Campylobacter* No paired batches (No paired samples)	No (%) samples positive FBO	No (%) samples positive CA	Wilcoxon Mann-Whitney test for paired data[c]	$P < 0.05$	Contextual sampling for *Salmonella* No paired batches (No paired samples)	No (%) samples positive FBO	No (%) samples positive CA	Wilcoxon Mann-Whitney test for paired data[c]	$P < 0.05$
1/H	30 (150)	26 (17.3)	58 (38.7)	$V = 153$; $P = 0.00986$; moderate effect size	CA>FBO	30 (150)	42 (28)	69 (46.0)	$V = 186$; $P = 0.00647$; large effect size	CA>FBO
2/H	22 (110)	8 (7.3)	19 (17.3)	$V = 33.5$; $P = 0.103$; small effect size	-	22 (110)	26 (23.4)	51 (46.4)	$V = 80.5$; $P = 0.00732$; moderate effect size	CA>FBO
3/H	26 (130)	36 (27.7)	40 (30.8)	$V = 12.5$; $P = 0.102$; small effect size	-	30 (150)	46 (30.7)	58 (38.7)	$V = 51$; $P = 0.00742$; moderate effect size	CA>FBO
4/H	9 (45)	6 (13.3)	24 (53.3)	$V = 21$; $P = 0.0178$; large effect size	CA>FBO	9 (45)	0 (0.0)	11 (24.4)	$V = 6$; $P = 0.0868$; large effect size	-
5/L	15 (75)	7 (93.3)	9 (12.0)	$V = 11$; $P = 0.5$; small effect size	-	15 (75)	6 (8.0)	8 (10.7)	$V = 3$; $P = 0.173$; moderate effect size	-
6/L	15 (75)	23 (30.7)	33 (44.0)	$V = 47.5$; $P = 0.102$; moderate effect size	-	16 (80)	8 (10.0)	8 (10.0)	$V = 3$; $P = 0.607$; small effect size	-
7/L	6 (30)	0 (0.0)	4 (13.3)	$V = 3$; $P = 0.186$; large effect size	-	11 (55)	15 (27.3)	25 (45.5)	$V = 22$; $P = 0.1$; small effect size	-
8[d]/L	-	-	-	-	-	-	-	-	-	-
9/L	8 (40)	6 (15.0)	1 (2.5)	$V = 0$; $P = 0.972$; effect size	-	8 (40)	0 (0.0)	0 (0.0)	$V = 0$; $P = 1$; effect size	-

[a] Slaughterhouse.
[b] Throughput: high (H); low (L).
[c] V: test statistic; P: *p*-value; effect size is an evalution of the magnitude of the result.
[d] No contextual sampling was carried out between FBO and CA.

