ARTICLE

A comparative study of culture-based and PCR-based protocols for *Salmonella enterica* detection in meat, environmental swabs, and commercial animal feed

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ABSTRACT

A polymerase chain reaction (PCR)-based assay targeting the *Salmonella*-specific *invA* gene was validated for the detection of *Salmonella enterica* in beef, chicken, animal feed, and sponge swabs. Assessed by a third-party proficiency testing service, the protocol was rated 'Satisfactory' across all matrices. It consistently demonstrated effective in detecting *S. enterica* not only in meat but also in non-meat sources, such as animal feed and environmental swabs. To compare performance with the conventional culture-based method outlined in the Bacteriological Analytical Manual (BAM) and ISO 6579-1:2017(E), parallel testing was conducted by the National Meat Inspection Service (NMIS), Department of Agriculture, Philippines, and the Pathogen-Host-Environment Interactions Research Laboratory (PHEIRL), University of the Philippines Diliman. Results showed that 84 of 95 samples (88%) yielded consistent outcomes between culture and PCR. However, 11 samples (12%) tested negative by culture but positive by PCR, likely due to atypical colonies that lacked characteristic morphology on the culture media. Findings confirm the PCR assay's superiority for *S. enterica* detection across diverse food and non-food matrices, regardless of colony morphology. Moreover, the assay provides results within two days, compared to five to seven days required by traditional methods, highlighting its value for rapid diagnostics. Notably, the study also reports the detection of *S. enterica* in commercial feed, underscoring its potential role as a disease vector. This is timely and significant, as the Philippines currently lacks microbiological quality standards for finished feed products, with testing limited to raw materials. The study provides critical groundwork for establishing microbiological standards for finished feed products in the country.

INTRODUCTION

Salmonella enterica is recognized as a significant foodborne enteric pathogen, with a considerable impact on public health in the United States, resulting in an estimated 1.35 million illnesses, approximately 26,500 hospitalizations, and around 420 deaths each year (US Centers for Disease Control and

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Prevention, 2020). The prevalence of these cases is particularly associated with typhoid fever, which is caused by the serotypes *Salmonella* Typhi and *S.* Paratyphi, as well as salmonellosis, a condition primarily caused by non-typhoidal *Salmonella*. *S. enterica* can contaminate a wide variety of food products, especially fresh and processed items like poultry, eggs, and raw meat. Consequently, the Food and Drug Administration (FDA) has established strict regulations to monitor and control the presence of *Salmonella* in these essential food categories, thereby protecting consumer health.

In the current local setting, *Salmonella* detection relies on conventional culture-based methods, as outlined in the Bacteriological Analytical Manual (BAM; US Food and Drug Administration 2024) and ISO 6579-1:2017(E) (International Organization for Standardization 2017), which is considered the gold standard for *Salmonella* detection in food samples. Unfortunately, these methods typically require five to seven days to yield results. These delays can hinder timely interventions and exacerbate the risk of *Salmonella*-related outbreaks, particularly in light of the rapid turnover of perishable products, such as poultry, eggs, and meat. Therefore, there is an urgent need for more rapid and sensitive detection methodologies that can facilitate quicker responses to potential health threats, thereby enhancing food safety measures across the industry.

Rapid methods for detecting *Salmonella* have significantly enhanced food safety and clinical diagnostics by drastically reducing the time needed for results compared to conventional culture-based methods. These advancements can be broadly categorized into three main types: immunological, spectroscopy-based, and molecular methods, each of which utilizes distinct biological principles for rapid and accurate identification (Awang et al. 2021). Immunological methods depend on the specific interaction between an antigen, such as a protein found on the surface of the *Salmonella* cell, and its corresponding antibody (Patel et al. 2024). These assays tend to be user-friendly, cost-effective, and suitable for high-throughput screening. Examples include the enzyme-linked immunosorbent assay (ELISA), lateral flow immunochromatographic assay (LFIA), and immunomagnetic separation (IMS). The primary advantages of immunological methods are their speed and simplicity, which make them ideal for on-site testing (Younes et al., 2024). However, they can suffer from cross-reactivity with non-*Salmonella* species that share similar antigens, leading to false-positive results (Raman 2017). Additionally, their sensitivity may be lower than that of molecular methods, and they often require a pre-enrichment step to boost bacterial levels to detectable amounts (Younes et al. 2024).

