THE EVALUATION OF BROTH-MEDIA EFFICIENCY AND RELATIVE RISK ASSOCIATED WITH *SALMONELLA* IN CULLED LAYERS AND THEIR ENVIRONMENT

Raja Arief Deli Raja Nasharuddin
Food Science & Technology Research Centre, MARDI Headquarters,
Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia
Email: del@mardi.gov.my

Syahrin Suhaimee
Socio-Economy, Market Intelligence and Agribusiness Research Centre,
MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia
Email: syahrin@mardi.gov.my

ABSTRACT

Salmonellosis is a type of food poisoning that has been implicated in many foodborne outbreaks globally, characterized by acute gastroenteritis with short incubation period, and is caused by Gram negative bacteria belonging to the genus Salmonella. Our preliminary studies have shown the overall prevalence of Salmonella was 14.0% (66/470) from 470 culled layers including environmental samples (feed, faeces, litter and drinking water) isolated from culled layers from farms and wet market in Penang and Kedah, Malaysia. Following to that, we were interested to investigate the efficiency of several enrichment broths and selective media used during the experiment. Interestingly, the isolation of Salmonella was observed more effective with higher isolation rate at 66.7% and 57.6% from Rappaport-Vassiliadis (RV) enrichment broth and Xylose Lysine Tergitol-4 (XLT-4) selective media, respectively. In addition, the incorporation between Salmonella prevalence data from previous study and relative risk (RR) value for culled layers and their environment was also evaluated to determine risk factors. In this study, RR value was calculated based on the ratio of incidence between layer farms and wet market to indicate risk of disease more likely or less likely to occur. From the prevalence study, there was a significant difference between farms and wet market ($p<0.05$) and RR value was found higher at wet market comparable to farms. Therefore, the exposure assessment tracks the pathogen from farm to the point of retail within this study, taking into account RR value. Our findings provide clearer perspective on the most efficient method of Salmonella isolation for detection accuracy. Furthermore, this study indicated baseline information on the distribution of Salmonella relative risk in culled layers and environment to form a basis for risk assessment and future interventions.

Keywords: *Salmonella*, efficiency, relative risk, culled layers, environment.

1.0 INTRODUCTION

The genus *Salmonella* consist of 2,463 serovars of *Salmonella* that divides into *S. enterica* and *S. bongori* (Tindall *et al*., 2005). From this, non-typhoidal *Salmonella* has continued to be the most frequently associated causes of foodborne illness such as gastroenteritis, fever, nausea, vomiting including death (Coburn *et al*., 2007), after *Campylobacter* species (Mead *et al*., 1999). *Salmonella* may inhabit the gastrointestinal tract, ovary, oviduct and the rearing environments. Various researchers have recorded foods of poultry origin as majority sources of *Salmonellas* such as broilers (Rusul *et al*., 1996; Zhu *et al*., 2014; Wang *et al*., 2015), layers especially older layers (Castellan *et al*., 2004; Namata *et al*., 2008, Raja Arief Deli & Adzitey, 2017) including poultry-based foods (Arumugaswamy *et al*., 1995; Modaressi & Thong, 2010). For culler layers (*Gallus gallus*), it is defined as the removal of the non-laying or low-producing hens from laying flocks, normally 24 to 36 months old after completing 2 laying cycles (Rahman & Shanmugavelu, 2008). Market demand for culled layers is favored not only as live or dressed chickens but also as secondary products such as chicken *rendang* (Malay traditional cuisine) and ayami (surimi-like processed chicken) (Babji & Song, 1994). Based on Malaysia AgroFood In Figures (2021), the local production of chicken meat are apparently 1,583,210.5 metric tonnes worth millions of ringgit in agriculture and food security.

Nonetheless, there has been continuous efforts among scientists relating to the need for recommended enrichment broth and selective media for recovering of *Salmonella* from poultry, essentially for diagnostic and intervention purposes (Srijan *et al*., 2015). The possibility of false-positive results is technically possible due to the overgrowth of nuisance organisms that could affect the recovery of a desired specific organism or species. Therefore, broth-media selection is essential to differentiate *Salmonella* spp. from other members of the *Enterobactericiae* in food products, hence the use selective broth medium and chromogenic media is suggested (Rambach, 1990; Ruiz *et al*., 1996). In addition, WHO (2012) has reported risk assessment as the scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazard. The systematic process of microbial risk assessment can be qualitative or quantitative (Lammerding & Fazil, 2000) and various reviews regarding the relative importance of different food handling practices and associated risks have been summarized (Griffith, 2000). Therefore, the evaluation of risk relative value from the data obtained from prevalence study is essential in understanding the dynamics of *Salmonella* persistence from farm to retail level. Thus, the application of risk assessment to minimize cross contamination of *Salmonella* in poultry meat has seen a significant research (Oh *et al*., 2023).

