

# Assessing the efficacy of conventional methods for detecting *Salmonella enterica* serovar enteritidis (ATCC 13076) in artificially contaminated food matrices

*Gustaf Eifel* Silalahi<sup>1</sup>, *Muhammad* Iqbal<sup>2</sup>, *Irzaman* Irzaman<sup>3</sup>, *Febdian* Rusydi<sup>4</sup>, *Laila Nur* Fatimah<sup>1</sup>, *Maria* Avina<sup>5</sup>, and *Widagdo* Sri Nugroho<sup>1\*</sup>

<sup>1</sup>Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

<sup>2</sup>Advanced Functional Materials Laboratory, Faculty of Industrial Technology, Institut Teknologi Bandung, Ganesha 10, Bandung, 40132, Indonesia

<sup>3</sup>Department of Physics, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, 16680, Indonesia

<sup>4</sup>Department of Physics, Faculty of Science and Technology, Universitas Airlangga, Jl. Mulyorejo, Surabaya, 60115, Indonesia

<sup>5</sup>Disease Investigation Center Wates, Jalan Raya Jogja-Wates Km 27 Po Box 18, Wates, Yogyakarta, 55602, Indonesia

**Abstract.** Conventional diagnostic methods often face challenges related to their efficacy, particularly in terms of sensitivity. This study aims to evaluate the contamination of selected animal products with *Salmonella enterica* serovar enteritidis (ATCC 13076) and compare the effectiveness of conventional diagnostic methods in milk, chicken meat, albumin, and yolk. The goal is to optimize detection protocols to improve food safety surveillance. A total of 360 samples were analyzed, comprising four food matrices: milk, chicken meat, egg albumin, and egg yolk, with 90 samples from each matrix. Each sample was artificially contaminated with *Salmonella enterica* serovar enteritidis (ATCC 13076) at three different concentrations ( $10^1$ ,  $10^2$ , and  $10^3$  CFU/mL). Re-isolation was conducted using conventional culture methods to evaluate detection efficacy across the various food matrices. The study demonstrated the effectiveness of the conventional diagnostic method for *Salmonella enterica* serovar enteritidis (ATCC 13076) in animal-derived foods, with efficacy rates exceeding 100% in milk, ranging from 36.7% to 70% in chicken, and 96.7% in albumin and yolk. Traditional culture-based methods effectively detect *Salmonella enterica* serovar enteritidis (ATCC 13076) in animal products, offering a reliable and cost-effective solution for food safety surveillance.

---

\* Corresponding author: [weesnugroho@ugm.ac.id](mailto:weesnugroho@ugm.ac.id)

## 1 Introduction

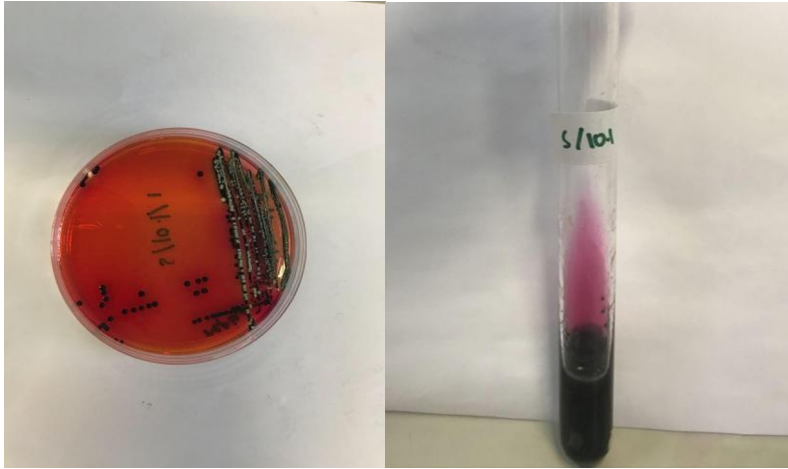
Animal-derived food products are frequently implicated in outbreaks of Salmonellosis, largely due to contamination by *Salmonella* spp., with chicken, milk, and eggs being particularly susceptible. Conventional methods for isolating and identifying these pathogens are notably time-intensive and are often complicated by the interference of competing, spoilage-causing bacteria in field samples [1]. This study aims to evaluate the effectiveness of conventional diagnostic methods for detecting *Salmonella enterica* serovar enteritidis (ATCC 13076) in chicken meat, eggs, and milk, focusing on improving detection efficiency.

