

Application of loop-mediated isothermal amplification to detect *Salmonella* spp. in egg samples

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ABSTRACT

The objective of the study was to evaluate the use of loop-mediated isothermal amplification (LAMP) technique for detection of *Salmonella* spp. in chicken eggs. Eight *Salmonella* strains and 26 non-*Salmonella* enteric strains were used to verify the specificity of the LAMP assay for detection of *Salmonella*. The Sal4 primer set was used to specifically amplify the target sequence on the *invA* gene of *Salmonella* and the hydroxyl naphthol blue was used as the indicator to read the reaction results. Blank egg samples were spiked with serial 10-fold diluted *Salmonella* suspensions to determine the limit of detection at 50% (LOD₅₀) of the assay. Egg samples collected from multiple locations in Ho Chi Minh City were tested for the presence of *Salmonella* using both LAMP method and the traditional culture method to compare the effectiveness of these two methods. Among 34 bacterial strains tested by LAMP assay, false-positive or false-negative results were not observed. The assays of spiked egg samples showed that the LOD₅₀ of the LAMP method was less than 2 CFU/25 g sample. These results indicated a high specificity and sensitivity of the LAMP assay in detection of *Salmonella* in egg samples, even with those of low levels of contamination. Upon testing collected egg samples (n = 42), the LAMP assay produced the same results of *Salmonella* detection as the culture method. *Salmonella* was detected in 4 out of 42 samples. This study showed that the LAMP method is highly effective and would be suitable for use in detection of *Salmonella* in egg samples. The assay has several advantages such as saving time and labor compared to traditional culture methods.

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1. Introduction

Non-typhoidal *Salmonella* is an important zoonotic pathogen relating to food and feed safety concerns. In 2017, there were an estimation of 95.1 million cases of enterocolitis caused by this pathogen worldwide, with 50.771 deaths and 3.1 million cases of disability-adjusted life-years (Stanaway et al., 2019). Most cases of *Salmonella* infection in humans have been associated with the consumption of contaminated eggs, poultry, pork, beef or dairy products (Park et al., 2014;

EFSA & ECDC, 2021). Among them, eggs and egg products are the most important source of *Salmonella* infection (EFSA & ECDC, 2021).

To ensure food safety, it is important to use reliable and effective methods of testing for the presence of *Salmonella* before delivering foods to markets. Usually, *Salmonella* is present in food in low quantities (< 100 CFU/g) along with millions of other bacteria in the sample (Velusamy et al., 2010). Therefore, to detect this bacterium, it is necessary to use a method that has high sensitivity and is not affected by inhibitors in samples.

The conventional culture method instructed by FDA (FDA, 2015) and ISO (ISO, 2017) is recognized as the “gold standard” for the detection of this bacterium in foods. However, this method is labor-intensive, and time-consuming, thus reducing the shelf life of tested foods due to inspecting time (Lin et al., 2020).

Loop-mediated isothermal amplification (LAMP) assay was first introduced by a Japanese research group in 2000 (Notomi et al., 2000). Briefly, in a LAMP assay, 2 - 3 pairs of primers are designed to specifically detect 6 - 8 regions of target gene by using a DNA polymerase with high strand displacement activity (Nagamine et al., 2002). The assay has an efficiently amplified property, which could produce 10^9 copies of the amplified DNA within an hour (Notomi et al., 2000). The technique has been proved to have various advantages such as simplicity, carrying out reactions in an isothermal condition, requiring little of equipment, low cost of chemicals, short processing time, and directly observing of the results by the naked eye (Notomi et al., 2015). Nevertheless, LAMP primers have been said to be a key factor determining success to the assay. Recently, FDA has approved a new set of primers which have been used for detection of *Salmonella* from a variety of food samples including cantaloupe, beef, tomato, etc. (Yang et al., 2016) and animal feeds (Domesle et al., 2020) but eggs. Therefore, this study was conducted to test the applicability of LAMP using these primers to detect *Salmonella* in egg samples.