Presently, the information on the broth-media efficiency and relative risks of *Salmonella* in culled layers in Malaysia is still fragmented. Therefore, the aim of the present study was to evaluate the efficiency of several enrichment broths and selective media used for *Salmonella* isolation in culled layers including their environment. In addition, this study also focused on the

evaluation of risk relative value to indicate the routes of transmission of *Salmonella* for better implementation of effective preventive measures.

2. MATERIALS AND METHODS

2.1 Sampling methods

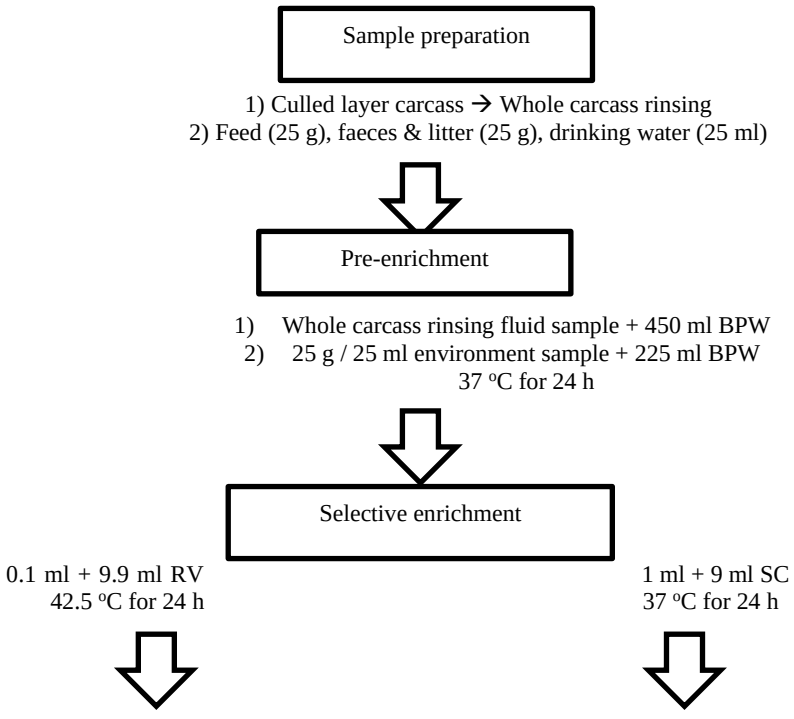
A total of 470 samples were collected from layers' farms and wet market in Penang and Kedah, Malaysia. For this purpose, 150 culled layers were taken at random from three different small-scale open-house farms with capacity of 7,000 to 10,000 birds per farm that consisted of seven to ten houses, and 50 culled layers were sampled randomly from wet market. The total samples were culled layers (200), environmental such as feed (90), faeces and litter (90) and drinking water (90). All samples were transported in a polystyrene box containing ice and analysed immediately upon reaching the laboratory. Samples were analysed for the presence of *Salmonella* by a modified method from the Food and Drug Administration-Bacteriological Analytical Manual (US-FDA, 2007).

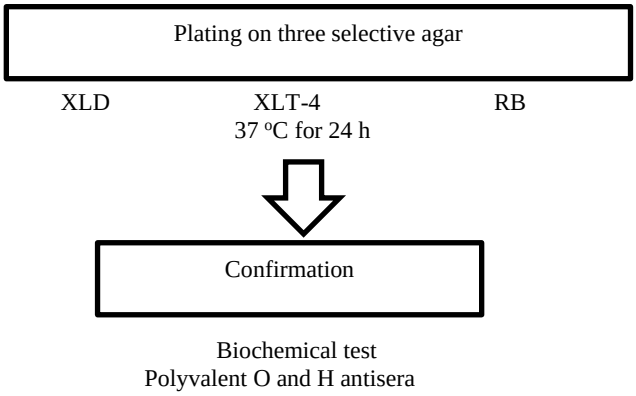
For culled layer samples, each bird was manually slaughtered and defeathered, and evaluated for *Salmonella* by using whole carcass rinsing technique (Cox *et al*., 1981). Each carcass was placed in a sterile plastic bag containing 450 ml of sterile buffered peptone water (BPW) (Merck, Germany) as the diluent and shaken for 2 min. The carcass was suspended to allow the diluent to drain back into the plastic bag and the diluent was incubated at 37 ºC for 24 h ± 2 h. For environmental samples, feed was collected from feed bins and conveyer belts. Faeces and litter (mixed) were collected from floors using sterile spoons, whereas drinking water was collected from the nipple drinker using sterile universal bottles. An amount of 25 g of feed, faeces and litter and 25 ml of drinking water were pre-enriched in 225 ml of BPW, respectively at 37 ºC for 24 h ± 2 h.

