



eISSN 2239-7132

Italian Journal of Food Safety

<https://www.pagepressjournals.org/index.php/ijfs/index>

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Ital J Food Saf 2026 [Online ahead of print]

Please cite this article as:

Conter M, Rega M, Bacci C, Bonardi S. **Process hygiene criteria for *Salmonella* in pig carcasses: comparing food business operator self-monitoring and official sampling in an Italian high-throughput slaughterhouse.** *Ital J Food Saf* doi:10.4081/ijfs.2026.14646

Submitted: 12-11-2025

Accepted: 23-01-2026

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Process hygiene criteria for *Salmonella* in pig carcasses: comparing food business operator self-monitoring and official sampling in an Italian high-throughput slaughterhouse

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Key words: official control, self-monitoring, process hygiene criteria, swine, *Salmonella* prevalence.

Contributions: Mauro Conter, Silvia Bonardi: conceptualization. Mauro Conter: formal analysis. Martina Rega: data curation. Mauro Conter: writing - original draft preparation. Silvia Bonardi, Martina Rega, Cristina Bacci: writing - review and editing. Silvia Bonardi: supervision. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: the authors declare that they have no competing interests

Ethics approval and consent to participate: not applicable.

Availability of data and materials: the datasets used and/or analysed during the current study are available upon reasonable request from the corresponding author.

Abstract

This study compares *Salmonella* monitoring data obtained from food business operator (FBO) self-monitoring and competent authority (CA) official sampling on pig carcasses in a high-throughput Italian slaughterhouse over ten years (2012-2021 for FBO; 2014-2021 for CA). A total of 1560 FBO and 377 CA samples were analyzed. *Salmonella* prevalence was 3.46% [95% confidence interval (CI): 2.6-4.5] for FBO and 10.34% (95% CI: 7.3-13.5) for CA, with statistically significant yearly differences in 2015, 2017, 2018, and 2020 ($p < 0.05$). Derby was the predominant serovar (22.6%); however, 20.4% of the FBO isolates were classified only as 'No Typhimurium and Enteritidis', highlighting gaps in serotyping protocols. Discrepancies likely reflect differences in sampling frequencies, carcass origins, and the use of different accredited laboratories by FBO and CA (private and official ones, respectively). The study identifies regulatory ambiguities, including a mistranslation in the Italian Ministerial Note, and recommends enhanced farm-level controls and harmonized surveillance.

Introduction

Salmonella spp. remains a leading zoonosis in the European Union (EU), representing a persistent public health challenge (ECDC, 2020; Pinedo *et al.*, 2022; EFSA & ECDC, 2025). While control programs in poultry production have achieved substantial reductions in *Salmonella* incidence, pork remains an important vehicle for transmission to humans (Mu *et al.*, 2023). The farm environment is a key reservoir of contamination, and apparently healthy pigs act as asymptomatic carriers, spreading the pathogen along the production chain (Borch *et al.*, 1996; Bonardi, 2017; Soliani *et al.*, 2023). Several studies, including those conducted in Italy, have confirmed the persistent detection of *Salmonella* from farm to slaughter and processing, emphasizing their role in cross-contamination (Piras *et al.*, 2014; Bonardi *et al.*, 2016; Bonardi *et al.*, 2021; Primavilla *et al.*, 2021; Lauteri *et al.*, 2022; Peruzy *et al.*, 2022; Soliani *et al.*, 2023; Visciano *et al.*, 2020).

The European legislative framework has progressively introduced and reinforced process hygiene requirements aimed at controlling *Salmonella* in pig abattoirs. Regulation (EC) No 2073/2005 established mandatory microbiological criteria for food business operators (FBOs), requiring routine self-monitoring of *Salmonella* spp. on swine carcasses using prescribed sampling protocols (European Commission, 2005). This regulation places responsibility for food safety and process hygiene on operators, demanding systematic data collection and corrective actions in response to non-compliance (EFSA, 2010). However, until 2014, systematic official sampling and surveillance by competent authorities (CAs) were not consistently applied in pig slaughterhouses throughout Europe. This changed with Regulation (EC) No 218/2014, which formally introduced official controls for *Salmonella* in pig carcasses, subsequently harmonized and consolidated by Regulation (EU) No 2019/627 (European Commission, 2014, 2019). These new regulatory provisions resulted in a dual monitoring approach: both self-checks by FBOs and independent control by CAs.