Spectroscopy-based methods represent an innovative and emerging category in the analysis of microorganisms, offering a distinct biochemical 'fingerprint' by examining the chemistry of samples (Mandal et al. 2022). These techniques assess how a sample interacts with electromagnetic radiation (Odularu 2020). The advantages of spectroscopy include its rapid and non-destructive nature, as well as its requirement for minimal sample preparation (Pandian et al. 2023). This method can analyze intact cells and potentially provide species-level identification without extensive biochemical or genetic analysis (Alexandrakis et al. 2012; Pandian et al. 2023). Notably, identifying pathogens without a pre-enrichment step is a significant advantage (Li et al. 2017). However, a major drawback of spectroscopy-based methods is the high cost of equipment and the necessity for specialized expertise to operate and interpret the results (Awang 2021). The accuracy of these methods also heavily relies on the quality of the spectral database used for comparison and the refinement of data analysis algorithms, which often require advanced machine learning techniques (Pandian et al. 2023; Patel et al. 2024).

Molecular methods represent advanced techniques for precisely identifying unique genetic material (DNA or RNA) from *Salmonella*, providing a level of specificity and sensitivity that far surpasses conventional detection methods (Patel et al. 2024). Molecular methods offer exceptional accuracy and sensitivity, enabling the detection of very low bacterial counts that are often undetectable by traditional culture methods. Moreover, the design of molecular assays allows for the simultaneous detection of multiple genetic targets, including various serotypes of *Salmonella* and co-occurring pathogens (Zhang et al. 2022). The heightened sensitivity of molecular methods stems from their capacity to target and amplify unique genetic sequences, such as the *invA* gene, which serves as a marker for the presence of *S. enterica* (Buehler et al. 2019). This sensitivity allows for precise detection in complex sample matrices and often negates the necessity for extensive pre-enrichment steps, further expediting the diagnostic process from several days to mere hours (Chirambo et al. 2021). As the availability of commercial kits and automated platforms increases, these technologies become more accessible, although they still require a certain level of technical expertise to ensure accurate results (Awang 2021, Younes 2023, Patel 2024). Overall, molecular methods represent a powerful approach for precisely identifying *Salmonella* and other relevant pathogens, significantly advancing the field of microbial detection.

In 2015, Soguilon-Del Rosario and Rivera developed a PCR-based *S. enterica* detection protocol. This method utilizes the *invA* gene, which is specific to *S. enterica*, thereby enabling precise detection. The PCR-based approach has been successfully implemented across a variety of food matrices, including raw and processed beef, pork, and chicken, demonstrating its versatility and effectiveness.

The current study aimed to further validate this PCR-based protocol through both proficiency and parallel testing, including assessing its efficacy and robustness for detecting *S. enterica* in traditional food matrices and other critical sources, such as animal feed and sponge swabs. Furthermore, this research seeks to compare the effectiveness of the PCR-based protocol against the conventional culture-based method. Thus, this study may also serve as the foundation for developing microbiological quality assessment protocols and standards to evaluate the quality of animal feeds released for livestock, contributing to overall food safety and public health. This is particularly timely and significant, as the Philippines currently lacks mandatory, routine microbiological quality standards or analysis for finished feed products.