## 2 Materials and methods

*Salmonella enterica* serovar enteritidis (ATCC 13076) isolates from Disease Investigation Centre Wates, Yogyakarta were prepared by initially culturing the bacteria in Rappaport-Vassiliadis broth (Oxoid) and incubating at 37°C for 24 hours. Following enrichment, aliquots of the culture were streaked onto Xylose Lysine Deoxycholate (XLD) agar (Oxoid) and incubated for an additional 24 hours at 37°C, as recommended by USDA FSIS [2]. On XLD agar, *Salmonella enterica* serovar enteritidis (ATCC 13076) was identified by the presence of round, black colonies. These presumptive colonies were further inoculated onto Triple Sugar Iron Agar (TSIA; Oxoid) for confirmatory identification. On TSIA, *Salmonella* spp. typically exhibit a red alkaline slope and a yellow acidic butt, with gas production and the generation of hydrogen sulfide (H<sub>2</sub>S), which is visualized as a black precipitate. For standardization, confirmed *Salmonella enterica* serovar enteritidis (ATCC 13076) isolates were suspended in 10 mL of Buffered Peptone Water (BPW; Merck) and adjusted to the appropriate turbidity using a McFarland standard. Serial dilutions of *Salmonella enterica* serovar enteritidis (ATCC 13076) were then prepared to yield concentrations of 10<sup>1</sup>, 10<sup>2</sup>, and 10<sup>3</sup> CFU/mL. A total of 360 serial dilutions of *Salmonella enterica* serovar Enteritidis (ATCC 13076) were used in this study to artificially contaminate four food matrices: milk, chicken meat, egg albumin, and egg yolk. For each matrix, 90 serial dilutions were divided into three categories based on three concentration levels, with 30 replicates for each concentration. Specifically, 1 mL from each dilution was combined with 100 mL of samples of milk, chicken meat, and eggs as well. Following contamination, all samples were stored under refrigeration for 24 hours prior to analysis using the conventional methods described (enrichment in Rappaport-Vassiliadis broth, and plating on XLD and TSIA). Data were analyzed descriptively to assess the efficacy of these conventional diagnostic methods. As no live animal subjects were involved, ethical approval was not required for this study.

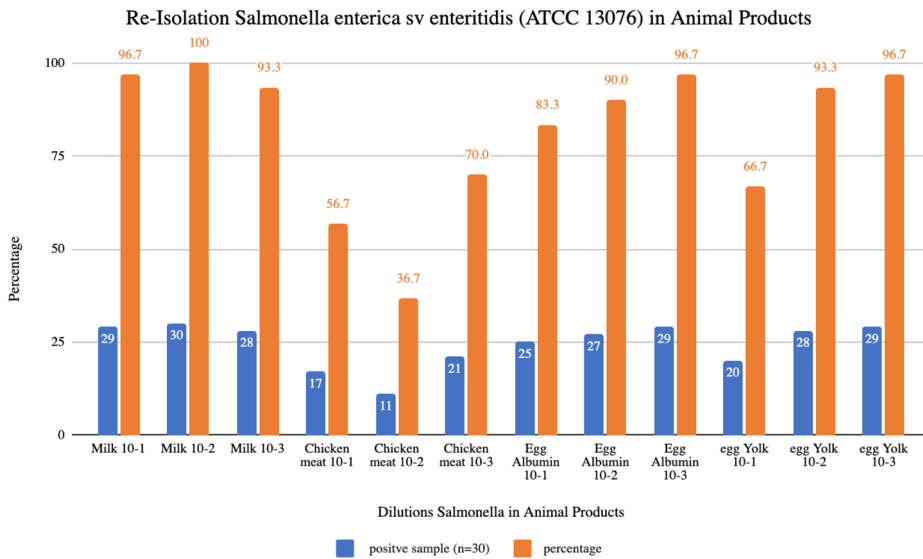
## 3 Results

In this study, the strain of *Salmonella* bacteria used was *Salmonella enterica* serovar enteritidis (ATCC 13076), which appears in Figure 1.