2. Materials and Methods

2.1. Bacterial strains and culture conditions

The strains used in this study were divided into 2 groups of *Salmonella* (8 strains) and non-*Salmonella* enteric bacteria (26 strains). The strains were either kindly provided by VPH Lab (Faculty of Animal Science and Veterinary Medicine, Nong Lam University HCMC) or isolated from chicken fecal samples in this study (Table 1).

Isolation of *Salmonella* was conducted following instructions by VS (2017). All *Salmonella* strains were confirmed by a slide agglutination test with *Salmonella* O polyvalent antisera (Pasteur Institute, HCMC).

Non-*Salmonella* strains were isolated by cul-

turing freshly collected fecal samples from farm chickens onto MCA agar (MacConkey Agar; Oxoid, CM0115) and/or EMB agar (Eosin Methylene Blue Agar; Oxoid, CM0069). These isolates were roughly identified by the IMViC tests and kept in TSB (Tryptone Soya Broth; Oxoid, CM0129) containing 30% glycerin at -20°C for later use.

2.2. Bacterial enrichment and DNA extraction

Bacterial strains were recovered from frozen stocks by streaking onto MCA plates and incubated at $37^{\circ}\text{C}/24$ h. One typical colony of strain was transferred onto a TSA slant (Tryptone soya agar; Oxoid, CM0131) and incubated at $37^{\circ}\text{C}/24$ h, which was then kept in a refrigerator during the study. For DNA extraction, a loop of bacteria on TSA slant of each strain was transferred into a 1.5 mL Eppendorf tube containing 500 μL of purified NFW (nuclease-free water; Promega, P1197), and vortexed thoroughly to obtain a homogeneous suspension. The sample was centrifuged at 3000 rpm/10 min and the supernatant was gently discarded (washing step). Then, 200 μL of NFW was added, and the precipitate was dissolved by pipetting. The tube was heated in a water bath at $96^{\circ}\text{C}/10$ min, then placed on ice for at least 20 min, and centrifuged at 14000 rpm/10 min. Finally, 100 μL of the supernatant solution (containing DNA) was transferred to a sterilized Eppendorf tube. Bacterial DNA templates were used immediately or stored in a refrigerator for use within 2 - 3 days.

For DNA extraction from egg samples, yolk and white of eggs were pooled into a clean plastic bag, and homogenized by a sterilized glass stick. For each sample, 25 g of egg blend was pre-enriched with 225 mL BPW (Buffered Peptone Water; Himedia, RM001) at 37°C for 16 h. One mL of pre-enrichment sample in BPW was transferred to a sterilized 1.5 mL tube and centrifuge at 3000 rpm/10 min. The supernatant was discarded followed by adding 500 μL of NFW and vortexed thoroughly. Then, samples were washed and extracted DNA as described above.

2.3. *Salmonella*-LAMP assay

The Sal4 primer set (Table 2) and the thermal condition of reactions were adapted from Yang et al. (2016). LAMP reactions were carried out in 200 μL Eppendorf tubes with a total volume

Table 1. Bacterial strains (n = 34) used for optimization of the *Salmonella*-LAMP assay¹

Species/groups	Origin	Numbers of strains	Codes
<i>Salmonella</i> strains	VPH lab, Nong Lam University HCMC	4	NLS001, NLS002, NLS003, NLS004
	From chicken fecal samples in this study	4	NLS005, NLS006, NLS007, NLS008
<i>E. coli</i> strains	VPH lab, Nong Lam University HCMC	1	NLE001
	From chicken fecal samples in this study	9	NLE002 to NLE010
Coliforms	From chicken fecal samples in this study	10	NLC001 to NLC010
<i>Proteus</i> spp.	From chicken fecal samples in this study	3	NLP001 to NLP004
<i>Shigella</i> spp.	From chicken fecal samples in this study	3	SHI001 to SH003