MATERIALS AND METHODS

Sample collection

Beef and chicken samples were obtained in duplicate from the reference collection at the National Meat Inspection Service Laboratory (NMIS), Department of Agriculture. Animal feed samples were collected from agricultural and veterinary supply stores in wet markets throughout Quezon City, Metro Manila, Philippines. For the swab samples, 10 cm^2 surface areas of tables, weighing trays, display trays, butcher knives, chopping boards, and other equipment and utensils in selected Metro Manila markets were swabbed. These swabs were placed in tubes containing 10 mL of sterile 1X phosphate-buffered saline (PBS; Sigma-Aldrich, MA, USA) as a transport medium. The use of 1X PBS as a transport medium represents an intentional modification of the Bacteriological Analytical Manual (BAM) protocol, which typically recommends Dey-Engley broth (D/E) for its superior neutralizing capabilities against residual sanitizers (Li et al., 2020). However, PBS was selected due to its suitability and cost-effectiveness in a resource-constrained environment, as it provides adequate short-term transport

conditions and supports the viability of *Salmonella* in environments without heavy antimicrobial or disinfectant use, such as wet markets. All samples were transported to the laboratory in ice boxes, maintained at a temperature between 4-8°C.

Pre-enrichment broth culture method

For beef, chicken, and feeds, 25 g of sample was aseptically weighed and placed onto sterile Rollbags® (Interscience, France) before adding 225 mL sterile buffered peptone water (BPW; Difco, Beckton Dickinson). While the BAM recommends Lactose Broth for meat samples, BPW was selected because it is the standard pre-enrichment broth routinely utilized by the NMIS for *Salmonella* detection, and it aligns with the ISO 6579-1 protocol, which was used for parallel testing alongside the BAM-referenced method. The samples were homogenized using BagMixer® 400 SW (Interscience, France) for 1 min. For swab samples, the swabs and transport medium were aseptically transferred into 215 mL BPW. For this pre-enrichment step, samples were incubated at 37°C for 18–24 h.

Selective enrichment broth culture method

Selective enrichment was done by inoculating 100 µL of the BPW cultures into 10 mL Rappaport-Vassiliadis R10 broth (RVB; Difco, Beckton Dickinson) and incubating at 42°C for 18-24 h. Note that for this modified selective enrichment, only RVB was used. Omitting the second selective medium (Tetrathionate broth) constitutes a modification of the full BAM and ISO protocols, driven by the need to reduce reagent cost and streamline the workflow in a resource-constrained environment. The acceptability of this reduced culture step is supported by the high performance of the downstream *invA* PCR protocol, which was rated 'Satisfactory' in independent proficiency testing.

DNA extraction

Genomic DNA was extracted using the boil lysis method. Cells were pelleted from the RVB culture by centrifugation at 15,330 x g for 5 min. The resulting cell pellets were subsequently washed with 1 mL of 1X PBS solution. The cell suspension was then centrifuged again at 15,330 x g for an additional 5 min. Following this, the supernatants were discarded, and the pellets were resuspended in 50 µL of Tris-EDTA (TE) buffer (Vivantis Technologies Sdn Bhd, Malaysia) before being boiled at 100°C for 10 min using a dry heating block. After lysis, the cells were centrifuged at 6,000 rpm for 5 min, and the resulting supernatant was transferred to a new microcentrifuge tube for storage at -20°C until further use. Additionally, for all samples, cells were pelleted from the BPW (pre-enrichment) culture to assess the detectability of *S. enterica* after 24 h without selective enrichment.

