Occurrences of *Salmonella* spp. and *Escherichia coli* in chicken meat, intestinal contents and rinse water at slaughtering place from traditional market in Surabaya, Indonesia

R Yulistiani¹², D Praseptiangga³, Supyani⁴, Sudibya⁵

¹ Doctoral Program of Agricultural Science, Graduate School Program of Sebelas Maret University (UNS), Jalan Ir. Sutami 36 A, Kentingan, 57126, Surakarta, Indonesia
² Department of Food Technology, Faculty of Engineering, Universitas Pembangunan Nasional Veteran Jawa Timur, Jalan Raya Rungkut Madya Gunung Anyar 60294, Surabaya, Indonesia
³ Department of Food Science and Technology, Faculty of Agriculture, Sebelas Maret University (UNS), Jalan Ir. Sutami 36 A, Kentingan 57126, Surakarta, Indonesia
⁴ Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University (UNS), Jalan Ir. Sutami 36 A, Kentingan 57126, Surakarta, Indonesia
⁵ Department of Animal Husbandry, Faculty of Agriculture, Sebelas Maret University (UNS), Jalan Ir. Sutami 36 A, Kentingan 57126, Surakarta, Indonesia

Email: ratnayulistiani@yahoo.co.id

Abstract. The purpose of this study was to compare the presence of *Salmonella* spp. and *Escherichia coli* in chicken meat, intestinal contents and rinse water at slaughtering place from traditional markets in Surabaya, Indonesia. A total of 120 samples (chicken meat, intestinal contents and rinse water, each of the 40 samples) were collected from the slaughtering place from traditional markets in Surabaya. The samples were analyzed for the presence of *Salmonella* spp. and *Escherichia coli* in chicken meat, intestinal contents and rinse water. Isolation and identification *Salmonella* spp. and *Escherichia coli* were carried out according to the conventional culture-based method (ISO 6579:2002). This study showed *Salmonella* spp. was isolated by 85.00%, 57.50%, 52.50% of chicken meat, intestinal contents and rinse water, respectively. While *E. coli* was isolated by 77.50%, 65.00%, 75.00% of chicken meat, intestinal contents and rinse water, respectively. Prevalence of *Salmonella* spp. and *E. coli* in chicken meat is higher than intestinal contents and rinse water. Thus, it is indicated that the contamination of *Salmonella* spp. and *Escherichia coli* in chicken meat not only comes from intestinal contents and rinse water.

1. Introduction
The process of poultry slaughtering includes the following phases: stunning and bleeding, scalding, defeathering, evisceration, rinsing/washing and chilling [1]. Chicken carcasses have higher
pathogenic and spoilage bacteria counts than most other foods, where carcass can be contaminated at several points of processing operations such as during scalding, defeathering and evisceration as well as cross contamination from other birds and processing equipment. During chicken slaughtering, carcass can be contaminated with fecal matters from the chicken intestine.

Contamination of poultry meat with foodborne pathogens is an important public health problem, where many food poisoning bacteria contaminate chicken meat [2]. *Salmonella* spp. and *Escherichia coli* (*E. coli*) causes health problems in the world because they are the two most important foodborne pathogens of *Enterobacteriaceae* family as a cause of foodborne illness which transmitted through poultry meat. *Salmonella* is the second most common cause of foodborne illness, it is responsible for millions of cases of foodborne illness every year [3]. *Salmonella* could infect and caused food poisoning in humans through the handling of raw carcasses and their products, or through the consumption of undercooked poultry meat [4]. *E. coli*, a natural inhabitant of the human intestinal tract and warm-blooded animals, is used as an indicator bacterium because these bacteria acquires antimicrobial resistance faster than other conventional bacteria [5]. According to [6], diarrheal diseases caused by *E. coli* account for more than 4% of the total daily global disease burden every day and about 1.8 million deaths occur every year, of which 90% are children.