¹LAMP: loop-mediated isothermal amplification.

of 20 μ L including the following reactants: 2 μ L of IAB II, 10X (Isothermal Amplification Buffer; New England Biolabs, M0538S); 1.2 μ L $MgSO_4$ (100 mM); 0.8 μ L BST (*Bacillus stearothermophilus*) 3.0 polymerase (8000 U/mL) (New England Biolabs, M0538S); 2.4 μ L dNTP Mix (25 mM) (New England Biolabs, N0447S); 1.5 μ L dimethyl sulfoxide; 1.8 μ M FIP/BIP primer; 0.1 μ M primer F3/B3; 1.0 μ M Loop-F/B primer (Integrated DNA Technologies); 1.0 μ M HNB (300 mM) (hydroxy naphthol blue; Alpha Chemika, 63451-35-4), 2 μ L of sample DNA extraction and added NFW to 20 μ L. The reactions were performed at 65°C for 45 min using a PCR machine (Benchmark, TC-32). The reaction was stopped at 80°C/10 min.

The result of the LAMP reaction was read based on the color change of the hydroxy naphthol (HNB) indicator. Positive results were obtained when the reaction turned blue color; while, negative samples still remained violet color (Goto et al., 2009).

2.4. Determination of specificity of the *Salmonella*-LAMP

The specificity of the *Salmonella*-LAMP assay was performed with 8 strains of *Salmonella* spp. and 26 strains of non-*Salmonella* bacteria (Table 1). The specificity results of the LAMP assay were verified by the number of reactions that gave positive results with *Salmonella* strains and the number of reactions that gave negative results with non-*Salmonella* strains. In addition,

to interpretation of the color change of each reaction, the LAMP products were electrophoresed on 1.5% agarose gel to confirm the results.

2.5. Determination of sensitivity of *Salmonella*-LAMP

To prepare blank samples, the yolk and white of each egg were homogenized by a sterilized glass stick and 25 g of the egg blend was taken for detection of *Salmonella* by culture following the instructions of VS (2017). Samples from which *Salmonella* was not detected were used as negative control, which were stored in refrigerator at 4°C for using within 3 to 4 days. The suspensions of *Salmonella* strains NLS001 and NLS002 were serial 10-fold diluted to concentrations of 10^0 - 10^4 CFU/mL. One mL of each bacterial dilution was spiked into each of 5 blank samples (25 g egg blend/sample), the suspension was homogenized by shaking sample bags. Each spiked sample was added 225 mL PBW and incubated at 37°C/24 h. DNA was extracted and *Salmonella*-LAMP reactions were performed. The limit of detection at 50% (LOD₅₀) was determined following ISO guidelines (ISO, 2016).

2.6. Detection of *Salmonella* by LAMP versus culture method

Commercial eggs were purchased from 7 locations that are mini-supermarkets and 7 locations that are individual business households at wet markets in Ho Chi Minh City for *Salmonella* de-

Table 2. The Sal4 primers set for *Salmonella*-LAMP¹

Primers	Sequence (5' – 3')	bp
FIP	GCGCGGCATCCGCATCAATATCTGGATGGTATGCCCGG	38
BIP	GCGAACGGCGAAGCGTACTGTCTGCACCGTCAAAGGAAC	38
F3	GAACGTGTCTGCGGAAGTC	18
B3	CGGCAATAGCGTCACTT	18
Loop-F	TCAAATCGGCATCAATACTCATCTG	25
Loop-B	AAAGGGAAAGCCAGCTTTACG	21

¹Yang et al. (2016); LAMP: loop-mediated isothermal amplification.

tection by using both conventional culture and *Salmonella*-LAMP methods. Samples from ten eggs were pooled as one sample for analysis. At each site, three pooled samples were collected. In this study, total 42 pooled samples were used for detecting the presence of *Salmonella*. To collect the egg content, egg shells were disinfected with 1% iodine alcohol solution for 1 minute and dried at room temperature. Ten disinfected eggs were cracked, pooled into a clean plastic bag, and homogenized by a sterilized glass stick. An amount of 25 g of each sample was pre-enriched with 225 mL BPW at 37°C/16 - 24 h. Pre-enriched samples were analyzed for *Salmonella* by both LAMP assay as described in sections 2.2 and 2.3 and culture method as described in VS (2017). Results of *Salmonella* detection from both methods were compared.