PCR-based *Salmonella enterica* detection

PCR-based detection of *S. enterica* targeting the *invA* gene was conducted following the methodology described by Soguilon-Del Rosario and Rivera (2015). A 12.5 µL PCR reaction volume was prepared with the following components: 6.25 µL of 2X Promega Green master mix (Promega Corporation, WI, USA), 0.5 µL each of 10 µM forward ('5-AC AGTGCTCGTTTACGACCTGAAT-3') and reverse ('5-AGACGACTGGTACTGATCGAT AAT-3') primers, 1 µL of DNA template, and 4.25 µL of nuclease-free water. The amplification of the *invA* gene was carried out under the following conditions: an initial denaturation at 95°C for 3 min; followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec; concluding with a final extension at 72°C for 5 min. Amplicons were analyzed via agarose gel electrophoresis at 280 V for 35 min. Five microliters of the amplicons were loaded into 1.5% agarose gels stained with 10,000X GelRed® in water (Biotium, CA, USA), using 1X Tris-Acetate-EDTA (TAE; Vivantis Technologies Sdn Bhd, Malaysia) as the running buffer. For each run, a positive control (KWIK-STIK™ 0363K derived from *S.* Typhimurium ATCC® 14028), a no-template control (NTC), and a negative control (KWIK-STIK™ 0335K derived from *E. coli* ATCC® 25922) were included. The gels were visualized using a BIO-PRINT ST4 (Vilber-Lourmat) UV transilluminator. Band sizes were estimated using a 1 kb DNA ladder (Promega Corporation, WI, USA) as a molecular weight marker. Amplicons displaying band sizes of approximately 244 bp were considered positive for the *invA* gene, indicating the presence of *S. enterica*.

Proficiency testing

The PCR-based protocol was subjected to proficiency testing to assess the protocol's effectiveness and the laboratory's performance in *S. enterica* detection across four matrices, namely, chicken, beef, animal feeds, and swabs. The proficiency testing service was obtained from Fapas®, Fera Science Ltd., York, United Kingdom. The four food matrices were analyzed using the PCR-based protocol within the timeframe required by Fapas®. Results were then submitted through an online platform, and assessment reports were sent electronically to participating laboratories.

Parallel testing

Parallel testing was done in collaboration with the NMIS. Both laboratories analyzed duplicates of the same samples, with PHEIRL using the PCR-based protocol and the NMIS laboratory using the standard culture-based *Salmonella* detection in meat samples as described by the BAM and ISO 6579-1:2017(E). Beef and chicken samples were obtained from reference samples of NMIS. Animal feeds were bought from agricultural and veterinary stores, while swabs were obtained from pork, beef, and chicken stalls in wet markets around Metro Manila. Ninety-five samples (18 beef, 25 chicken, 25 swabs, and 27 feeds) were analyzed, and results from both protocols were compared.

RESULTS AND DISCUSSION

Proficiency testing

The effectiveness of the PCR-based protocol in detecting *S. enterica* across four food matrices—beef, chicken, animal feeds, and sponge swabs—was assessed and rated 'Satisfactory.' Detailed reports of the results and assessments can be accessed on the Fapas® website, specifically in the Fapas®–Food Microbiology Proficiency Test Report 226 (October 2017; animal feed), Report 227 (November 2017; beef), and Report 230 (February–March 2018; chicken and sponge swabs.

S. enterica has been identified in both BPW and RVB cultures as part of the proficiency test workflow (Figure 1).

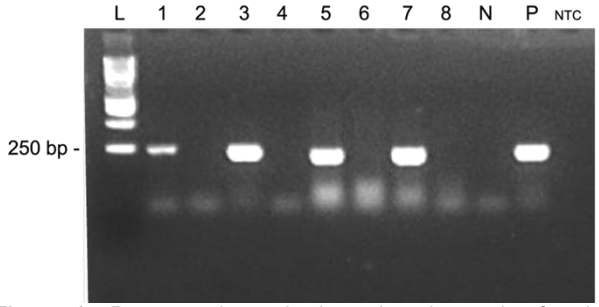

Figure 1: Representative gel electrophoresis results for the proficiency test for chicken (lanes 1–4) and sponge swab (lanes 5–8) samples using the PCR-based *Salmonella* detection protocol. Bands at ~244 bp correspond to the *invA* gene from DNA extracts obtained from BPW (lanes 1-2; 5-6) and RVB (lanes 3-4; 7-8) cultures. (L: ladder; 1: positive BPW culture, chicken; 2: negative BPW culture, chicken; 3: positive RVB culture, chicken; 4: negative RVB culture, chicken; 5: positive BPW culture, swab; 6: negative BPW culture,

swab; 7: positive RVB culture, swab; 8: negative RVB culture, swab; N: negative control, KWIK-STIK™ 0335K derived from *E. coli* ATCC® 25922™; P: positive control, KWIK-STIK™ 0363K derived from *S.* Typhimurium ATCC® 14028™; NTC: no template control.)