**Fig. 1.** The results of isolation and identification of *Salmonella enterica* serovar enteritidis (ATCC 13076) by conventional methods. Isolation of XLD (A) and TSIA (B).

Figure 1 shows that *Salmonella enterica* serovar enteritidis (ATCC 13076) grows on XLD media as black, circular, rough-edged colonies. While the isolation of *Salmonella enterica* serovar enteritidis (ATCC 13076) implanted into TSIA media has a red alkaline slope and a yellow acidic butt, and produces H<sub>2</sub>S gas, indicated in black. The isolates were implanted into 30 chicken meat samples, eggs, and milk. Re-isolation results of *Salmonella enterica* serovar enteritidis (ATCC 13076) in each type of animal product and each concentration are shown in Figure 2.



**Fig. 2.** The amount of re-isolation of *Salmonella enterica* serovar enteritidis (ATCC 13076) after clearing in animal products with various dilutions. (n=30).

Figure 2 shows that *Salmonella enterica* serovar enteritidis (ATCC 13076) is most frequently re-isolated from milk, with a rate of 100%. In contrast, chicken meat has a lower re-isolation rate, ranging from 36.7% to 70%. Higher *Salmonella enterica* serovar enteritidis (ATCC 13076) levels in milk samples compared to chicken meat are due to conditions inhibiting *Salmonella* growth in chicken and spoilage bacteria. The existence of putrefactive bacteria prevents *Salmonella* from being able to compete effectively, thereby inhibiting the growth of *Salmonella* [3].

## 4 Discussion

*Salmonella* is a pathogenic bacterium responsible for foodborne illnesses, prevalent in both developed and developing countries. It poses significant economic challenges due to animal mortality and risks associated with animal products. In Surabaya, Indonesia, *Salmonella* contamination in chicken meat was found to be 85%, while in Iran, the prevalence in milk and eggs was 21.3% and 7.49% in eggshells, respectively [4,5,6,7]

The process of re-isolating samples is performed using XLD, specifically designed to support the growth of certain bacteria while inhibiting others. We employ TSIA to accurately identify the samples, which provides valuable information on the metabolic characteristics of the organisms present. Based on the results showed that there were circular black colonies on the surface of XLD media, indicating the production of H<sub>2</sub>S gas. *Salmonella* bacteria's trisulfate metabolism produces H<sub>2</sub>S, which forms circular black colonies [8]. In Figure 1B, the suspected positive test shows a black precipitate. The black precipitate is formed from a specific reaction of *Salmonella* bacteria that desulfurizes amino acids and methionine to produce H<sub>2</sub>S gas, which then reacts with iron (Fe<sup>+</sup>) contained in the media [9].

Results from testing chicken meat, milk, and egg samples indicated that milk contained the highest levels of *Salmonella enterica* serovar enteritidis (ATCC 13076), followed by chicken meat and eggs. The nutrient-rich composition of milk, along with its neutral pH and high water content, makes it particularly susceptible to microbial contamination. Poor milking practices and inadequate cleaning can further encourage the growth of *Salmonella*. This pathogen thrives in milk at temperatures ranging from 15°C to 37°C [10]. *Salmonella* can grow and develop on eggshells during the oviposition process because the lower reproductive tract of chickens has been infected with *Salmonella* spp. [11]. In addition, infection of the eggshells can also occur due to contamination from outside, both fecal and environmental contamination. Unless bacteria can penetrate the vitelline membrane and contaminate the yolk, their initial numbers remain limited, which prevents proliferation and growth.

Meat is a favorable medium for the growth of *Salmonella* spp., which thrives at warmer temperatures, leading to more frequent infections in summer. Improper meat preparation can also contribute to the proliferation of these bacteria, resulting in illness [12].

*Salmonella* growth is significantly influenced by environmental factors, with temperature being key. It thrives and multiplies rapidly in warm conditions, especially between 4°C and 60°C. The acidity level (pH) of food also affects *Salmonella* survival. *Salmonella* can survive at different pH levels, but an acidic environment can inhibit the growth of these bacteria. The naturally acidic pH of eggs and milk can provide protection against *Salmonella*. However, Succinic acid can reduce *Salmonella* levels on chicken meat, though an acidic pH alone may not be sufficient to stop *Salmonella* growth [13]. The nutritional content of eggs, chicken, and milk can also affect the growth of *Salmonella*. These bacteria grow better when they get enough nutrients like protein and carbohydrates. Proper handling and storage, including low temperatures, can reduce the growth of these bacteria.