The dynamic of the diffusion of *Salmonella* in the pork chain is often reported with an emphasis on the serotype most frequently involved, their origin, and correlation to animal source (Marín *et al.*, 2019; Primavilla *et al.*, 2021; EFSA and ECDC, 2025). According to EFSA, the three most common serovars detected in pigs are serovar 4,[5],12:i:- (Typhimurium monophasic variant) (27.9%), Derby (23.4%), and Typhimurium (16.2%) (Primavilla *et al.*, 2021; EFSA and ECDC, 2025). In Italy, an agreement between the Government, the Regions, and the Autonomous Provinces of Trento and Bolzano was set on 31/03/2016 (Ministry of Health, 2016) and it established that, in case the FBO detects 'relevant' *Salmonella* serovars (defined as *Salmonella* Enteritidis and *Salmonella* Typhimurium, including its monophasic variant), hygienic interventions at the slaughterhouse shall be implemented. For this reason, *Salmonella* isolates detected by the FBO must be serotyped. Moreover, regarding the process hygiene criteria, Regulation (EU) No 627/2019 establishes only that "where the FBO fails on several occasions to comply with the process hygiene criterion, the CA shall require it to submit an action plan and shall strictly supervise its outcome". This leaves considerable discretion

to individual CAs and operators regarding both legislative interpretation and appropriate interventions, and consequently, a single EU-wide critical limit for *Salmonella* is lacking.

A further limitation is the absence of an EU-mandated, harmonized *Salmonella* control program at the pig farm level. Although a minority of Member States (MSs) have instituted some form of risk-based on-farm monitoring plans for pigs (Correia-Gomes *et al.*, 2021), such plans remain fragmented across the EU. This regulatory gap is problematic, as contamination events at slaughter are often rooted in primary production, with infected herds sending infected pigs to slaughter (Chalias *et al.*, 2022; Rizzotto *et al.*, 2022; García-Díez *et al.*, 2023; Viltrop *et al.*, 2023).

All data collected across MSs since the implementation of FBO and CA sampling for *Salmonella* spp. under Regulation (EC) No 2073/2005 and Regulation (EU) No 218/2014 have consistently documented huge discrepancies between the results of FBO self-monitoring and those from CA official control programs (EFSA, 2010; EFSA and ECDC, 2025). Official control samples frequently report a statistically higher prevalence of *Salmonella*-positive pig carcasses in comparison with FBO self-monitored own-checks (EFSA and ECDC, 2025). Similar patterns have been described in many countries, including Italy (Martelli *et al.*, 2021; Peruzy *et al.*, 2022). Variability in sampling strategies, laboratory methodologies, and reporting protocols, along with potential bias in sample collection and timing, have been proposed as contributing factors for these discrepancies (Primavilla *et al.*, 2021; Moura-Alves *et al.*, 2022; Gross *et al.*, 2025).

The aim of the present study is to compare the results of CA and FBO sampling for *Salmonella* spp. in pig carcasses in an industrial Italian slaughterhouse over a 10-year period. By critically assessing parallel control programs in a high-throughput abattoir setting, the study attempts to provide insight into the current challenges and opportunities for improving *Salmonella* risk management in the pork supply chain, ultimately contributing to enhanced public health protection (Bonardi *et al.*, 2021; Carvajal *et al.*, 2024; Saenkankam *et al.*, 2025).

Materials and Methods

Data collection

The study was conducted in a high-throughput abattoir located in the Lombardy region (Northern Italy). The abattoir had a daily output of about 3000 heavy pigs, mainly destined for the Italian Protected Designation of Origin (PDO) products chain and slaughtered at a live weight of ~170 kg and at least 9 months of age. The slaughterhouse receives heavy pigs from approximately 800 farms located predominantly in the Po Valley (mainly Lombardy, Emilia-Romagna, Veneto, and Piedmont regions), thus processing a significant portion of animal suitable for the Italian PDO pork production. The pig carcass samples were collected by the FBO between January 2012 and December 2021, following Regulation (EC) No 2073/2005, and by the CA between November 2014 and December 2021, following Regulation (EC) No 218/2014 and the Italian Ministerial Note 31817-P-05/08/2014 (Ministry of Health, 2014).