Parallel testing

To evaluate the comparative efficacy of PCR-based methods for *Salmonella* detection against traditional culture-based techniques, reference samples obtained from the NMIS Central Laboratory were subjected to parallel testing alongside newly collected samples sourced from various markets in Metro Manila. This approach aimed to determine the reliability and accuracy of PCR-based detection for identifying *Salmonella* in diverse matrices. Ninety-five samples (18 beef, 25 chicken, 25 swabs, and 27 feeds) were analyzed using the standard culture-based and PCR-based methods. Overall, 88% (84/95) of samples yielded concordant results between the two methods (Table 1). By matrix, 96% (24/25) of chicken samples, 94.4% (17/18) of beef samples, 68% (17/25) of swab samples, and 96.3% (26/27) of feed samples yielded concordant results. All discordant samples followed the same pattern: they tested *Salmonella*-positive using the PCR-based protocol but *Salmonella*-negative using the standard culture-based protocol.

The lower concordance observed in the environmental swab samples (68%) compared to meat and feed matrices highlights the inherent difficulty of applying conventional culture methods to environmental samples. By the nature of their collection method, swabs often capture lower initial levels of the target organism, which can add to the difficulty of detecting them using standard, culture-based protocols. Discordance in this matrix is likely due to the culture method's failure to detect present *Salmonella* (false negatives) because of two main factors. First, complex environmental matrices present high background microflora, which can competitively inhibit *Salmonella* growth even in selective media. Second, *Salmonella* may enter a viable-but-not-cultivable (VBNC) state due to environmental stresses (e.g., desiccation, sanitizer exposure, or prolonged cold storage). The use of PCR, which detects the target gene (*invA*) irrespective of the cell's cultivability, overcomes these limitations. Numerous comparative studies support this finding, demonstrating that molecular assays consistently yield higher detection rates than culture-based methods in complex environmental samples and food matrices. For instance, studies by Ríos-Castillo et al. (2022) and Hariri (2022) found that PCR significantly increased the detection of *Salmonella* in environmental and food samples, attributing the difference to the culture method's inability to recover VBNC cells efficiently.

Furthermore, Lin et al. (2020) and Patel et al. (2024) concluded that molecular methods offered superior speed and sensitivity for *Salmonella* screening in animal feed production environments. Crucially, the optimized *invA* PCR protocol was independently rated as 'Satisfactory' by a third-party proficiency testing laboratory, affirming its high sensitivity and accuracy in blind, controlled samples across all matrices. This proficiency result provides strong evidence that the 32% discordance in swab samples represents false-negatives from the culture method rather than false-positives from the PCR.

Table 1: Percent concordance of PCR-based and culture-based detection methods for *Salmonella* in different sample matrices

Matrix	Concordance (%)	Discordance (%)
Beef	94.4	5.60
Chicken	96.0	4.00
Swab	68.0	32.0
Animal Feed	96.3	3.70
Total	88.0	12.00

PCR-based *Salmonella enterica* detection for various food and non-food matrices

The PCR-based protocol, adapted from Soguilon-del Rosario and Rivera (2015), targets the *S. enterica*-specific *invA* gene and offers several advantages, including increased sensitivity, specificity, cost-effectiveness, and efficiency. Although this protocol was previously tested only on raw and processed meat samples, it can also be applied to non-meat matrices, such as animal feed and sponge swabs, demonstrating its potential for broader use across diverse sample types. Furthermore, the PCR-based protocol can deliver results within two to three days (assuming no re-analyses are required), which is significantly faster than the minimum five-day turnaround time of the conventional culture-based method. While culture-based protocols are reliable, they tend to be labor-intensive, time-consuming, and expensive, as they require various enrichment and selective media, along with biochemical and serological tests (Zhang et al., 2011). Notably, the PCR-based detection protocol can be further expedited, as DNA extracted from BPW cultures has consistently yielded results comparable to those from RVB cultures. This suggests that *S. enterica* does not require selective cultivation on separate media for reliable detection, and that the PCR-based method can effectively detect the pathogen even in the presence of high levels of background microflora.