3. Results and Discussion

3.1. Specificity of the *Salmonella*-LAMP

All 8 *Salmonella* strains gave positive results with the LAMP assay whereas no color change (negative results) was observed from all 26 samples of non-*Salmonella* strains. This agreed with the summary of the meta-analysis study by Yang et al. (2018) of which many LAMP reactions used for detecting different *Salmonella* strains had specificity rates of 97.4 – 100% with positive control samples, and 100% with negative controls. When electrophoresis was conducted for LAMP products of the control strains, all 8 *Salmonella* strains showed ladder-like pattern of LAMP products and all non-*Salmonella* samples did not have the presence of LAMP products (Figure 1).

3.2. Sensitivity of *Salmonella*-LAMP

Limit of detection at 50% obtained by the *Salmonella*-LAMP on egg samples was less than 2 CFU/25 g (Table 3). This agreed with the results of 10⁰ CFU/25 mL by Yang et al. (2013), and 1.63 - 4.18 CFU/25 g by Hu et al. (2018). Meanwhile, LOD₅₀ obtained by the traditional culture method on the dried egg powder samples (with confidence interval 95%) was 6.0 (4.7 - 7.7) CFU/25 g (the annex attached to ISO (2017) for detecting *Salmonella*). Thus, *Salmonella*-LAMP showed a better detection of *Salmonella* at low levels of contamination in samples comparing to the culture method.

3.3. Efficacy of LAMP assay in detection of *Salmonella* from egg samples

3.3.1. Detection of *Salmonella* in egg samples

A total of 42 pooled egg samples were tested for the presence of *Salmonella* by both LAMP and culture methods, from which the results were similar (Table 4). This would indicate a strong recommendation to use LAMP for detection of *Salmonella* in egg samples over the culture method.

The high similarity in results of *Salmonella* detection by the two methods have been noted in many studies (Zhang et al., 2011; D'Agostino et al., 2016; Hu et al., 2018). Wang et al. (2015) showed similar results obtained by LAMP and culture without and with a pre-enrichment step (89.58% and 100%, respectively). In a recent study (Ge et al., 2019), the comparison of LAMP and culture methods to detect *Salmonella* in multiple laboratories concluded that LAMP method rigorously met the validation of AOAC, FDA, and ISO guidelines. Ge et al. (2019) also proposed the use the LAMP assay to screen samples for *Salmonella* and only those with positive results