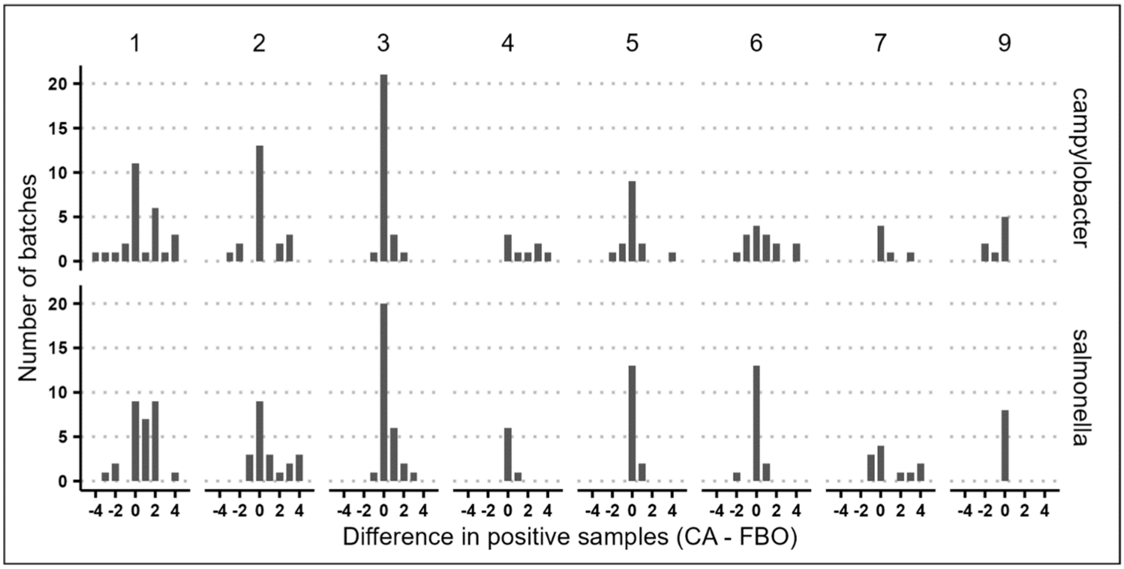

Fig. 1. Distribution of the differences in positive units found in paired controls between food business operators (FBOs) and competent authorities (CAs) in neck skin samples.
The numbers 1-9 are identifiers of slaughterhouses. The unit of analysis is the controlled batch. The analyzed variable is the difference between the positive units (CA - FBO).

Breach of process hygiene criteria (PHC)

Fig. S1, graphs 1–9C, illustrate breaches in the moving window PHC, based on data collected by FBOs. The results highlight substantial variability in hygienic performance across slaughterhouses over the three-year period, with some facilities (i.e. slaughterhouses 1, 2, 3 and 6) exhibiting particularly large fluctuations. Notably, slaughterhouse 3, which implements a reduced sampling frequency for *Campylobacter*, failed to comply with PHC for a duration of 11 weeks between week 15 and week 78. The final column of Table 5 shows the number of non-compliant weeks per slaughterhouse over three years. FBOs must evaluate PHC based on samples collected weekly or at a reduced frequency, depending on their throughput. However, incorporating results from contextual sampling conducted by both FBOs and CAs could offer valuable insights into how varying outcomes may affect the duration of PHC breaches (Fig. S1).

Salmonella status at supplying farms

The dataset comprises 239 broiler farms in Emilia-Romagna. Between 2021 and 2023, only 2030 of 14,072 consignments (23.7 %) tested negative for *Salmonella* spp. within three weeks prior to slaughter, ideally one week for optimal relevance. Remaining consignments originated from farms with varying levels of *Salmonella* positivity: 45.1 % of farms had 0.7–20.0 % positive consignments, 17.8 % ranged from 20.0 to 40.0 %, and 13.4 % exhibited high prevalence (40.0–90.0 %). These consignments represent only part of the throughput at the region's nine slaughterhouses, as additional supplies came from neighboring regions. Due to restricted access to interregional data via the SISalm system (Italian Republic, 2022), the analysis relied on available data as a representative sample of the production process. We focused on six slaughterhouses that sourced at least 7.5 % of their slaughtered batches from farms in Emilia-Romagna (Fig. 2). This data concern environmental samples taken prior to the delivery of chickens to the slaughterhouse. Under EC Regulation 2073/2005, FBOs are required to test broiler flocks for *Salmonella* before slaughter, typically within three weeks before slaughter, but ideally one week prior to ensure relevance. Apart from slaughterhouse 9, a low-throughput facility that received birds only from two farms in Emilia-Romagna, the data for slaughterhouses 1, 2, 3, 4 and 5 represent only 19.7 %, 21.7 %, 18.3 %, 14.2 % and 7.5 % of their respective supplying farms. Although these samples can be useful for gathering data on the *Salmonella* status of farms supplying birds to these slaughterhouses, which impacts contamination events occurring during slaughtering. Data for slaughterhouse 1, 2 and

Table 5
Data on slaughterhouses including total suppliers, regional broiler suppliers, *Salmonella* prevalence and weeks outside process hygiene criteria (PHC).