The detection of hydrogen sulfide (H<sub>2</sub>S) production remains a critical method for identifying *Salmonella*; however, recent studies highlight both progress and challenges in

this area. Advances in enrichment media, such as the use of ornithine-arginine-based broth, have improved the efficiency of Salmonella screening [14]. Nevertheless, the identification of non-H<sub>2</sub>S-producing Salmonella strains, as documented by Lee et al. [15], reveals the limitations of traditional diagnostic methods, emphasizing the need for alternative detection strategies.

The re-isolation of Salmonella enterica serovar enteritidis (ATCC 13076) varied across different food matrices, with milk showing consistently high recovery rates (96.7–100%), indicating its suitability for bacterial survival and detection. Egg albumin also demonstrated high detection (83.3–96.7%), reinforcing its potential as a contamination vector. In contrast, chicken meat had lower and more variable recovery rates (36.7–70%), likely due to competing microflora affecting detection sensitivity. Egg yolk exhibited moderate to high detection (66.7–96.7%), with reduced recovery at lower bacterial loads.

The study revealed consistently high Salmonella enterica serovar enteritidis (ATCC 13076) recovery rates in milk and egg albumin, whereas detection in chicken meat and egg yolk was less reliable, particularly at lower contamination levels. These findings emphasize the need for matrix-specific adjustments to improve the sensitivity of conventional diagnostic methods. Additionally, they reinforce the necessity of rigorous surveillance programs and strict contamination control measures in the dairy and poultry industries. Relying solely on traditional culture-based methods may underestimate contamination risks in certain food products, highlighting the importance of integrating complementary detection techniques.

The authors appreciate to Universitas Gadjah Mada for facilitating this study by Indonesia Research Collaboration Program (Riset Kolaborasi Indonesia)-World Class University Grant, with the Grant Number 806/UN1.DITLIT/DIT-LIT/PT/2021. Salmonella enterica serovar enteritidis (ATCC 13076) isolates from Disease Investigation Centre Wates, Yogyakarta, Ministry of Agriculture, Indonesia.

Gustaf Eifel Silalahi (GES): data analysis, manuscript drafting, and review. Widagdo Sri Nugroho (WSN): Study design, Coordinating research activity, data analysis, manuscript drafting, and review. Muhammad Iqbal (MI): data analysis, manuscript drafting and review. Irzaman (I): manuscript drafting and review. Febdian Rusyidi (FR): manuscript drafting and review. Laila Nur Fatimah (LNF): Laboratory diagnosis, data collection. Maria Avina (MA): Laboratory diagnosis, data collection, manuscript drafting, and review.

The authors declare that there is no conflict of interest.

This research is funded by Indonesia Research Collaboration Program (Riset Kolaborasi Indonesia)-World Class University Grant funded this study with contract no. 806/UN1.DITLIT/DIT-LIT/PT/2021.

## References

1. Y. Rosmania, *Perhitungan jumlah bakteri di Laboratorium Mikrobiologi menggunakan pengembangan metode Spektrofotometri*. J. Penelit. Sains **22** (2), 76–86 (2020).
2. United States Department of Agriculture, Food Safety and Inspection Service, *Microbiology laboratory guidebook: Isolation and identification of Salmonella from meat, poultry, and egg products (MLG 4.15)*. (2024). Retrieved from: [https://www.fsis.usda.gov/sites/default/files/media\\_file/documents/MLG\\_4.15.pdf](https://www.fsis.usda.gov/sites/default/files/media_file/documents/MLG_4.15.pdf)
3. L. Screpanti, N. Desmaures, M. Schlüsselhuber, *Exploring resource competition by protective lactic acid bacteria cultures to control Salmonella in food: an Achilles' heel to target?* Crit. Rev. Food Sci. Nutr. 1–15 (2024). <https://doi.org/10.1080/10408398.2024.2416467>