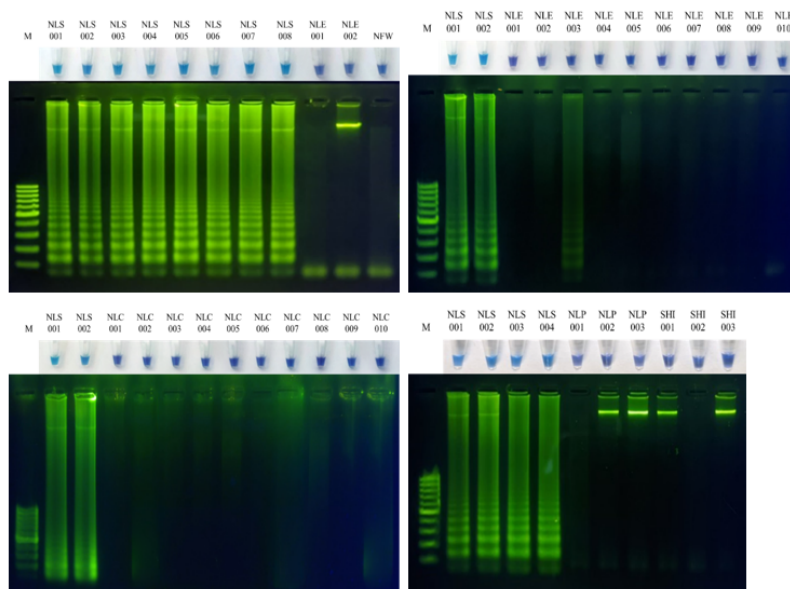


Figure 1. Results of *Salmonella*-loop-mediated isothermal amplification were obtained by hydroxy naphthol blue indicator and electrophoresis. Lane M, 100 bp DNA ladder (ThermoFisher, 15628019); Lane NFW, nuclease-free water; Lanes NLS: *Salmonella* strains; and others were non-*Salmonella* strains.

Table 3. Limit of detection of *Salmonella*-loop-mediated isothermal amplification for egg samples spiked with NLS001 and NLS002 strains

NLS001		NLS002	
Concentration	Positive	Concentration	Positive
0 CFU/25g	0/1	0 CFU/25g	0/1
0.95×10^0 CFU/25 g	4/5	1.14×10^0 CFU/25 g	3/5
0.95×10^1 CFU/25 g	4/5	1.14×10^1 CFU/25 g	5/5
0.95×10^2 CFU/25 g	5/5	1.14×10^2 CFU/25 g	5/5
0.95×10^3 CFU/25 g	5/5	1.14×10^3 CFU/25 g	5/5
LOD ₅₀ (*) (95% confidence interval)	1.91 (0.64 - 5.65)	LOD ₅₀ (*) (95% confidence interval)	0.86 (0.26 - 282)

(*): LOD₅₀ or limit of detection at 50% and 95% confidence interval calculated following the guidance of ISO (2016).

Table 4. Comparison of *Salmonella* detection in egg samples using the *Salmonella*-LAMP (loop-mediated isothermal amplification) and culture methods

		Culture	
		Positive	Negative
LAMP	Positive	100% (4/4)	0%
	Negative	0%	100% (38/38)
Total		4	38

should undergo bacterial isolation. This would reduce time, labor, and increase the efficiency of testing and isolating *Salmonella* from samples.

Salmonella was detected from 4 of 42 egg samples (9.5%), of which 3 samples were from mini-supermarkets and 1 sample was from a wet market. This was higher than those from previous reports. For example, the bacteria were found from 2 out of 52 egg yolk samples taken in Hau Giang province (Tran, 2012), while the bacteria were not detected from 115 egg samples collected from some retailers in Ha Noi (Ha et al., 2017).

Meanwhile, high detection rates (at over 25%) of *Salmonella* from poultry, pork and beef samples have been reported (Luu et al., 2013; Le et al., 2019). In a study in Ho Chi Minh City, 68.4% meat samples were detected with *Salmonella* (Nguyen et al., 2018). These results strongly indicate high prevalence of *Salmonella* presence in products of animal origins in Vietnam.

A report in Europe in 2020 presented low prevalence of *Salmonella* in egg and egg products (0.63%) and in fresh meat samples of all kinds (0.40 - 8.01%) (EFSA & ECDC, 2021). However, outbreaks of human salmonellosis in Europe have been most associated with the consumption of eggs and egg products. In the detail, 44% of 84 salmonellosis outbreaks in Europe in 2020 were associated with eggs and egg products, followed by pork and pork-related products with only 13.1% (EFSA & ECDC, 2021). This shows that although the prevalence of *Salmonella* in eggs is lower than in meat products, the role in food safety of this bacterial contamination in egg samples is of significant importance. Therefore, the high prevalence of *Salmonella* in eggs in this study represents a major health hazard to consumers.