SH[a]/T[b]	No total suppliers	No regional broiler suppliers (%)	No positive regional suppliers for *Salmonella*	% (95 % CI)	Weeks operating without meeting PHC (%)[c]
1/H	375	74 (19.7)	68	91.9 (83.2-97.0)	76 (51.7 %)
2/H	391	85 (21.7)	69	81.2 (71.2-88.9)	62 (37.8 %)
3/H	263	48 (18.3)	29	60.4 (45.3-74.2)	59 (40.1 %)
4/H	450	64 (14.2)	5	7.8 (2.6-17.3)	0 (0 %)
5/L	441	33 (7.5)	24	72.7 (54.5-86.7)	18 (17.0 %)
6/L	140	6 (4.3)	0	0.0 (0.0-4.6)	88 (82.0 %)
7/L	227	2 (0.9)	0	0.0 (0.0-8.4)	56 (40.9 %)
8/L	84	6 (7.1)	0	0.0 (0.0-4.6)	0 (0 %)
9/L	2	2 (100.0)	0	0.0 (0.0-0.8)	0 (0 %)

[a] Slaughterhouse.
[b] Throughput: high (H); low (L).
[c] Weeks during which the slaughterhouses failed to meet the hygiene standards as specified by EC Regulation 2073/2005.

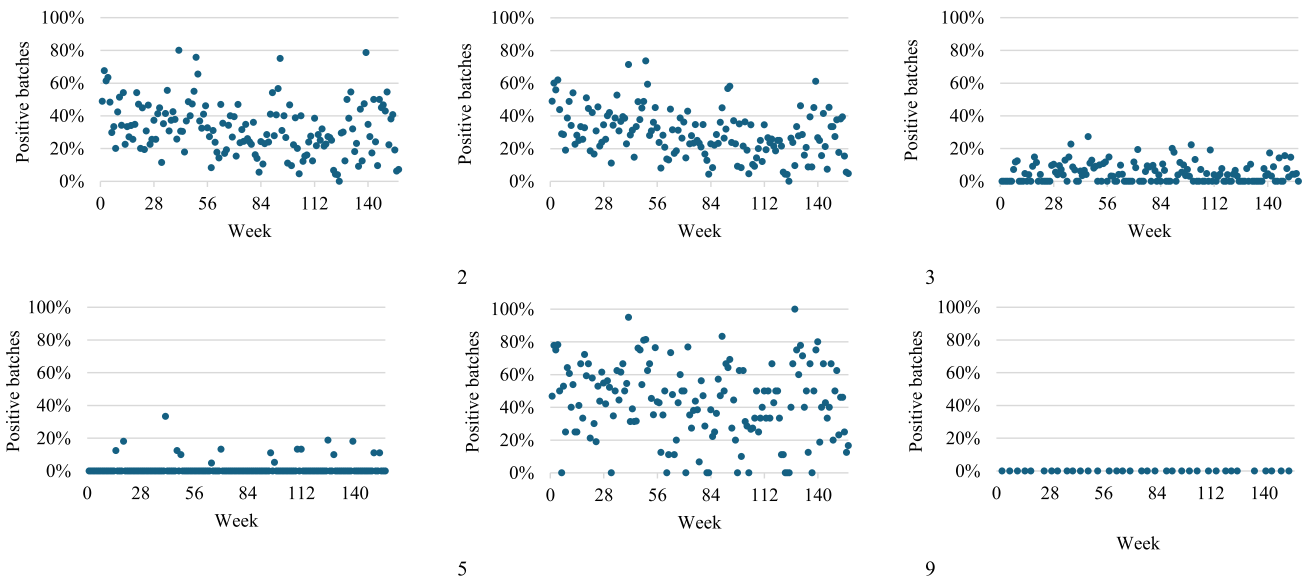

Fig. 2. Number of *Salmonella* positive batches supplying broilers to the slaughterhouses 1, 2, 3, 4, 5 and 9 in the period 2021-2023.

3, the highest throughput facilities, with an ADS between approximately 100,000 and 125,000 - show that 91.9 %, 81.2 %, and 60.4 % of the batches received from farms in Emilia-Romagna tested positive for *Salmonella spp*. A high number of batches supplied to the slaughterhouse 5, which has an ADS of ~12,600, resulted positive (72.7 %) while data for slaughterhouse 4, which has an ADS of 45,000 broilers, shows only 7.8 % of batches tested positive (Table 5).