2.2 Isolation and identification of *Salmonella*

An amount of 1 ml and 0.1 ml of the pre-enriched samples were transferred into 9 ml of Selenite Cystine enrichment (SC) broth (Merck, Germany) and 9.9 ml of Rappaport-Vassiliadis (RV) (Merck, Germany), and were incubated at 37 ºC and 42 ºC, respectively for 24 h ± 2 h. After enrichment, one loop of RV and SC broth cultures were streaked on Xylose Lysine Deoxycholate agar (XLD) (Merck, Germany), Xylose Lysine Tergitol-4 agar (XLT-4) (Oxoid, UK) and Rambach agar (RB) (Merck, Germany) were incubated at 37 ºC for 24 h to 48 h ± 2 h. Isolated colonies that showed typical reactions (XLD and XLT-4; dark red colonies with black centre, RB; bright red colonies) according to manufacturer's instruction were considered as presumptive *Salmonella* and purified by streaking onto nutrient agar plates (Merck, Germany). Well-isolated colonies were Gram stained and submitted to the following biochemical test; catalase, cytochrome oxidase, triple sugar iron, lysine iron, urease, indole and motility test. The biochemical test materials were obtained from Merck, Germany. *Salmonella* was confirmed by using polyvalent O and H antisera (BD, Franklin Lakes, USA) according to the Bacteriological Analytical Manual (US-FDA 2007). *Salmonella* isolates were stored in 5% glycerol plus Tryptic Soy Broth (TSB) (Merck, Germany) at -18 ºC. Protocol for isolation and identification of *Salmonella* in culled layers and environment is summarized as Figure 1.

Figure 1. Flowchart for the isolation and identification of *Salmonella*





2.3 Relative risk (RR) evaluation

In this study, RR analysis of *Salmonella* was performed by a method modified from Murchie *et al* (2008) and Bouzidi *et al* (2012) to screen the risk factors of culled layers including their environment from layer farms to wet market. Qualitative exposure assessment approach was taken to assess the safety of culled layers with the combination input from *Salmonella* risk factors. As outlined by Lammerding & Fazil (2000), the link between the organism in food matrix and adverse human health effects must be identified prior to the risk assessment being undertaken. Thus, *Salmonella* was identified as the microorganism concerned in the present study with related to food as identified as culled layers. Within this framework, the exposure assessment tracks the *Salmonella* from farms to the point of retail (wet market), taking into account the RR values and summary of the currently available literature. Chap (2003) defined RR as the ratio of incidence of disease in exposed individuals to the incidence of disease in non-exposed individuals as presented below:

$$RR = \frac{\text{Disease incidence in group 1}}{\text{Disease incidence in group 2}}$$

The relation of health risk among one group with the risk among another group was investigated by measuring group 2 as standard conditions such as non-exposure to a certain risk factor against group 1 as exposed conditions. To evaluate this, RR value of 1 means there is no difference in risk between the two groups. A relative risk greater than 1 (RR > 1) indicates harmful effects or means the event is more likely to occur in group 1 than in group 2 (the control group), or increased risk of disease among those that have been exposed. RR value below than 1 (RR < 1) indicates beneficial effects or means the event is less likely to occur in group 1 as comparable to group 2, or decreased risk of disease among those that have been exposed. Combining these types of pathogen data from isolation and prevalence study leads to risk factors association of *Salmonella* contamination in culled layers and their environment.

2.4 Statistical analysis

All experimental data were analyzed using the one-way Analysis of Variance (ANOVA) for the purpose to measure overall significance of collected samples. Differences among means of samples by location, enrichment broth, plating media and environment were determined by Duncan Multiple Range Test (DMRT) at $p < 0.05$ using the Statistical Analysis System (SAS, 2011) computing program.

3. RESULTS AND DISCUSSION

3.1 Broth-media efficiency for isolation of *Salmonella*

In this study, the efficiency of enrichment broths and selective plating media for the isolation of *Salmonella* is presented in Table 1. Isolation of *Salmonella* in Rappaport-Vassiliadis (RV) broth was significantly higher ($p < 0.05$) as 44 (66.7%) out of 66 positive samples were isolated after enrichment, compared to SC (Selenite Cystine) broth as 22 (33.3%) out of 66 positive samples, respectively. This might be explained by the synergistic effects of the presence of malachite green and magnesium chloride in RV concurrent with samples' incubation at 42 °C in inhibiting the growth of Gram positive bacteria and other indigenous microflora. The findings of this study was supported by Vassiliadis (1983); Valentine-Bon *et al* (2003); Singh *et al* (2010) that observed higher isolation rate from RV broth in comparison to Tetrathionate (TT) broth. The authors reported *Salmonella* isolates grew luxuriantly better in RV whereby TT indicated relatively slow and sometimes not appreciable results, even after longer period of incubation. Furthermore, Rall *et al*. (2005) also recommended RV broth medium for *Salmonella* recovery from low and highly contaminated food.