4. A. Abrar, T. Beyene, W. Furgasa, *Isolation, Identification and Antimicrobial Resistance Profiles of Salmonella from Dairy Farms in Adama and Modjo Towns, Central Ethiopia*. Eur. J. Med. Health Sci. **2** (1), 1–11 (2020). <https://doi.org/10.34104/ejmhs.02001011>
5. R. Yulistiani, D. Praseptianga, Supyani, D. Sudibya, D. Raharjo, T. Shirakawa, *Prevalence of antibiotic-resistance Enterobacteriaceae strains isolated from chicken meat at traditional markets in Surabaya, Indonesia*. IOP Conf. Ser. Mater. Sci. Eng. **193** (2017). <https://doi.org/10.1088/1757-899X/193/1/012007>
6. B. Hosseinzadeh, E. Berizi, M. Nader, S.M. Mazloomi, S. Hosseinzadeh, L. Ebrahimi et al., *Prevalence of Salmonella contamination in consumed eggs in Iran: A systematic review and meta-analysis study on published studies from 1996 to 2018*. Vet. World **13** (12), 2743–2751 (2020). <https://doi.org/10.14202/vetworld.2020.2743-2751>
7. A. Bedassa, H. Nahusenay, Z. Asefa, T. Sisay, G. Girmay, J. Kovac et al., *Prevalence and associated risk factors for Salmonella enterica contamination of cow milk and cottage cheese in Ethiopia*. Food Saf. Risk **10** (2) (2023). <https://doi.org/10.1186/s40550-023-00101-3>
8. Suripto, C. Alfani, *Identification of pathogenic bacteria in traditional packaged donuts at Ampenan Market using Xylose Lysine Deoxychoalate (XLD) media*. J. Pengabd. Magister Pendidik. IPA **5** (2), 313–319 (2022). <https://doi.org/10.29303/jpmpi.v3i2.1770>
9. H. Maritsa, F. Aini, D.S. Nurhakim, G.M. Sihombing, A. Saputra, *Isolasi dan identifikasi cemaran bakteri Salmonella sp. pada daging ayam dan ikan mentah*. Biosite **3** (3), 47–70 (2017). <https://doi.org/10.22437/bs.v3i2.4427>
10. R.A.A. Putri, W. Tyasningsih, F. Fikri, *Uji cemaran Salmonella sp. pada susu segar kambing Sapera di Kecamatan Siliragung Kabupaten Banyuwangi*. In: Proc. Sem. Nas. Pembang. Pendid. Vokasi Pertanian **2** (1), 186–197 (2021). <https://doi.org/10.47687/snppvp.v2i1.186>
11. V.V. Pande, R.L. Devon, P. Sharma, A.R. McWhorter, K.K. Chousalkar, *Study of Salmonella Typhimurium infection in laying hens*. Front. Microbiol. **7**, 203 (2016). <https://doi.org/10.3389/fmicb.2016.00203>
12. A. Darmawan, L. Muslimin, S. Arifah, H. Mahatmi, *Kontaminasi Salmonella spp. pada daging ayam broiler yang dijual di beberapa pasar tradisional di Makassar*. Indones. Med. Vet. **9** (2), 168–176 (2020). <https://doi.org/10.19087/imv.2020.9.2.168>
13. M. Radkowski, B. Zdrodowska, M. Gomółka-Pawlicka, *Effect of succinic acid on elimination of Salmonella in chicken meat*. J. Food Prot. **81** (9), 1491–1495 (2018). <https://doi.org/10.4315/0362-028X.JFP-17-446>
14. J. Saranak, A. Deepatana, A. Thipayarat, *Combined effect of amino acid decarboxylation on the improvement of hydrogen sulfide enrichment media for presumptive screening of thiosulfate-reducing Salmonella*. Retrieved from: <https://web.archive.org/web/20200808123949/https://ph02.tci-thaijo.org/index.php/swujournal/article/download/241315/164122>
15. K.S. Lee, D. Kim, H. Lee, K. Lee, D. Yong, *Isolation of non-hydrogen sulfide-producing Salmonella enterica serovar Infantis from a clinical sample: the first case in Korea*. Ann. Lab. Med. **40**, 334–336 (2020).