Serotype distribution of Salmonella isolate

Table 6 report *Salmonella* serotypes detected in broiler farms (all registered producers in the Emilia-Romagna region) by the farmers and within the framework of hygiene performance monitoring plans by FBOs and the CAs during the period 2021 to 2023. Among the 2,403 *Salmonella* isolates from birds' litter, *S.* Infantis was the most prevalent serotype (72.0 %), followed by *S.* Livingstone (14.3 %). Similarly, of the 257 serotyped isolates from neck skin samples collected by CAs, *S.* Infantis remained the most common (69.3 %), with *S.* Bredeney (12.1 %) and *S.* Mikawasima (9.7 %) identified as the next most frequent serotypes.

Serotyping data collected from FBOs appear to be underestimated, as high-throughput slaughterhouses 1 and 2 performed analyses mainly to exclude relevant *Salmonella* serogroups. Overall, 193 untyped *Salmonella* were classified into serogroups B, C1, C2 and E (Table 6). *Salmonella* strains isolated from broiler samples tested by CAs show that 39 out of 69 (56.5 %) isolates detected in slaughterhouse 1 were of the serotype *S.* Infantis. This serotype was also prevalent in samples from supplying farms, with 1,092 out of 1,565 isolates (69.8 %).

With regard to serotypes under specific surveillance in broiler farms, *S.* Enteritidis was identified in one CA sample collected from slaughterhouse 5, along with *S.* Infantis (Fig. S1, graph 5A). More precisely, two out of five sample units tested positive for *S.* Enteritidis, while one unit tested positive for *S.* Infantis. This indicates that different serovars can coexist within a single batch of birds at slaughter, and their detection is influenced by their prevalence as well as the number of colonies selected from plates for identification and analysis. The serovar *S.* Typhimuruim was detected in one sample taken by the FBOs at the slaughterhouse 6 (Fig. S1, graph 6A). Because these are target serovars for reduction programs in broiler farms and considered of relevance for public health (EC, 2010), their detection was followed by a traceback investigation at the supplying farms and implementation of corrective actions to control and eliminate the contamination.

Table 6
Salmonella serotypes detected in broiler farms by the farmers and within the framework of hygiene performance monitoring plans by food business operators (FBOs) and the competent authorities (CAs) during the period 2021 to 2023.

Salmonella serotypes isolated from the farms[a]	No	*Salmonella* serotypes and serogroups isolated from the slaughterhouse			
		FBO	No	CA	No
S. Infantis	1,730	*S.* Infantis	28	*S.* Infantis	178
S. Livingstone	344	*S.* Isangi	6	*S.* Bredeney	31
S. Senftenberg	54	*S.* Bredeney	4	*S.* Mikawasima	25
S. Agona	24	*S.* Mikawasima	4	*S.* Anatum	2
S. Anatum	22	*S.* Brandenburg	3	*S.* Agona	10
S. Bredeney	18	*S.* Kentucky	3	*S.* Isangi	3
S. Mbandaka	18	*S.* Agona	1	*S.* Enteritidis	2
S. Cerro	12	*S.* Virkow	1	*S.* Give	1
S. Thompson	9	*S.* Typhimurium	1	*S.* Kentucky	1
S. Blockley	7	Serogroup C1	161	*S.* Litchfield	1
S. Liverpool	6	Serogroup B	28	*S.* Mbandaka	1
S. Lagos	5	Serogroup E	4	*S.* Senftenberg	1
Other serotypes[b]	154			*S.* Thompson	1

[a] All registered producers in the Emilia-Romagna region.
[b] Less than 5 isolates.

Corrective actions adopted by FBOs

Based on the questionnaire responses, FBOs implemented corrective actions to address non-compliances exclusively associated with the detection of *S.* Enteritidis and *S.* Typhimurium. These pathogens were identified on three occasions at slaughterhouses 5 and 6 (Fig. S1), although they were not detected in the corresponding litter samples collected from the source farms.

Extraordinary measures, such as intensified sanitation protocols or the seizure of carcasses from the affected batches, were enacted immediately following the results of confirmatory tests that were only available 72 hours post-sampling. It is important to note that although enhanced sanitation procedures were subsequently carried out at the two implicated slaughterhouses, these interventions did not influence the subsequent PHC outcomes.