Our study also revealed that the isolation of *Salmonella* from culled layers in Xylose Lysine Tergitol-4 (XLT-4) agar (57.6%) was significantly higher ($p < 0.05$) followed by Rambach (RB) (24.2%) and Xylose Lysine Desoxycholate (XLD) agar (18.2%). These results are similar to El-Sheriff Amal & Elmossalami (1998) compared the use of Rambach agar to detect *Salmonella* excelled over the use of XLD agar in meat products. The study also reported 33.3% and 83.3% false negative cases for raw meat

products and ready-to-eat meat products, respectively on XLD agar plates. In addition, Miller & Tate (1990) observed XLT-4 media significantly improved the recovery of *Salmonella* from chicken and farm environmental drag swab samples. According to Dusch & Altwegg (1995), XLT-4 media had a nearly 100% specificity and clinically usable for stool samples screening of non-typhoid *Salmonella* Comparatively, other selective media such as Hektoen Enteric (HE), Rambach (RB), SM-ID medium (SM), XLT-4, Novobiocin-Brilliant Green-Glycerol-Lactose (NBGL) agar were also examined in the study by Dusch & Altwegg (1995).

Overall, our study underlines a better understanding on the selection of enrichment broth and selective media that can ensure the highest possible recovery of *Salmonella*. This is particularly essential when overgrowth of *Proteus*, *Pseudomonas* and *Citrobacter* in isolation media can dramatically interfere with the detection of *Salmonella*. As many selective agar media have been developed, not one is considered perfect. For instance, XLD contains xylose that differentiates *Salmonella* and other enteric pathogens from *Shigella* species because *Shigellas* are unable to ferment xylose to produce acid (Anonymous, 2011a). RB contains nutritive substrates that enable *Enterobactericeae* to multiply readily, sodium desoxycholate to inhibit the growth of accompanying Gram positive bacteria, chromogene to differentiate *Salmonella* from coliform (Anonymous, 2011b). In addition, XLT-4 contains enzymatic digest of animal tissue as the source of complex nitrogen compounds together with yeast extract, xylose, lactose, sucrose, lysine, ferric ions, sodium thiosulfate, sodium chloride, phenol red and tergitol 4 (Miller & Tate, 1990). Therefore, our study of RV enrichment broth in combination with XLT-4 selective media showed the most efficient results, thus recommended for the isolation of *Salmonella* in culled layers and environment. Noteworthy, the outcome of the broth-media efficiency evaluation compliments with the prevalence study showed in Table 2 by Raja Arief Deli & Adzitey (2017).

Table 1. Efficiency of enrichment broths (RV & SC) and selective plating media (XLD, XLT-4 & RB) for the isolation of Salmonella from culled layers and their environment

Location	Number of positive samples (% efficiency)				
	Enrichment broth		Plating media		
	RV	SC	XLD	XLT-4	RB
Farm 1	16 / 23 (69.6)^{aA}	7 / 23 (30.4)^{aB}	6 / 23 (26.0)^{aB}	12 / 23 (52.2)^{bA}	5 / 23 (21.7)^{aB}
Farm 2	12 / 18 (66.7)^{aA}	6 / 18 (33.3)^{aA}	4 / 18 (22.2)^{aB}	9 / 18 (50.0)^{bA}	5 / 18 (27.8)^{aB}
Farm 3	6 / 9 (66.7)^{aA}	3 / 9 (33.3)^{aA}	1 / 9 (11.1)^{aB}	8 / 9 (88.9)^{aA}	1 / 9 (11.1)^{bB}
Wet market	10 / 16 (62.5)^{aA}	6 / 16 (37.5)^{aB}	1 / 16 (6.3)^{aC}	9 / 16 (56.2)^{bA}	5 / 16 (31.3)^{aB}
Overall	44 / 66 (66.7)^{A}	22 / 66 (33.3)^{B}	12 / 66 (18.2)^{B}	38 / 66 (57.6)^{A}	16 / 66 (24.2)^{B}

Statistical analysis was done separately between enrichment broths and selective plating media (% efficiency)

Means followed by the superscript of the same lower case letter in the same column were not significantly different from each other
($p > 0.05$)

Means followed by the superscript of the same upper case letter in the same row were not significantly different from each other ($p > 0.05$)