Food safety is an issue of growing public health concern [7], especially foodborne diseases associated with the consumption of poultry meat and its processed products [8]. During and after slaughtering, the bacteria from animal microbiota, the slaughterhouse environment, and the equipment used contaminate carcasses, their subsequent cuts, and processed meat products. Carcasses and cuts after animal killing could be contaminated by animal microbiota and the slaughterhouse environment [9]. Some of these bacterial contaminants can grow or survive during food processing and storage. Previous research [10], indicated that *Salmonella* spp. and *E. coli* found in chicken meat at traditional markets in Surabaya were resistant to some antibiotics. Thus, it is important to evaluate the source of *Salmonella* spp. and *E. coli* contamination at slaughtering place from traditional market. The purpose of this study was to compare the presence of *Salmonella* spp. and *Escherichia coli* in chicken meat, intestinal contents and rinse water at slaughtering place from traditional market in Surabaya, Indonesia.

2. **Experimental**

A total of 120 samples (chicken meat, intestinal contents and rinse water, each of 40 samples) was collected from chicken slaughtering places at traditional market locations in Surabaya, Indonesia. The collected samples were packed in a sterile plastic bag, transferred directly to the laboratory in the ice box for an immediate bacteriological testing.

The chicken meat, intestinal contents and rinse water samples were processed immediately for isolation and identification of *Salmonella* and *E. coli*. Isolation and identification *Salmonella* spp. from all samples were carried out according to the horizontal method [11] with modification. From each sample (25 gram of chicken meat/intestinal content samples or 25 ml rinse water samples) were pre-enriched in 225 ml buffered peptone water (BPW, Oxoid), and incubated at 37°C for 24 h. One ml of BPW pre-enrichment step transferred to 10 ml Selenite Cystine Broth (SCB, Oxoid) for selective enrichment, and incubated at 37°C for 24 h. A loopful of inoculum from selective enrichment broth was streaked onto Xylose-Lysine Deoxycholate (XLD) agar plate and incubated at 37°C for 24 h. For confirmation, single colonies that growing on the XLD agar surface with specific criteria for *Salmonella* spp. (pink colonies with black centers) was evaluated to gram staining and biochemical tests (TSI and IMViC test) were carried out [12].

Isolation and identification *E. coli* from all samples were carried out according to ISO 6579 : 2002. From each sample (25 gram of chicken meat/intestinal content samples or 25 ml rinse water samples) were chopped aseptically and homogenized with 225 ml of 0.1% Buffered Pepton Water (BPW, Oxoid); and then incubated at 37°C for 24 h. A loop full pre-enrichment broth was streaked on MacConkey agar plates (MCA, Oxoid) and incubated at 37°C for 24 h. For confirmation, single
colonies with pink colour colonies on the MacConkey agar surface (specific criteria for *E. coli* spp.) was evaluated to gram staining and biochemical tests (TSI and IMViC test) were carried out [12]. The flow diagram in this research as shown in Figure 1.

![Flow Diagram](image-url)

Figure 1. The flow diagram of chicken slaughtering in traditional markets with detailed description of *Salmonella* spp. dan *E. coli* sampling point

3. Results and Discussion

| Bacteria          | Biochemical test | Motility | Indole production test | Methyl-red | Voges-Proskauer test | Simmon’s citrate test |
|-------------------|------------------|----------|------------------------|------------|----------------------|-----------------------|
| *Salmonella spp.* | A/A gas +, H₂S+ or K/A, or K/A gas + or K/A, H₂S or K/A gas, H₂S | +        | -                      | +          | -                    | ±                     |
| *Escherichia coli*| A/A, or A/A gas + or K/A, or K/A gas + | +        | +                      | +          | -                    | -                     |

Notes: K, Alkali (red) ; A, Acid (yellow)
+ , positive; - negative ; ±, positive/negative
Salmonella spp and E. coli isolates were obtained met the criteria for microscopic examination (short rod form, gram negative) and positive biochemical test results (TSI and IMViC) for Salmonella spp and E. coli (Table 1). TSI (Triple Sugar Iron) tests were carried out to check their ability to ferment glucose, lactose and sucrose sugars, gas and H2S production. The IMViC test includes Indole production, Methyl Red, Voges-Proskauer, and Simmon Citrate utilization [13] [14].