Another significant distinction between traditional culture-based methods and modern PCR-based assays lies in their respective levels of identification. In the absence of subsequent serotyping, conventional culture techniques can typically identify *Salmonella* only at the genus level, often resulting in reports that indicate "*Salmonella* spp." While such identification confirms the organism's presence, it does not furnish specific information regarding subspecies or serovars, which are critical for effective epidemiological tracking and public health response initiatives. Conversely, while the specific *invA* PCR assay used in this study is limited to the detection of *S. enterica* species at the species level, molecular methods in general can be tailored for high specificity by targeting genes unique to specific *S. enterica* subspecies. Notably, the DNA extract obtained using this protocol remains available and suitable for subsequent molecular serogrouping and/or serotyping assays, thus maximizing the utility of a single extraction step. This is a significant advantage over conventional methods.

Differences in results from the conventional culture-based and PCR-based protocols during parallel testing may be attributed to the traditional methods' reliance on colony morphology and colorimetric media reactions (BAM, 2024), which can be highly subjective. The test samples were also obtained from retention samples stored at the NMIS Central Laboratory at -20°C for an extended period. Under cold storage conditions, the viability of *Salmonella* is significantly reduced, potentially leading to a transition into a VBNC state (Gruzdev et al., 2012)—this reduction in viability results in lower recovery rates when using conventional culture-based methods.

The PCR-based detection method offers substantial advantages, particularly for testing perishable products, due to its faster turnaround time and straightforward interpretation of results. Another noteworthy advantage of PCR-based methods is their scalability and adaptability. Unlike specific traditional methodologies that yield only qualitative outcomes, PCR has evolved to include quantitative PCR (qPCR), allowing researchers to determine the concentration or load of pathogen DNA within a sample. Such quantitative data is invaluable for food safety assessments and environmental monitoring, as it provides insights into the severity of contamination and informs risk management decisions. Moreover, the molecular framework is particularly amenable to multiplexing, which permits a single assay to detect multiple genes or even distinct microorganisms

simultaneously. For example, a single qPCR assay could be designed to detect *Salmonella*, *E. coli* O157:H7 (Delgado, 2022), and other pathogens concurrently, thereby significantly enhancing throughput and efficiency in high-volume testing environments. This capability to assess multiple targets simultaneously renders molecular methods exceptionally well-suited for comprehensive food safety evaluations and clinical diagnostic panels.

In addition, implementing the validated PCR-based methodology for *S. enterica* detection fulfills its primary objective. It introduces significant ancillary applications, as DNA extracts from the protocol can be stored for extended periods. These nucleic acid samples are invaluable for further molecular characterization, providing critical insights essential to epidemiological investigations and outbreak management. For instance, the DNA extracts can be employed to screen for antibiotic resistance genes, including but not limited to *bla* genes associated with *β*-lactamase production and *tet* genes linked to tetracycline resistance. The identification of such resistance markers is crucial for informed clinical treatment strategies and enhancing public health surveillance related to antimicrobial resistance.

Furthermore, researchers can target virulence gene sequences, particularly those situated within pathogenicity islands, to assess the pathogenic potential of various *S. enterica* strains (Wu et al., 2021). This comprehensive genetic characterization is crucial for differentiating between highly pathogenic strains and those exhibiting lower virulence potential. Moreover, the DNA extracts obtained through these methodologies also enable molecular serotyping and serogrouping, offering a rapid and culture-independent alternative to traditional serological techniques for classifying *S. enterica*. The ability to extract extensive genetic data from a single sample greatly enhances the overall value and applicability of the initial rapid detection technique.