Table 2. Prevalence of Salmonella in culled layers and environmental samples

Location	Number of positive samples (%)				
	Culled layers	Environment			Total
	Carcass	Feed	Faeces and litter	Drinking water	
Farm 1	11 / 50 (22.0)^{ba}	6 / 30 (20.0)^{a}	7 / 30 (23.3)^{a}	0 / 30 (0.0)	24 / 140 (17.1)^{a}
Farm 2	9 / 50 (18.0)^{ba}	5 / 30 (16.7)^{ab}	5 / 30 (16.7)^{a}	0 / 30 (0.0)	19 / 140 (13.6)^{ba}
Farm 3	6 / 50 (12.0)^{b}	1 / 30 (3.3)^{b}	1 / 30 (3.3)^{a}	0 / 30 (0.0)	8 / 140 (5.7)^{b}
Wet market	15 / 50 (30.0)^{a}	NA	NA	NA	15 / 50 (30.0)^{a}
Overall	41 / 200 (20.5)^{A}	12 / 90 (13.3)^{BC}	13 / 90 (14.4)^{BAC}	0 / 90 (0.0)	66 / 470 (14.0)

NA = Not available

Means followed by the superscript of the same lower case letter in the same column were not significantly different from each other
($p > 0.05$)

Means followed by the superscript of the same upper case letter in the same row were not significantly different from each other ($p > 0.05$)

3.2 Relative risk analysis of *Salmonella* in culled layers and environment

As summarized in Table 2, *Salmonella* was isolated from 66 samples among 470 samples with the overall prevalence of 14.0%. The prevalence for *Salmonella* was highest in carcass rinse (20.5%), followed by faeces and litter (14.4%) and feed (13.3%). Table 3 and Figure 2 indicated that carcass obtained from all three layer farms (combined) were less affected by *Salmonella* than carcass at wet market because the RR value was less than 1 (RR = 0.58). This also showed that the likelihood of *Salmonella* doubled from farms to wet market. The higher prevalence of *Salmonella* in samples from the wet market could be attributed to the stress induced during transportation of live layers to retail chain in overcrowded baskets. Stress has already been shown to have an

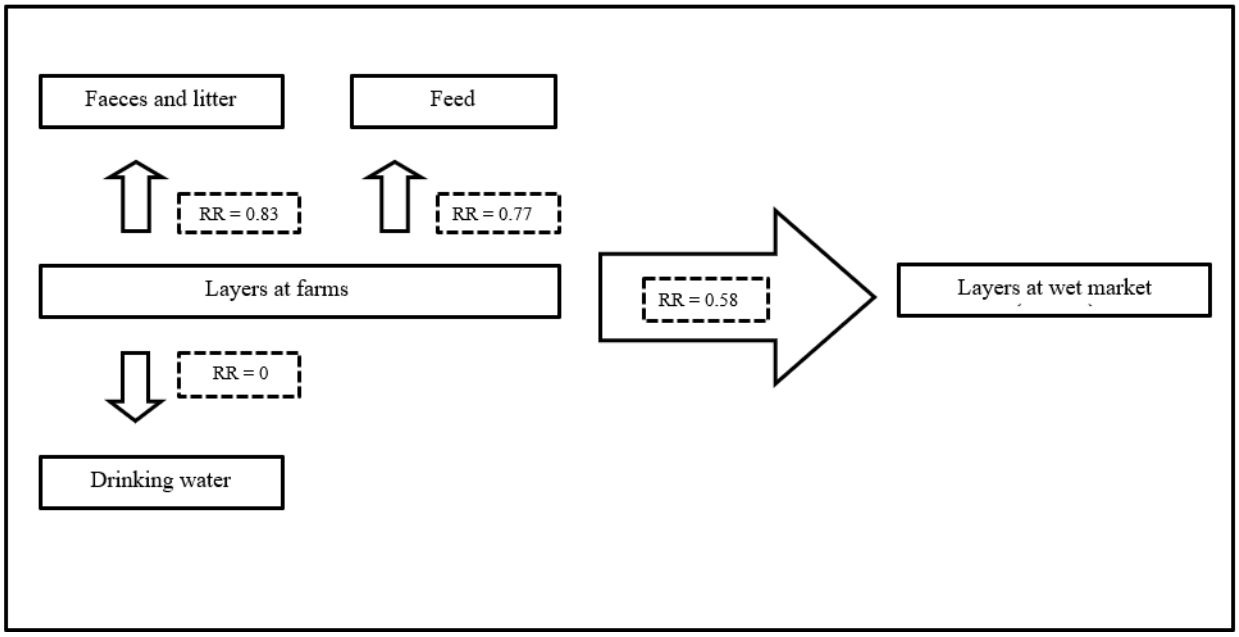
immunosuppressive effect in chickens (El-lethey *et al*., 2003; Humphrey, 2006). Cross contamination of *Salmonella* can also occur during scalding and plucking of the culled layers at the wet market during processing steps such as dipping of chicken in hot water and defeathering of chickens by machines (Hafez *et al*., 1997; Shirota *et al*., 2001). For environmental samples, the likelihood of *Salmonella* was highest in faeces and litter (RR = 0.83) followed by feed (RR = 0.77) with no harmful effects likely to occur from drinking water (RR = 0). This could be explained by the relatively high prevalence of faeces and litter from previous literature (Bailey *et al*., 2001; Liljeljebke *et al*., 2005) and the ability of *Salmonella* to survive for a prolong periods under dry conditions such as feed (Whyte *et al*., 2003; Adzitey & Nurul, 2011). In contrast, *Salmonella* was rarely reported in water-borne outbreaks despite it being frequently detected in surface waters (Levantasi *et al*., 2012).