The sampling plan was defined according to Regulation (EC) No 2073/2005 and the Italian State-Regions Agreement of 03/03/2016 (Ministry of Health, 2016) for the FBO, and according to Regulation (EC) No 218/2014 and Regulation (EU) No 627/2019 for the CA. Under routine conditions, the FBO sampled five carcasses every fifteen days, for a total of approximately 130 samples per year, while the CA collected an average of 49 samples annually. FBO sampling frequency was intensified to weekly sampling from mid-2017 to mid-2019 (following exceedance of Process Hygiene Criteria) and again in 2021 (following multiple CA-positive samples), with detailed yearly sampling data reported in *Supplementary Table 1*.

A non-destructive method with sterile premoistened sponges was used in both cases on four specific sampling sites before chilling, according to the cited regulations. The sponge samples were transported to the laboratory in thermal boxes, stored at +4°C, and processed within 24 h. The CA analyses for detection and serotyping of *Salmonella* spp. were performed by the official laboratory *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna* (IZSLER) following the UNI EN ISO 6579-1:2017 and ISO/TR 6579-3:2014 standards, respectively. For FBO samples,

Salmonella spp. detection was performed by private accredited laboratories following the UNI EN ISO 6579-1:2017 standard. Serotyping was carried out using agglutination protocols according to the ISO/TR 6579-3:2014 method, or through serological tests targeting specific antigenic markers (A67/Vi+, Group B O:4,5-, Group D O:9-, PolyH+), thereby excluding the presence of Typhimurium and Enteritidis serovars. Although the FBO could have changed laboratories over the years, all of them were accredited and followed the same standardized methodology, minimizing potential methodological bias.

Statistical analysis

Calculation of 95% confidence intervals (CIs) was performed using the Clopper-Pearson (exact) method based on the binomial distribution. Fisher's exact test was used for yearly comparisons between FBO and CA prevalence, with significance set at $p < 0.05$. Given the exploratory nature of the study and the limited number of yearly comparisons, no formal correction for multiple testing was applied.

All the statistical analyses were performed by using the IBM SPSS Statistics 28.0 software (IBM Corp. Released 2020, IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY, USA: IBM Corp).

Results

A total of 1560 samples were analyzed by the FBO and 377 samples were analysed by the CA. It should be noted that, in 2017, the FBO exceeded the limit of 3 positive results/50 samples laid down in Regulation (EC) No 2073/2005. Consequently, the FBO increased the frequency from sampling every two weeks to weekly sampling for 30 weeks, until the prevalence fell back within the limits established by the Regulation and the Italian State-Regions Agreement of 03/03/2016. In 2021, following positive results detected by the CA "on several occasions", the FBO adopted, in agreement with the CA, a weekly sampling regime for 30 weeks. For these reasons, the total number of samples collected by the FBO generally exceeded the expected 130 samples per year, reaching up to 260 samples annually in the years when the sampling frequency was increased. Detailed yearly sampling and prevalence data are reported in *Supplementary Table 1*.

Overall, the prevalence of *Salmonella* spp. recorded by the FBO from 2012 to 2021 was 3.46% (95% CI 2.6-4.5) (54 positive samples out of 1,560), while the prevalence detected by the CA from 2014 to 2021 was 10.34% (95% CI 7.3-13.5) (39 positive samples out of 377). The distribution of prevalence over the years is shown in Figure 1.

Serotyping data (Table 1) revealed that the most frequently detected serovars, in *S. enterica* subspecies *enterica* isolates, were Derby (22.6%), Bredeney (11.8%), Rissen (11.8%), Give (10.8%), and Choleraesuis (8.6%). Prevalence of *S. enterica* subspecies *arizonae* isolates was 7.5%. Both CA and FBO reported Derby as the predominant serovar.

It is important to note that 20.4% of the FBO isolates were assigned to the "No Typhimurium and Enteritidis" category. This reflects the protocol applied by some FBO-contracted laboratories that test the isolates for Typhimurium and Enteritidis serovars only.