Table 2. The prevalence (%) of Salmonella spp. and E. coli in chicken meat, intestinal contents and rinse water

| Bacteria            | Prevalence (%) | Chicken meat (n=40) | Intestinal contents (n=40) | Rinse water(n=40) |
|---------------------|----------------|---------------------|----------------------------|-------------------|
| Salmonella spp.     |                | 34(85.00)           | 23(57.50)                  | 21(52.50)         |
| Escherichia coli    |                | 31(77.50)           | 25(65.00)                  | 30(75.00)         |

Notes: n = sample numbers collected

As shown in Table 2 and Figure 2, the prevalence of Salmonella spp. 34 (85.00 %) in chicken meat was higher than Escherichia coli 31 (77.50 %). High prevalence of Salmonella in chicken meat, not only indicating poor sanitation conditions during slaughtering but also indicates the health status of poultry as carriers of Salmonella [15], where poultry is the most important reservoir of Salmonella [16]. The unhygienic handling during poultry slaughtering and processing of chicken meat using unclean equipment and contaminated water were the risk factors associated with the presence of Salmonella with chicken meat due to cross contamination [17]. Poor sanitary and hygiene conditions and high humidity at slaughterhouses are ideal for the formation of Salmonella biofilms. This biofilm can last for a long time and tends to protect Salmonella from sanitizers, so it is very risky to cross contamination of Salmonella in chicken meat at the slaughterhouse [18].

Poultry food products are important sources of E. coli, because at the time of slaughter, faecal contamination from the intestines contaminated the carcass. As a result, poultry meat can be contaminated with fecal material or ingesta and with bacteria associated with these contaminants [19]. Poultry carcasses can be contaminated with intestinal contents which E. coli can spill out as a contaminant. Ref. [20] found the prevalence of E. coli in healthy broiler chickens was 88%, 38% and 25%, respectively, from the samples of caecum, ileum and duodenum (intestinal contents). Broiler chicken meat, intestinal contents and chicken carcasses are the main sources of E. coli contaminants in meat and warm-blooded animals. Presence of E. coli in meat is a good indicator of fecal contamination [21].

Figure 2. The comparison of Salmonella spp. and E. coli in chicken meat, intestinal contents and rinse water.
Our study (Figure 2), demonstrated Salmonella spp. was isolated by 85.00%, 57.50%, 52.50% of chicken meat, intestinal contents and rinse water, respectively. While E.coli was isolated by 77.50%, 65.00%, 75.00 % of chicken meat, intestinal contents and rinse water, respectively. Evisceration is the first stage of the clean part of the slaughter process. It is consisting of several stages, evisceration starts with head removal followed by the opening of the body cavity, removal of intestines, and ends with the cleaning of the carcass [22]. From the hygienic point of view, attention is paid to the removal of the intestines and the prevention of cross-contamination with faecal material. Prevalence of Salmonella spp. and E. coli in chicken meat are higher than intestinal contents and rinse water. Thus, it indicates that contamination of Salmonella spp. and Escherichia coli in chicken besides come from the intestinal contents and rinse water, but also can come from others, such as equipment surfaces, animal microbiota and the slaughtering place environment. During slaughtering, bacterial contamination may occur from equipment surfaces, water, and animal microbiota. Chicken carcasses can be contaminated by the slaughterhouse environment [9].

4. Conclusion
The presence of Salmonella spp. and Escherichia coli in chicken meat are higher than intestinal contents and rinse water. Thus, it is indicated that the contamination of Salmonella spp. and Escherichia coli in chicken meat not only comes from intestinal contents and rinse water. The need of implementing a stricter hygiene and sanitation standard in slaughtering place to reduce the incidence of Salmonella and E. coli.