From the joint analysis (Figure 2), our results showed that there are multiple factors that could influence *Salmonella* contamination in culled layers and their environment at different likelihood of RR values. However, it is worth mentioning that the RR values can vary remarkably from one country to another depending on local production practices. Principally, the evaluation of microbial risks can help in control strategies for decision makers in minimizing the health consequences of *Salmonella*. Kelly *et al* (2003) reported that research might not be necessary to develop a full farm-to-fork assessment, if resources are not available, thus a simpler approach may suffice. Risk relative evaluation relies heavily on the availability of local valid data and informed scientific knowledge. Our study provided a preliminary risk-based approach to gain insights on *Salmonella* interventions by comparing culled layers and environment dataset with coherent categorization of food safety issues. Proactive measures are likely to be taken by authority to reduce the transmission of *Salmonella* both vertically and horizontally for better refinement of national government policy.

Table 3. Relative risk evaluation for *Salmonella* in culled layers and environment

Group	Location	Prevalence (%)	Risk ratio	RR values
Culled layers	Exposure pathway: Farms to wet market			
Carcass	Wet market	30.0	Control group	
	All farms	17.3	17.3 / 30.0	0.58
Environment	Exposure pathway: Farms only			
Carcass	Farm 1	22.0	Control group	
	Farm 2	18.0		
	Farm 3	12.0		
	All farms	17.3		
Feed	Farm 1	20.0	20.0 / 22.0	0.91
	Farm 2	16.7	16.7 / 18.0	0.93
	Farm 3	3.3	3.3 / 12.0	0.28
	All farms	13.3	13.3 / 17.3	0.77
Faeces and litter	Farm 1	23.3	23.3 / 22.0	1.06
	Farm 2	16.7	16.7 / 18.0	0.93
	Farm 3	3.3	3.3 / 12.0	0.28
	All farms	14.4	14.4 / 17.3	0.83
Drinking water	Farm 1	0	0 / 22.0	0
	Farm 2	0	0 / 18.0	0
	Farm 3	0	0 / 12.0	0
	All farms	0	0 / 17.3	0

Figure 2. Exposure pathway with relative risk value associated with Salmonella in culled layers and environment



4. CONCLUSIONS

In summary, our analysis observed that the isolation of *Salmonella* from culled layers and environment in RV broth (66.7%) was significantly better than SC broth (33.3%). Following to that, our study revealed the positivity of XLT-4 agar (57.6%) was significantly higher than RB (24.2%) and XLD (18.2%), respectively. Moreover, the prevalence and risk relative (RR) evaluation showed the likelihood of *Salmonella* doubled from farms to wet market. For environmental samples, the likelihood of *Salmonella* was highest in faeces and litter, followed by feed with much lesser risk from drinking water. Our results established better understandings on the best isolation methods for *Salmonella* and local perspective of relative risk associated with *Salmonella* in culled layers and their environment. Data can compliment local authorities in implementing better government policy in foodborne pathogens control.

ACKNOWLEDGEMENTS

The authors are grateful to Food Science & Technology Research Centre at MARDI and School of Industrial Technology, Universiti Sains Malaysia for their overall laboratory, technical and financial support during the study.

REFERENCES

Adzitey, F. & Nurul, H. (2011) Salmonellas, poultry house environments and feeds: a review. *Journal of Animal and Veterinary Advances*, 105(5), 679-685.

Anonymous (2011a) Xylose lysine deoxycholate (XLD) agar. Available at: http://www.pmlmicro.com/assets/TDS/815.pdf.

Anonymous (2011b) Differential-diagnostic culture medium for identifying non-typhi *Salmonella* in foodstuffs and clinical samples. Available at: http://85.238.144.18/analytics/Micro_Manual/TEDISdata/prods/1_07500_0003.html.