Occurrence of *Salmonella* in commercially available animal feed

In the Philippines, animal feed ingredients must undergo microbiological assessment before entering the production line. These raw materials, especially animal and marine protein sources, as well as milk and whey, should be negative for *Salmonella* (PNS-BAFS 372-2023). However, quality assessment of finished feeds often only includes proximate analysis, evaluation of nutritional value and formulation, and detection of chemical contaminants such as pesticides, heavy metal residues, and different types of toxins (PNS-BAFS 282-2019; PNS-BAFS 194-2022). Microbiological assessment of animal feeds, which is often limited to the detection of *Escherichia coli* and *Salmonella*, is only performed upon request by the feed manufacturer and is not a mandatory prerequisite for release. However, several studies have established a link between salmonellosis and feed contaminated with *Salmonella*. *Salmonella* has been shown to persist in farms and feed mills, with contamination often traceable to high-protein feed ingredients, such as soy and rapeseed. In addition, *Salmonella* can be introduced to feed mills and farms by infected animals, including rodents, birds, pets, human workers, and visitors (EFSA, 2008; Alali & Ricke, 2012; Rönnqvist *et al*., 2017).

In this study, 27 samples of various commercial swine and poultry feeds were purchased from different local agricultural and veterinary stores in Metro Manila. The feeds included starter, grower, and finisher feeds for swine, as well as chick booster, breeder, and high-protein pellets for fowl. In the parallel testing performed, three out of 27 (11.11%) of the sampled feeds tested positive for *S. enterica* using the PCR-based method, while two out of 27 (7.41%) were positive for *Salmonella* spp. using the culture-based method. The positive samples include locally-manufactured breeder pellets, high-protein pellets, and stag developer pellets—all of which are fowl feeds. These breeder and high-protein pellets from the same local brand have crude protein levels of 17% and 22%, respectively. These feeds are formulated with various protein sources, including soybean meal, fish meal, pork meal, and meat and bone meal. Similarly, stag developer pellets under another local brand also have protein sources as ingredients, namely, soybean and rapeseed meal. These protein-rich ingredients may be a source of *a* contamination. Although the *Salmonella* incidence in this study is low, it is of great significance since *Salmonella* contamination can be traced back to feed mills, feed manufacturing plants, and even raw materials, potentially contaminating various batches of finished feeds. On these grounds, it is imperative to include microbiological assessment of finished feeds before commercial release. Acceptance and rejection criteria for finished feeds must be standardized to ensure that they are safe for animal consumption.

CONCLUSION

The PCR-based *S. enterica* detection protocol developed by Soguilon-del Rosario and Rivera (2015) utilizes the *S. enterica*-specific *invA* gene. It has been evaluated as 'Satisfactory' for use in both meat (such as chicken and beef) and non-meat matrices (including feed and sponge swabs). This method is faster than the conventional culture-based technique, which typically requires a turnaround time of about seven days; the PCR protocol produces results in just two to three days. Moreover, this protocol reduces the subjectivity often encountered with conventional culture-based detection methods, such as interpreting colony morphology and color reactions in the media. We recommend that the versatility and usability of the PCR-based detection protocol be tested with additional sample matrices, including milk, dairy products, confectionery, fats, oils, fruits, vegetables, and eggs, to broaden its application across various industries. While PCR-based pathogen detection methods are commonly utilized globally, only a limited number of laboratories in the Philippines have adopted this technology due to budgetary constraints and restricted access to essential equipment and expertise. Therefore, it is imperative to focus efforts on making these advanced diagnostic tools more accessible and affordable throughout the Philippines.

Lastly, given that *S. enterica* has been detected in commercially available animal feed, scaling up sampling and analysis is recommended to better assess its prevalence and incidence, as it poses a threat to both animal and food safety. In foresight, this study could serve as a baseline for developing microbiological assessment protocols and standards for finished feeds in the Philippines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

JM: Conceptualization, Data curation, Formal analysis, Investigation, Writing–original draft, Writing–review & editing. PS: Conceptualization, Formal analysis, Writing–original draft, Writing–review & editing. RM: Formal analysis. WR: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing–original draft, Writing–review & editing.

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