Among the surveyed facilities, only the representative from slaughterhouse 4 reported the implementation of a scheduled slaughtering policy for batches containing non-relevant *Salmonella* serovars. These batches are processed either at the end of the slaughtering day or following a cleaning procedure prior to the resumption of operations. Additionally, this slaughterhouse enforces a stricter biosecurity measure by rejecting consignments originating from farms that have tested positive for *S.* Enteritidis or *S.* Typhimurium. Other slaughterhouses indicated that, in such cases, they opt for segregated slaughtering of the *S.* Enteritidis and *S.* Typhimurium positive batches, followed by extraordinary sanitation procedures. These procedures predominantly involve the use of chlorinated compounds, but also include amphoteric surfactants (e.g., TEGO^{TM}) and glutaraldehyde.

Regarding carcass sampling practices, most respondents stated that carcasses visibly contaminated with fecal material are typically excluded from sampling.

Discussion

The study underscores the importance of robust sampling protocols for comparative assessments by FBOs and CAs. While sampling bias may arise, such as from including highly contaminated birds or excluding problematic consignments like final loads from depopulated barns, no evidence of such issues was observed in this study. Discrepancies between FBO and CA sampling results, as reported by EFSA, are in this study largely attributed to the lower sampling frequency employed by CAs, which likely captures only a fraction of the microbial variability. Recurrent breaches of PHC underscore the ineffectiveness of current interventions in controlling microbial loads. Furthermore, the reduced frequency of the sampling plans negatively affected the monitoring windows, leading to delays in recognizing issues and assessing the potential effects of the subsequent management options implemented. Similar data were reported in a study conducted in United Kingdom (Hutchison et al., 2016). These findings emphasize the necessity for comprehensive control measures across the production chain, including surveillance of emerging serovars and monitoring of antimicrobial resistance. The asymmetrical distribution of some differences between the results of FBOs and CAs illustrated in Fig. 1, and the significant differences stressed out by the Wilcoxon-Mann-Whitney test, indicates that certain CAs may overestimate the number of sample units exceeding the M limits, or that FBOs may systematically underestimate these figures. Therefore, implementing strict sampling protocols should be integral to the operational check manual in slaughterhouses, and similar protocols must be developed by CAs. It is essential for both sampling plans to accurately represent the broader production within each slaughterhouse. With this aim FBOs must submit their scheduled sampling events in advance to the CAs, enabling the CAs to collect samples from the same batches on the same day. This coordination ensures that paired samples are representative and temporally aligned. According to EFSA (2017), when a PHC is not met, the FBO, sometimes in collaboration with the CA in cases involving national prevalence targets, is expected to implement corrective actions. Notably, the FBOs did not implement corrective actions in response to breaches of the PHC, with the sole exception of slaughterhouse 4, and extraordinary sanitation procedures were applied only in the limited instances where *S.* Enteritidis and *S.* Typhimurium were detected on broiler carcasses. On the other hand, these interventions had no discernible impact on PHC trends.

The study underscores the need to enhance hygiene management not only when targeted *Salmonella* serotypes are detected in birds accepted by slaughterhouses, but also when *Salmonella* spp. are identified through PHC sampling. When FBOs prioritize maintaining throughput levels, they may be hesitant to restrict the acceptance of birds from suppliers. Consequently, this can impact PHC data, but this study identified slaughterhouses with persistent non-compliance, including also low-throughput facilities such as slaughterhouses 6 and 7, operating at line speeds of 1,000-1,500 broilers per hour. Repeated breaches of PHC suggest that implemented measures to control *Campylobacter* and *Salmonella* contamination were ineffective. In this context, discrepancies between own-check and official verification results may appear secondary. Regarding *Salmonella*, the identified non-compliances are likely attributable to the high prevalence of positive test results among bird suppliers, as illustrated by the data in Fig. 2. Data from six slaughterhouses clearly show that when *Salmonella*-positive batches are rare or absent, as in slaughterhouses 4 and 9, or scheduled slaughtering of batches with non-relevant serovars is applied, PHC values remain within acceptable limits. Under these conditions, no significant discrepancies are observed between FBO monitoring data and CA verification results. This is also evidenced in the monitoring data reported by EFSA regarding PHC data in the MSs that has a very low prevalence of *Salmonella* infection at farm level (EFSA and ECDC, 2022, 2023, 2024).