3.3.2. Efficiency of the *Salmonella*-LAMP assay

The LAMP assay for detection of *Salmonella* consumed approximately 20 h, of which most of the time (16 h) was spent on the pre-enrichment phase. Techathuvanan et al. (2012) noted that the limit of detection of *Salmonella* by LAMP for pre-enriched samples at 6 h, 12 h, & 16 h were 10^6 , 10^4 , & 10^0 CFU/25 mL, respectively. Therefore, a pre-enrichment step for 16 h would be optimal to obtain reliable results, especially with samples that have low levels of contamination. In other words, it would take less than a day to get the

results for *Salmonella* detection in food samples using LAMP assay comparing 5 - 7 days by the standard culture method. LAMP is more suitable for testing *Salmonella* in products having a short shelf life such as eggs and ready-to-eat foods (Lin et al., 2020). Furthermore, the assay meets the need of quickly testing pathogens in hatched eggs for breeding in poultry husbandry (Trampel et al., 2014).

4. Conclusions

The *Salmonella*-LAMP assay demonstrated advantages including convenience, simplicity in chemical preparation, and saving time and labor when compared with the standard culture method. Therefore, it would be strongly recommended as an alternative approach for detection of *Salmonella* in egg samples.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

- D'Agostino, M., Robles, S., Hansen, F., Ntafis, V., Ikonopoulou, J., Kokkinos, P., Alvarez-Ordóñez, A., Jordan, K., Delibato, E., Kukier, E., Sieradzki, Z., Kwiatek, K., Milanov, D., Petrović, T., Gonzalez-Garcia, P., Lazaro, D. R., Jackson, E. E., Forsythe, S. J., O'Brien, L., & Cook, N. (2016). Validation of a loop-mediated amplification/ISO 6579-Based method for analysing soya meal for the presence of *Salmonella enterica*. *Food Analytical Methods* 9, 2979-2985. <https://doi.org/10.1007/s12161-016-0602-7>.
- Domesle, K. J., Young, S. R., Yang, Q., & Ge, B. (2020). Loop-mediated isothermal amplification for screening *Salmonella* in animal food and confirming *Salmonella* from culture isolation. *Journal of Visualized Experiments* 159. <https://doi.org/10.3791/61239>.
- EFSA & ECDC (European Food Safety Authority & European Centre for Disease Prevention and Control). (2021). The European union one health 2020 zoonoses report. *EFS A Journal* 19(12), 6971. <https://doi.org/10.2903/j.efsa.2021.6971>.
- FDA (Food and Drug Administration). (2015). *Guidelines for the validation of analytical methods for the*