Arumugaswamy, R.K., Rusul, G., Abdul Hamid, S.N. & Cheah, C.T. (1995) Prevalence of *Salmonella* in raw and cooked foods in Malaysia. *Food Microbiology*, 12, 2-8.

Babji, A. S. & Song, K.G. (1994) Changes in sarcoplasmic and myofibrillar proteins of spent hen and broiler meat during the processing of surimi-like material (ayami). *Journal of Tropical Agriculture*, 17(2), 117-123.

Bailey, J.S., Stern, N.J., Fedorka-Cray, P., Craven, S.E., Cox, N.A., Cosby, D.E., Ladely, S. & Musgrove, M.T. (2001) Sources and movement of *Salmonella* through integrated poultry operations: A multistate epidemiological investigation. *Journal of Food Protection*, 64, 1690-1697.

Bouzidi, N., Aoun, L., Zaghdoudi, M., Bensouilah, M., Elgroud, R., Oucief, I., A. Granier, S., Brisabois, A., Desquilbet, L. & Millemann, Y. (2012). *Salmonella* contamination of laying-hen flocks in two regions of Algeria. *Food Research* International, 45, 897-904.

Castellan, D.M., Kinde, H., Kass, P.H., Cutter, G., Breitmeyer, R.E. & Bell, D.D. (2004). Descriptive study of California egg layer premises and analysis of risk factors for *Salmonella* enterica serotype *Enteritidis* as characterized by manure drag swabs. *Avian Diseases*, 48, 550-561.

Chap, T. L. (2003). Introductory Biostatistics. New Jersey, USA. Wiley Interscience. p.18-19.

Coburn, B., Grass, G.A. & Finlay, B.B. (2007) *Salmonella*, the host and disease: A brief review. *Immunology and Cell Biology*, 85, 112-118.

Cox, N.A., Thomson, J.E. & Bailey, J.S. (1981) Sampling of broiler carcasses for *Salmonella* with low volume water rinse. *Poultry Science*, 60, 768-770.

Dusch, H. & Altwegg, M. (1993) Comparison of Rambach agar, SM-ID medium and Hektoen Enteric agar for primary isolation of non-typhi *Salmonella* from stool samples. *J. Clin. Microbiol*, 31, 410-412.

El-Lethey, H., Huber-Eicher, B. & Jungi, T. W. (2003) Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses. *Veterinary Immunology and Immunopthology* 95, 91-101.

El-Sherif Amal, M. & Elmossalami, M.K. (1998). Rambach agar as a new plate differential medium for the identification of some enteric pathogens in meat products. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, 207, 160-163.

Griffith, C.J. (2000) Food safety in catering establishments. In: Farber, J.M. & Todd, E.C. (eds). *Safe handling of foods*, New York, USA: Marcel Dekker.

Hafez, H. M., Stadler, R. & Kosters, J. (1997) Surveillance of *Salmonella* in turkey flocks and processing plants. *Deutsche Tieraerztliche Wochenschrift*, 104, 33-35.

Humphrey, T. (2006) Are happy chickens safer chickens? Poultry welfare and disease susceptibility. *Poultry Science*, 47, 379-391.

Kelly, L. A., Hartnett, E., Gettinby, G., Fazil, A., Snary, E. & Wooldridge, M. (2003). Microbiological safety of poultry meat: risk assessment as a way forward. *World's Poultry Science Journal*, 59, 495-507.

Lammerding, A. & Fazil, A. (2000). Hazard identification and exposure assessment for microbial food safety risk assessment. *International Journal of Food Microbiology*, 58, 147-157.

Levantesi, C., Bonadonna, L., Briancesco, R., Grohmann, E., Toze, S. & Tandoi, V. (2012) *Salmonella* in surface and drinking water: Occurrence and water-mediated transmission. *Food Research International*, 45, 587-602.

Liljebjelke, K.A., Hofacre, C.L., Liu, T., White, D.G., Ayers, S. & Young, S. (2005) Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. *Foodborne Pathology and Diseases*, 2, 90-102.

Malaysia Agrofood in Figures (2021) Main information of chicken and eggs (2016-2021). Table 3.6.1. p.80. *Ministry of Agriculture and Food Industries*.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S. & Shapiro, C. (1999) Food-related illness and death in the United States. *Emerging Infectious Disease*, 5, 607-625.

Miller, R.G. & Tate, C.R. (1990) XLT-4: A highly selective plating medium for the isolation of *Salmonella*. *Maryland Poultryman*, April: 2-7.

Murchie, L., Xia, B., Madden, R. H., Whyte, P. & Kelly, L. (2008) Qualitative exposure assessment for *Salmonella* spp. in shell eggs produced on the island of Ireland. *International Journal of Food Microbiology*, 125, 308-319.