This study was limited by the use of data from the National Information System of Zoonoses (SINZOO), which only provides own-check and official control data from our region, representing a partial view of regional slaughterhouse activity. Greater transparency and data sharing could enhance oversight and support improved hygiene practices.

It was observed that the circulation of *Salmonella* serovars that are not considered relevant for human health and subject to control in poultry farms was notably high among the groups of broilers (Table 6).

It is important to recognize that the flock-level prevalence of *Salmonella* in broiler farms may be related to the flock-level prevalences observed in breeding hens and other potential sources of infection. A recent study reported a prevalence of *Salmonella* in Italian breeding hens of 2.6 % in 2017 and 1.8 % in 2018. Among the detected isolates, *S.* Veneziana comprised 21.0 %, followed by *S.* Mbandaka at 17.6 %, *S.* Kedougou at 6.2 %, *S.* Infantis at 5.5 %, *S.* Typhimurium at 4.0 %, and *S.* Napoli at 3.9 % (Leati et al., 2021).

A longitudinal study in Brazil found that spatial, temporal, structural, and management factors significantly influenced Salmonella presence in litter (Machado Junior et al., 2020). Consistent with other studies, intestinal colonization was the main risk factor for carcass contamination, especially when gut integrity was compromised during processing. In Spain, 69.4 % of broiler batches and 46.3 % of carcasses tested positive for *Salmonella* in 2015 (Marin et al., 2022). High genetic similarity among isolates from carcasses and the environment suggests persistent strain recirculation, likely due to ineffective sanitation and biofilm-forming *Salmonella* on transport crates.

S. Infantis, frequently detected in broiler farms and carcasses (Table 6), is also notable in human cases reported regionally (IZSLER, 2024) and by ECDC (EFSA and ECDC, 2024). Unlike *S.* Enteritidis and *S.* Typhimurium, often linked to egg consumption, *S.* Infantis is primarily associated with chicken meat. In Emilia-Romagna, it was the most common serovar in minced poultry meat (Rosamilia et al., 2023) and was the leading serovar in EU broiler samples from 2018 to 2020 (EFSA and ECDC, 2021a, 2021b).

Spread of *S.* Infantis through Belgium, Poland, and Italy, occurred between ~1992 and 2000 (Guzinski et al., 2024). A recent study assessed the prevalence and antimicrobial resistance of *S.* Infantis in the United States from 2013 to 2020, revealing a concerning prevalence of 16.7 % in chickens, along with 82.5 % of strains exhibiting multi-drug resistance (Sohail and Varga, 2025). The significance of *S.* Infantis, detected in isolates from Italian poultry farms and meat samples, has been highlighted in recent research (Di Marcantonio et al., 2022; Di Taranto et al., 2025; Proietti et al., 2020), particularly regarding resistance to critically important antimicrobials (hpCIAs). The emergence of Multi-Resistance-Strains (MRS), particularly those harboring the pESI-like megaplasmid, directly contributes to their enhanced ability to persist and spread within the broiler sector. Hatcheries, feed mills, processing factories, mobile equipment and personnel are clear potential routes for biosecurity breaches. The dissemination and importation of *S.* Infantis may also occur via poultry by-products (Cawthraw et al., 2025). These findings should guide stakeholders in selecting serovars for inclusion in national *Salmonella* surveillance programs for broilers.

Salmonella-positive rates in broiler carcasses reported by CAs were 30.7 %, slightly below Italy's national average (31.8 %) reported for the year 2023, but well above the EU average (17.8 %) (EFSA and ECDC, 2024). FBO data showed lower rates: 8.7 % in Emilia-Romagna, 9.7 % in Italy, and 2.6 % in the EU. Despite efforts to control *S.* Enteritidis and *S.* Typhimurium, *Salmonella* prevalence remains high, suggesting biosafety gaps. Annual EU reports show Italy's rates consistently exceed the EU average, with 95 % CIs of 5.8–7.3 (2021), 8.1–9.6 (2022), and 8.9–10.5 (2023).