References
[1] Escudero-Gilete ML, Gonzalez-Miret ML and Heredia FJ 2005. Multivariate study of the decontamination process as function of time, pressure and quantity of water used in washing stage after evisceration in poultry meat production. J. Food Eng. 69 245-51.
[2] Mbata TI 2005. Poultry meat pathogens and its control. Internet J Food Safety 7 20-28.
[3] FSIS 2008. FSIS Issues Public Health Alert for Frozen, Stuffed Raw Chicken products.
[4] Panisello PJ, Rooney R, Quantick PC and Stanwell-Smith R 2000. Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. Int. J. Food Microbiol. 59 221-234.
[5] Miranda, JM, Vázquez BI, Fente CA, Barros-Velázquez J, Cepeda A and Franco CM 2008. Evolution of resistance in poultry intestinal Escherichia coli during three commonly used antimicrobial therapeutic treatments in poultry. Poult. Sci. 87 1643-1648.
[6] WHO 2004. The World Medicines Situation. Geneva, Switzerland:World Health Organization
[7] Nata Menabde. 2015. Message from WHO representative to India on World Health Day 2015. http://www.searo.who.int/india/about/who_representative
[8] Bhaisare DB, Thyagarajan D, Churchil RR and Punniamurthy N 2014. Bacterial pathogens in chicken meat: Review. Int J Life Sci Res 2 1-7.
[9] Firildak G, Asan A and Goren E 2015. Chicken carcasses bacterial concentration at poultry slaughtering facilities. Asian J. Biol. Sci 8 16-29
[10] Yulisatiani R, Praseptianaga D, Raharjo D, Supyani, Sudibya and Shirakawa, T 2017. Prevalence of antibiotic-resistance Enterobacteriaceae strains isolated from chicken meat at traditional markets in Surabaya, Indonesia. IOP Conference Series: Materials Science and Engineering 193 (2017) 012007. IOP Publishing. doi:10.1088/1757-899X/193/1/012007
[11] International Organization for Standardization (ISO) 2002. Microbiology of Food and Animal Feeding Stuffs : Horizontal Method for the Detection of Salmonella spp. ISO 6579 : 2002. Geneva, Switzerland.
[12] Roy SR, Rahman MB, Hassan J and Nazir KNH 2012. Isolation and identification of bacterial flora from internal organs of broiler and their antibiogram studies. Microbes and Health 1 72-75.
[13] Morello JA, Granato PA and Mizer HE 2002. Laboratory manual and workbook in microbiology. Applications to patient care. 7th Ed. ISBN :0-07-246354-0. WCB/McGraw-Hill.

[14] Paiao FG, Arisitides LGA, Murate LS, Vilas-Bôas GT, Vilas-Boas LA and Shimokomaki M 2013. Detection of Salmonella spp, Salmonella enteritidis and typhimurium in naturally infected broiler chickens by a multiplex PCR-based assay. Braz. J. Microbiol. 44 37-42

[15] Shah AH and Korejo NA 2012. Antimicrobial resistance profile of Salmonella serovars isolated from chicken meat. J. Vet. Anim. Sci. 2 40-46

[16] Balakrishnan S, Sangeetha A and Dhanalakshmi M 2018. Prevalence of Salmonella in chicken meat and its slaughtering place from local markets in Orathanadu, Thanjavur district, Tamil Nadu. J. Entomol Zool. Stud 6 2468-2471

[17] Nidaullah, H., Abirami N, Shamila-Syuhada AK, Chuah LO, Nurul H, Tan TP, Abidin FWZ and Rusul G 2017. Prevalence of Salmonella in poultry processing environments in wet markets in Penang and Perlis, Malaysia. Vet. World 10 286 – 292

[18] Smith DP, Northcutt JK, Cason JA, A. Hinton JR, Buhr RJ and Ingram KD 2007. Effect of external or internal faecal contamination on numbers of bacteria on prechilled broiler carcasses. Poult. Sci. 86 1241–1244

[19] Seidavi A, Mirhosseini SZ, Shivazad M, Chamani M, Sadeghi AA and Pourseify R 2010. Detection and investigation of Escherichia coli in contents of duodenum, jejunum, ileum and cecum of broilers at different ages by PCR. Asia Pac. J. Mol. Biol. Biotechnol 18 321-6

[20] Mead GC 2000. Fresh and Further-Processed Poultry. In: The Microbiological Safety and Quality of Food, Lund BM, Baird-Parker TC and Gould GW(Eds.). Vol. 1, Chapter 20, Aspen Publication, Gaithersburg, MD., USA., ISBN-13: 9780834213234, pp: 445-471

[21] Cox JM and Pavic A 2010: Advances in Enteropathogen control in poultry production. J. Appl. Microbiol. 108 745-755

[22] Rouger A, Tresse O and Zagorec M 2017. Bacterial contaminants of poultry meat: sources, species, and dynamics. Microorganisms 5 50