Modaressi, S. & Thong, K.L. (2010) Isolation and molecular sub typing of *Salmonella* enterica from chicken, beef and street foods in Malaysia. *Scientific Research and Essays*, 5(18), 2713-2720.

Namata, H., Meroc, E., Aerts, M., Faes, C., Cortinas Abrahantes, J., Imberechts, H. *et al*. (2008) *Salmonella* in Belgian laying hens: An identification of risk factors. *Preventive Veterinary Medicine*, 83, 323-336.

Oh, H., Yoon, Y., Yoon, J.W., Oh, S.W., Lee, S., Lee, H. (2023) *Salmonella* risk assessment in poultry meat from farm to consumer in Korea. *Foods*, 12, 649.

Raja Arief Deli, R.N. & Adzitey, F. (2017) Prevalence and antibiotic resistance of *Salmonella* serovars isolated from spent hens and its environmental samples in Penang and Kedah, Malaysia. *J. Trop. Agric. and Fd. Sc.*, 45(1), 37-50.

Rall, V.L.M., Rall, R., Aragon, L.C., Silva, M.G. (2005) Evaluation of three enrichment broths and five plating media for *Salmonella* detection in poultry. *Brazillian Journal of Microbiology*, 36, 147-150.

Rambach, A. (1990) New plate medium for facilities differentiation of *Salmonella* spp. from *Proteus* spp. and other enteric bacteria. *Appl. Environ. Microbiol*., 56, 301-303.

Rahman, M.Y. & Shanmugavelu, S. (2008) Economic production. In: Chicken Layers Farming, 2nd Edition, p.84, MARDI Serdang.

Ruiz, J., Nunez, M.L., Diaz, J., Lorente, I., Perez, J. & Gomez, J. (1996) Comparison of five plating media for isolation of *Salmonella* species from human stools. *J. Clin. Microbiol*., 34, 686-688.

Rusul, G., Khair, J., Son, R., Cheah, C.T. & Yassin, R.M. (1996) Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia. *International Journal of Food Microbiology*, 33, 183-194.

SAS Institute (2011). SAS® Users Guide: Statistics Version 9.4 Cary. North Carolina, USA: SAS Institute Incorporated.

Shirota, K., Katoh, H., Murase, T., & Otsuki, K. (2001) Monitoring of layer feed and eggs for *Salmonella* in Eastern Japan between 1993 and 1998. *Journal of Food Protection*, 64, 734-737.

Singh, S., Yadav, A.S., Singh, S.M. & Bharti, P. (2010) Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Research International*, 43, 2027-2030.

Srijan, A., Wongstitwilairoong, B., Bodhidhatta, L., Mason, C. (2015) Efficiency of plating media and enrichment broths for isolating *Salmonella* species from human stool samples: A comparison study. *Open Journal of Medical Microbiology*, 5, 231-236.

Tindall, B.J., Grimont, P.A.D., Garrity, G.M. & Euzeby, J.P. (2005) Nomenclature and taxonomy of the genus *Salmonella*. *International Journal of Systematic and Evolutionary Microbiology*, 55, 521-524.

US-FDA. (United States-Food and Drug Administration)(2007) Bacteriological Analytical Manual Chapter 5: *Salmonella*. Available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm.

Valentine-Bon, I.E., Brackett, R.E., Sco, K.H., Mammock, T.S. & Andrews, W.H. (2003) Pre-enrichment versus direct selective agar plating for the detection of *S*. Enteritidis in shell eggs. *Journal of Food Protection*, 66(9), 1670-1674.

Vassiliadis, P. (1983) The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of *Salmonella*: An overview. *J. Appl. Bacteriol*., 54, 69-76.

Wang, Y., Yang, B., Cui, Y., Alali, W.Q., Xia, X., Xi, M., Wang, X., Shi, X., Wang, D. & Meng, J. (2015) Subtyping of *Salmonella* isolates on retail raw chicken in China by pulsed-field gel electrophoresis and plasmid analysis. *Food Control*, 47, 420-426.

WHO (World Health Organization)(2012) About risk analysis in food. Geneva, Switzerland: WHO. Available at: http://www.who.int/foodsafety/micro/riskanalysis/en.

Whyte, P., McGill, K., Collins, J.D. (2003) A survey of the prevalence of *Salmonella* and other enteric pathogens in a commercially poultry feed mill. *Journal of Food Safety*, 23, 13-24.

Zhu, J., Wang, Y., Song, X., Cui, S., Xu, H., Yang, B., Huang, J., Liu, G., Chen, Q., Zhou, G., Chen, Q. & Li, F. (2014) Prevalence and quantification of *Salmonella* contamination in raw chicken carcasses at the retail in China. *Food Control*, 44, 198-202.