- detection of microbial pathogens in foods and feeds* (2nd ed.). Washington DC, USA: FDA Foods Program Regulatory Science Steering Committee.
- Ge, B., Domesle, K. J., Yang, Q., Hammack, T. S., Wang, S. S., Deng, X., Hu, L., Zhang, G., Hu, Y., Lai, X., Chou, K. X., Dollete, J. R., Hirneisen, K. A., La, S. P., Richter, R. S., Rai, D. R., Yousefvand, A. A., Park, P. K., Wu, C. H., Eames, T., Kiang, D., Sheng, J., Wu, D., Hahn, L., Ledger, L., Logie, C., You, Q., Slavic, D., Cai, H., Ayers, S. L., Young, S. R., & Pamboukian, R. (2019). Multi-laboratory validation of a loop-mediated isothermal amplification method for screening *Salmonella* in animal food. *Frontiers in Microbiology* 10, 562. <https://doi.org/10.3389/fmicb.2019.00562>.
- Ha, T. T., Ngan, T. P., Huong, T. T. C., & Ha, T. T. C. (2017). Antibiotic resistance of *E. coli* and *Salmonella* isolated from poultry eggs sold at some markets in Ha Noi city. *Vietnam Journal of Agricultural Sciences* 15(6), 770-775.
- Hu, L., Ma, L. M., Zheng, S., He, X., Hammack, T. S., Brown, E. W., & Zhang, G. (2018). Development of a novel loop-mediated isothermal amplification (LAMP) assay for the detection of *Salmonella* ser. enteritidis from egg products. *Food Control* 88, 190-197. <https://doi.org/10.1016/j.foodcont.2018.01.006>.
- ISO (International Organization for Standardization). (2017). *Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. (ISO 6579-1:2017)*. Retrieved from February 20, 2022, from <https://www.iso.org/standard/56712.html>.
- ISO (International Organization for Standardization). (2016). *Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016)*. Retrieved from February 20, 2022, from <https://www.iso.org/standard/54870.html>.
- Le, P. H., Vo, C. M., Nguyen, H. M., Nguyen, T. T., Nguyen, C. T. K., & Bui, H. T. D. (2019). Survey on *Escherichia coli*, *Salmonella* contamination and residues of some antibiotics in pork and chicken meat in some provinces of Southwest region. *Journal of Veterinary Science and Technology* XXVI(7), 47-54.
- Lin, L., Zheng, Q., Lin, J., Yuk, H. G., & Guo, L. (2020). Immuno- and nucleic acid-based current technique for *Salmonella* detection in food. *European Food Research and Technology* 246(8), 373-395. <https://doi.org/10.1007/s00217-019-03423-9>.
- Luu, T. Q., Bui, H. M., & Nguyen, H. V. (2013). Evaluation of the risk of *Salmonella* contamination of pork in Hanoi. *Vietnam Journal of Preventative Medicine* XXIII (4), 10-17.
- Nagamine, K., Hase, T., & Notomi, T. (2002). Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and Cellular Probes* 16(3), 223-229. <https://doi.org/10.1006/mcpr.2002.0415>.
- Nguyen, N. T., Nguyen, V. T. B., Nguyen, C. V., Truong, D. T. Q., Tran, N. T., Tran, H. T. T., Nguyen, N. T. H., Bach, K. T., Vo, H. B., Pham, N. T., Campbell, J., Thwaites, G., & Carrique-Mas, J. (2018). Antimicrobial residues and resistance against critically important antimicrobials in non-typhoidal *Salmonella* from meat sold at wet markets and supermarkets in Vietnam. *International Journal of Food Microbiology* 266, 301-309. <https://doi.org/10.1016/j.ijfoodmicro.2017.12.015>.
- Notomi, T., Mori, Y., Tomita, N., & Kanda, H. (2015). Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. *Journal of Microbiology* 53(1), 1-5. <https://doi.org/10.1007/s12275-015-4656-9>.
- Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* 28(12), 63e. <https://doi.org/10.1093/nar/28.12.e63>.
- Park, S. H., Aydin, M., Khatiwara, A., Dolan, M. C., Gilmore, D. F., Bouldin, J. L., Ahn, S., & Ricke, S. C. (2014). Current and emerging technologies for rapid detection and characterization of *Salmonella* in poultry and poultry products. *Food Microbiology* 38, 250-262. <https://doi.org/10.1016/j.fm.2013.10.002>.
- Stanaway, J. D., Parisi, A., Sarkar, K., Blacker, B. F., Reiner, R. C., Hay, S. I., Nixon, M. R., Dolecek, C., James, S. L., Mokdad, A., Abebe, G., Ahmadian, A., Alahdab, F., Alemnew, B., Alipour V., Bakeshei, F. A., Debalkie, M., Ansari, F., Arabloo, J., Asfaw, E. T., Bagherzadeh, M., Bassat, Q., Belayneh, Y. M. M., Carvalho, F., Daryani, A., Mekonnen, F., Demis, A., Dubey, M., Ejeta, E., Dunachie, S., Eftekhari, A., Fernandes, E., Fard, R. F., Gedefaw, G., Meharie, B. G., Gibney, K. B., Hasanzadeh, A., Hong, L. C., Kasaeian, A., Teklemariam, Z., Molla, A., Malekzadeh, R., Melese, A., Mengistu, D. T., Meštrović, T., Miązowski, B., Mohammad, K. A., Mohammadian, M., Hafshejani, A. M., Nguyen, C. T., Hoang, L., Nguyen, S. H., Legesse, Y., Olagunju, A. T., Olagunju, T. O., Pourjafar, H., Qorbani, M., Rabiee, M., Rabiee, N., Rafay, A., Rezapour, A., Samy, A., Sepanlou, S., Shaikh, M. A., Sharif, M., Shigematsu, M., Tessema, B., Tran, B., Ullah, I., Yimer, E. M., Zaidi, Z., Murray, C. J. L., & Crump, J. (2019). The global burden of non-typhoidal *Salmonella* invasive disease: a systematic analysis for the global burden of disease study 2017. *The Lancet Infectious Diseases* 19(12), 1312-1324. [https://doi.org/10.1016/S1473-3099\(19\)30418-9](https://doi.org/10.1016/S1473-3099(19)30418-9).
- Techathuvanan, C., & D'Souza, D. H. (2012). Reverse-transcriptase loop-mediated isothermal amplification as a rapid screening/monitoring tool for *Salmonella* enterica detection in liquid whole eggs. *Journal of Food Science* 77(4), M200-M205. <https://doi.org/10.1111/j.1750-3841.2011.02601.x>.
- Trampel, D. W., Holder, T. G., & Gast, R. K. (2014). Integrated farm management to prevent *Salmonella* enteritidis contamination of eggs. *The Journal of Applied Poultry Research* 23(2), 353-365. <https://doi.org/10.3382/japr.2014-00944>.