Discussion

As reported by the EU One Health 2024 Zoonoses Report (EFSA and ECDC, 2025), *Salmonella* remains a leading zoonotic agent in the EU, with notable discrepancies in prevalence observed between samples collected by CAs and FBOs. Specifically, among the 27 MSs, the samples collected by CAs showed a higher proportion of *Salmonella*-positive results (3.0%) at pig slaughterhouses compared to those collected by FBOs (1.3%). Italy's figures (4.0% positivity among CA samples and 1.5% among FBO samples) were comparable to the European average, confirming that the pattern at national level aligns with the EU-wide trend. Several factors may explain the observed discrepancies between CA and FBO *Salmonella* prevalence. Firstly, sampling frequency differed substantially: the FBO collected approximately 130 samples annually under routine conditions, whereas the CA

collected an average of 49 samples per year. The lower sampling size by CA might result in greater variability in prevalence estimates. Secondly, the CA and FBO samples were collected from different carcasses, which could originate from different farms with heterogeneous *Salmonella* status. Thirdly, the use of separate laboratories could introduce inter-laboratory variability, despite adherence to standardized ISO methods. Additionally, potential selection bias in CA sampling could not be excluded: official controls could be intensified following previous positive findings or targeted at specific production days or suppliers perceived as higher risk, thereby inflating prevalence compared to routine FBO self-monitoring. These discrepancies were consistently confirmed in the present study over the entire decade considered, with most of the yearly differences (2015, 2017, 2018 and 2020) being statistically significant ($p < 0.05$). While the results from a single facility could be directly extrapolated to the national context, the high throughput, long observation period and relevance of this plant for PDO production provided meaningful insights into FBO-CA surveillance dynamics in a representative Italian setting.

This gap between CA and FBO results should be further investigated. Even though it has been highlighted by EFSA and ECDC (2025), it remains without definitive conclusions. Furthermore, the consequences of positive results from CA sampling lack clear regulatory definitions, contrasting with the well-defined thresholds for FBO sampling non-compliances. Adding complexity, the inaccurate Italian translation of the same note laid down in Regulation (EC) No 218/2014 and Regulation (EU) No 627/2019 has contributed to misunderstandings. The aforementioned note is the following: “If all samples are negative (here referring to 50 negative samples), 95% statistical certainty is provided that the prevalence is below 6%” was incorrectly translated in the Italian version as “if one or more carcasses are positive, it can be considered with 95% confidence that the prevalence of *Salmonella* in meat is equal to or greater than 6%”. The original EU note provides a one-sided upper confidence bound: if 50 samples test negative, the true prevalence can be stated with 95% confidence to be below 6% (based on the rule of three approximation for binomial CIs). This statement applies only to negative results and provides reassurance about low prevalence when no positives are found. The Italian mistranslation incorrectly inverts this logic, suggesting that even one positive sample implies prevalence $\geq 6\%$ with 95% confidence, which is statistically invalid. In reality, a single positive among 50 samples yields a point estimate of 2% prevalence (1/50), with a 95% CI approximately 0.05-10.6%, not a lower bound of 6%. This mistranslation can have direct consequences for FBOs. Specifically, when a single positive sample is found during official CA controls, the incorrect wording could lead to unjustified regulatory action by the CA regarding the prevalence threshold, potentially resulting in undue interventions towards the FBO. In such cases, only a careful verification of the efficacy of the FBO self-monitoring system by the CA is needed to ensure compliance with the regulatory limits (Ministry of Health, 2014).

It is well known that preventive measures during slaughter alone (e.g., using sanitary dressing procedures, improving evisceration practices, etc.) are insufficient to reliably control *Salmonella* contamination, given many critical points along the process that facilitate bacterial cross-contamination (Borch *et al.*, 1996; Berends *et al.*, 1997; Arguello *et al.*, 2012; Zeng *et al.*, 2021; Moura-Alves *et al.*, 2022; Brasileiro *et al.*, 2025). It is difficult to expect that, beyond improving hygienic conditions during slaughter, the abattoir could also take responsibility for intervening at farm level reviewing controls on the origin of animals or acting on biosecurity measures on farms, as mentioned in the section “Actions in case of unsatisfactory results” of Regulation (EC) No 2073/2005, as positive carcasses cannot be undoubtedly traced to specific herds due to common cross-contamination from lairage to evisceration. Stable prevalence trends in the EU confirm that slaughter controls are undermined without farm-level reduction strategies (EFSA and ECDC, 2025).