FBOs reported 24.1 % *Campylobacter*-positive broiler batches, with wide variation among slaughterhouses, from 0.0 % (slaughterhouses 7 and 8) to over 45.0 % (slaughterhouses 1, 3, and 6) (Table S1). In some cases, all five samples exceeded 1,000 CFU/g (Fig. S1). No farm-level comparison was possible due to the absence of systematic *Campylobacter* monitoring, though studies suggest high flock prevalence in Italy and the EU (Nastasijevic et al., 2020). Key risk factors include high animal density, depopulation practices, and uneven bird weights during automated evisceration (EFSA, 2008; Trevisani et al., 2024). CA compliance checks reported 27.6 % positivity in 2023, above Italy's national average (21.7 %) and the EU average (16.6 %). FBO data showed lower rates: 13.0 % in Emilia-Romagna, 14.1 % in Italy, and 9.0 % in the EU (EFSA and ECDC, 2024). From 2021–2023, the proportion of samples exceeding PHC limits remained stable, with Italy consistently above the EU average.

The EFSA biohazard panel estimated that a reduction higher than 50.0 % the public health risk associated with *Campylobacter* could be achieved if all poultry batches would comply with microbiological criteria with a critical limit of 1,000 CFU/g of neck, while in Italy 21.7 % and 14.1 % of all bactches tested by CAs and FBOs, respectively, do not comply with this criterion (EFSA BIOHAZ Panel, 2011). Broiler contamination is influenced by multiple factors, including transport and slaughterhouse operations (Hastings et al., 2011; Rasschaert et al., 2020; Rosenquist et al., 2003). A United Kingdom study estimated that on-farm factors are 3.5 times more influential than processing factors in determining *Campylobacter* levels on post-chill broiler carcasses (Hutchison et al., 2016). Despite this, only a few northern European countries have implemented national *Campylobacter* surveillance programs (Olsen et al., 2024).

Based on the official text of EC Regulation 2073/2005, particularly Annex I, Chapter 2, point 2.1.5, when PHC are not met, FBOs are required to take corrective actions (EC, 2005). These may include reviewing hygiene practices, process controls, and the origin and biosecurity status of the animals supplied (EC, 2019). Recent studies have discussed the risk categorization of slaughterhouses based on current PHC (Cegar et al., 2022). However, the risk categorization of slaughterhouses has not yet been standardized (Hauge et al., 2023). Managing the risks associated with FBOs that consistently fail to comply with PHC, as well as broader food legislation, poses a significant challenge for food safety. Inspectors often find the current control methods to be inadequate in addressing these issues (Kiviniemi et al., 2025).

Conclusions

Regulations aim to protect consumers from foodborne illnesses and play a crucial role in shaping poultry processing practices, driving improvements in safety, quality, and transparency throughout the industry. Adhering to food safety regulations can lead to increased costs for processing facilities, as they may need to invest in training, equipment, and infrastructure to meet compliance standards. This can affect overall operational practices and profitability.

Ethical statement

The authors have no ethical statement to declare.

Data availability

Due to the sensitive or confidential nature of the information collected survey respondents were assured some data would remain confidential and would not be shared. All other data will be made available upon request.

CRediT authorship contribution statement

Alfonso Rosamilia: Conceptualization, Data curation, Writing – original draft. **Giorgio Galletti:** Formal analysis, Writing – review & editing. **Gabriele Casadei:** Formal analysis, Writing – review & editing. **Giovanni Dell'Orfano:** Investigation, Data curation. **Margherita Ferrari:** Investigation, Data curation. **Elisa Di Carlantonio:** Investigation, Data curation. **Francesca Vergani:** Investigation, Data curation. **Nicola Riceputi:** Investigation, Data curation. **Francesco Zanchini:** Investigation, Data curation. **Lia Bardasi:** Investigation, Data curation. **Laura Fiorentini:** Investigation, Data curation. **Chiara Chiapponi:** Investigation, Data curation. **Michele Dottori:** Investigation, Data curation. **Anna Padovani:** Project administration, Supervision. **Marcello Trevisani:** Conceptualization, Data curation, Validation, Writing – original draft.

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.

Acknowledgments

The authors sincerely thank the official slaughterhouse veterinarians for their assistance in conducting this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105465.

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