- Tran, B. N. (2012). The prevalence of *Salmonella* infection in waterfowl and waterfowl products in Hau Giang province. *Can Tho University Journal of Science* 23a, 235-242.
- Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K., & Adley, C. (2010). An overview of food-borne pathogen detection: In the perspective of biosensors. *Biotechnology Advances* 28(2), 232-254. <https://doi.org/10.1016/j.biotechadv.2009.12.004>.
- VS (Vietnam Standards). (2017). *Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp (TCVN 10780-1:2017)*. Retrieved from February 20, 2022, from <https://luattrongtay.vn/ViewFullText/Id/2bbf2643-3fc2-4724-83d5-823e5f3baeef>.
- Wang, D. G., Brewster, J. D., Paul, M., & Tomaszula, P. M. (2015). Two methods for increased specificity and sensitivity in loop-mediated isothermal amplification. *Molecules* 20(4), 6048-6059. <https://doi.org/10.3390/molecules20046048>.
- Yang, Q., Chen, S., & Ge, B. (2013). Detecting *Salmonella* serovars in shell eggs by loop-mediated isothermal amplification. *Journal of Food Protection* 76(10), 1790-1796. <https://doi.org/10.4315/0362-028X.JFP-13-140>.
- Yang, Q., Domesle, K. J., & Ge, B. (2018). Loop-mediated isothermal amplification for *Salmonella* detection in food and feed: Current applications and future directions. *Food-borne Pathogens and Disease* 15(6), 309-331. <https://doi.org/10.1089/fpd.2018.2445>.
- Yang, Q., Domesle, K. J., Wang, F., & Ge, B. (2016). Rapid detection of *Salmonella* in food and feed by coupling loop-mediated isothermal amplification with bioluminescent assay in real-time. *BMC Microbiology* 16, 112. <https://doi.org/10.1186/s12866-016-0730-7>.
- Zhang, G., Brown, E.W., & Gonzalez-Escalona, N. (2011). Comparison of realtime PCR, reverse transcriptase real-time PCR, loop-mediated isothermal amplification, and the FDA conventional microbiological method for the detection of *Salmonella* spp. in produce. *Applied and Environmental Microbiology* 77(18), 6495-6501. <https://doi.org/10.1128/AEM.00520-11>.