The absence of systematic farm-level controls in Italy represents a critical gap in *Salmonella* prevention along the pork production chain. Evidence from MSs that have implemented national surveillance and control programs at the farm level (e.g., Denmark, Germany, Belgium) shows that measures such as serological monitoring, herd categorization according to *Salmonella* status and targeted biosecurity interventions can significantly reduce the proportion of infected pigs sent to

slaughter and, consequently, carcass contamination. A similar risk-based approach, such as the Danish system of serological monitoring and herd categorization, could realistically be adapted to the Italian context, although this would require targeted legislative adjustment and coordination between CA and FBO. In this context, the stable prevalence observed in our study over a ten-year period supports the view that slaughterhouse-based interventions alone are insufficient, and that an effective *Salmonella* control should focus on primary production.

Serotyping data from this study revealed that *S. enterica* subspecies *enterica* serovar Derby was predominant (22.58%), followed by Bredeney (11.83%) and Rissen (11.83%). The category 'no Typhimurium/Enteritidis' which accounts for 20.37% of the FBO isolates, highlights the importance of a harmonized and complete identification of serotypes in all laboratories. Moreover, the detection of uncommon serovars, such as London or *S. enterica* subspecies *arizonae*, underscores variability in *Salmonella* epidemiology in the pork chain. Our findings partially align with the latest EFSA & ECDC (2025) data for pigs and pig meat source in the EU, where the predominant serovars were monophasic *S. Typhimurium* (28.0%), Derby (24.8%), and Typhimurium (16.7%), together accounting for nearly 70% of the isolates. Studies from Italy and other European countries confirm that the *Salmonella* population in pigs and pork meat is dominated by a small number of serovars (mainly Derby, Typhimurium, and its monophasic variant), though other serovars such as Rissen, Infantis, Bredeney, and Choleraesuis are not infrequent (Piras *et al.*, 2014; Bonardi *et al.*, 2018; Primavilla *et al.*, 2021; Moura-Alves *et al.*, 2022; Peruzzy *et al.*, 2022; Mati Roasto *et al.*, 2023). Despite some differences in the serovar proportions, these results confirm that control strategies for *Salmonella* in the pork supply chain must account for both the dominant and the less common circulating serovars, as all of them are likely responsible for foodborne illness in consumers.

Conclusions

This study, conducted in a single high-throughput Italian slaughterhouse, confirms that FBO self-monitoring consistently detected a lower *Salmonella* prevalence (3.46%) in pig carcasses, compared to CA official sampling (10.34%) over a 10-year period. The discrepancies found in our study strongly reflect the trends observed in many EU countries for years. As it is well known, the role of the CA is to verify FBO compliance through official sampling. However, as discussed above, misconceptions around interpreting these statistics can lead to regulatory and practical ambiguities. Given that systematic *Salmonella* control programs at the farm level are not mandatory in the EU countries, the lack of reduction in *Salmonella* prevalence at farms limits the effectiveness of slaughterhouse controls alone. These findings suggest the need to review current hygiene controls and strengthen upstream interventions in primary production, while recognizing FBO self-monitoring as a valuable surveillance component of surveillance.

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Online supplementary material

Supplementary Table 1. Yearly *Salmonella* prevalence comparison between food business operator self-monitoring and competent authority official sampling in pig carcasses.

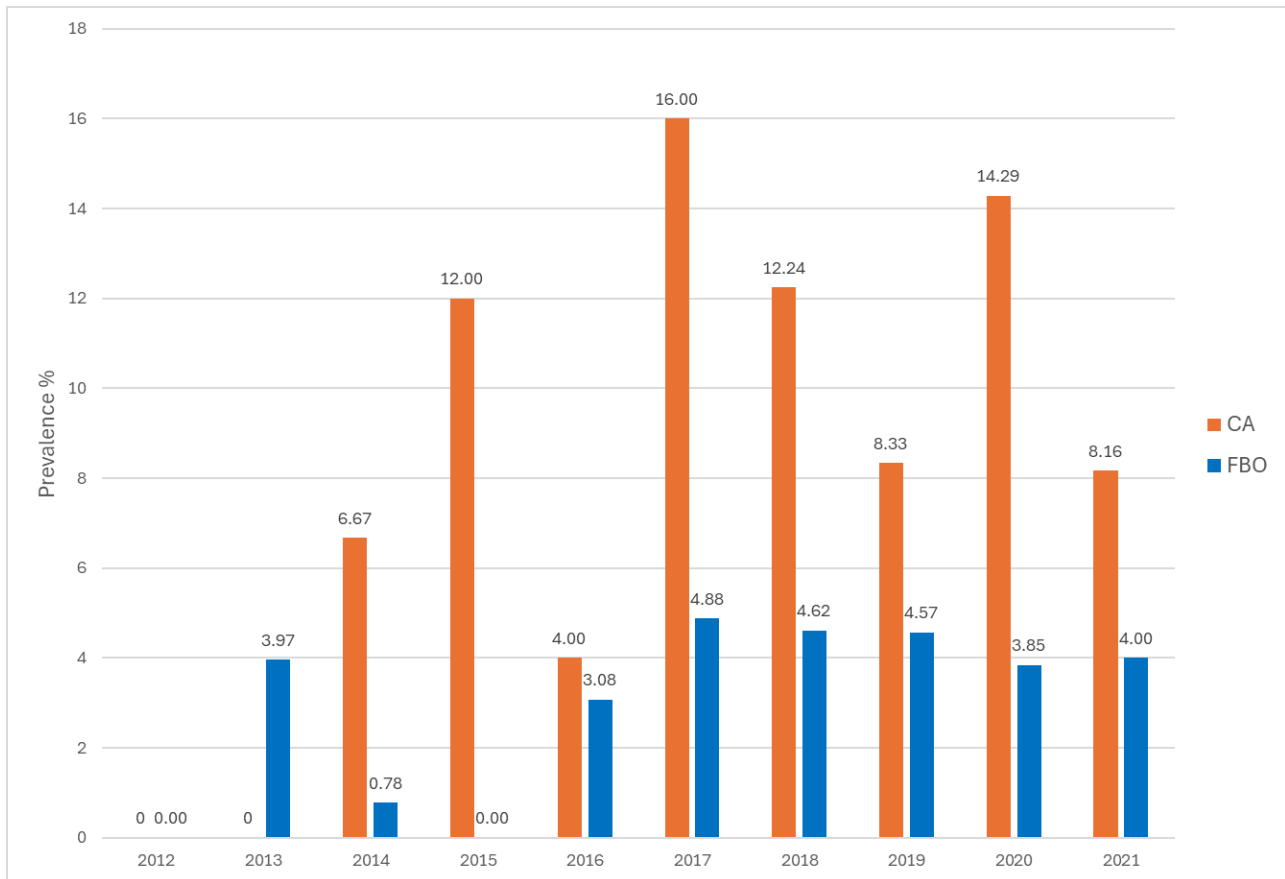


Figure 1. Distribution of *Salmonella* spp. prevalence over the years. FBO, food business operator; CA, competent authority.

Table 1. Percentage of *Salmonella* serovars isolated from pig carcasses sampled by food business operators and competent authorities.

Serovar	Overall % (n=93)	CA % (n=39)	FBO % (n=54)
<i>S. enterica</i> subspecies <i>enterica</i>			
Brandenburg	2.15	5.13	0.00
Bredeney	11.83	15.39	9.26
Choleraesuis	8.58	7.69	9.26
Derby	22.58	25.64	20.37
Give	10.75	12.82	9.26
Kapemba	1.08	2.56	0.00
London	3.23	0.00	5.56
Munchen	3.23	5.13	1.85
Rissen	11.83	12.82	11.11
Typhimurium monophasic 4,[5],12:i:-	5.38	7.69	3.70
“no Typhimurium and Enteritidis”	11.83	0.00	20.37
<i>S. enterica</i> subspecies <i>arizonae</i>	7.53	5.13	9.26
Total	100.00	100.00	100.00

CA, competent authority; FBO, food business operator; n, number of isolates.