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SALMONELLA DETECTION IN DIFFERENT TYPES OF PACKED RAW POULTRY MEAT BY CULTURE ELISA AND PCR METHODS

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ABSTRACT

Chicken meat is one of the most widely consumed nutrients worldwide. This study aimed to investigate the presence of Salmonella spp. in raw chicken meats by culture, ELISA, and PCR methods. A total of 200 raw chicken meat samples (80 whole chicken carcasses, 60 drumsticks, and 60 wings) were randomly collected from the butchers, supermarkets, and restaurants. The samples were analyzed by culture technique, ELISA, and PCR methods. Salmonella spp. was isolated from 34 of 200 (17 %) chicken meat samples, and 83 % were found negative. Salmonella spp. was isolated from the whole chicken carcass, drumstick, and wing samples as 15 %, 20 %, and 16.6 %, respectively. All samples were also investigated by ELISA and PCR at the same time. According to these results, Salmonella spp. was detected 13% and 16%, respectively. The high prevalence of Salmonella contamination of raw chicken meats may constitute a potential public health risk. To reduce contamination of Salmonella spp., it was stated that good manufacturing practices, good hygiene practices, and HACCP-based quality assurance systems should be applied.

KEYWORDS:
Raw chicken meat, *Salmonella* spp., ELISA, PCR

INTRODUCTION

Salmonella species are among the critical foodborne and environmental bacterial pathogens in the world [1, 2]. Today, many foods pose a risk due to the increase in fast-food consumption habits [3]. The contamination of poultry products with *Salmonella, Campylobacter* and *L. monocytogenes* are significant for public health, as it can lead to significant zoonoses [2, 4, 5, 6]. *Salmonella* agents are serotypes included in species belonging to the genus Salmonella in the Enterobacteriaceae family. They are gram-negative, non-spore forming, and motile microorganisms in the form of short rods with flagella except *S. Pullorum* and *S. Gallinarum*. The infectious *Salmonella* strains belong to the *S. enterica subsp. enterica* subgroup, and this group includes quite a few serotypes [5, 7].

Salmonella serotypes cause various infections in poultry. Pullorum disease caused by S. enterica subsp. enterica serovar Pullorum and fowl typhoid infection caused by *S. enterica subsp. enterica serovar Gallinarum* is an acute disease. These diseases cause significant economic losses by causing loss of egg yield and chick death [8, 9]. Since *Salmonella* species, which are essential for public health, are intracellular bacteria and the development of multiple antibiotic resistance in recent years. *Salmonella* control programs have increased in every stage of poultry production [10].

S. enterica subsp. enterica serovar Typhimurium and *S. enterica subsp. enterica serovar Enteritidis*, the most isolated bacteria from food poisoning caused by poultry products, cause paratyphoid infections in poultry and have a zoonotic character [5]. Usually, the asymptomatic enteric form of the paratyphoid disease that forms colonization in the intestinal tract is observed chiefly. This situation may cause carcass contamination in broilers and contamination of the eggshell in layers or agglomeration in their contents [5]. Regarding microbial safety of foods, many tests are developed and applied to detect Salmonella species that cause paratyphoid infections in poultry products [11].

Microbiological, molecular, and immunological methods are used for Salmonella spp. detection in raw chicken samples so far [4]. The culture method, which is accepted as the gold standard in diagnosing Salmonella spp., is still widely used to isolate Salmonella spp. from various sources. Polymerase Chain Reaction (PCR) has found widespread use in recent years due to its fast, simple, and highly specific nature. On the other hand, lateral flow assay and ELISA-based methods for antigen detection are frequently used in the food industry [1, 4, 12].

Various studies have been conducted in many countries to determine the prevalence of Salmonella spp. in raw chicken meats [14, 15, 16, 17]. However, very few studies investigate the presence of Salmonella spp. in raw chicken meats sold in Turkey. Therefore, the objective of this study was to investigate the occurrence of Salmonella spp. in raw chicken meats by culture, ELISA, and PCR methods.

MATERIALS AND METHODS

Sample collection. In the present study, 200 packed raw chicken meat samples (80 whole chicken carcasses, 60 drumsticks, 60 wings) were randomly collected from the butchers, supermarkets, and restaurants between January and August 2020 Aksaray province, Turkey. All raw chicken meat samples were immediately transferred to the laboratory under a cold chain then analyzed on the same day to detect *Salmonella* species.

Isolation and identification of *Salmonella* species. Under aseptic conditions, 25 g of raw chicken meat sample was transferred to sterile polyethylene bags containing 225 mL of buffered peptone water (Oxoid, Hampshire, UK) and homogenized by stomacher (Stomacher400, France) for 5 min. Subsequently, samples were incubated at 37 °C for 24 h for pre-enrichment. Then, 1 mL of homogenate was transferred into tubes containing 10 mL Tetrathionate broth (Oxoid, Hampshire, UK), and it was incubated at 37 °C for 24 h. After the enrichment process, 0.1 mL of the enriched samples were streaked onto duplicate plates of Xylose Lysine Deoxycholate agar (Oxoid, Hampshire, UK) and incubated at 37 °C for 48 h. The suspected colonies were selected and subcultured onto Trypticase Soy Agar (Oxoid, Hampshire, UK). For identification, Gram staining, characteristic colony morphology, and biochemical tests were performed [18]. After identification, *Salmonella* spp. isolates were stored at -80 C° in Brain Heart Infusion broth (Oxoid, Hampshire, UK) containing 15% of glycerol.

PCR. Genomic DNA of *Salmonella* isolates from chicken meat samples were extracted using the protocol provided in Vivantis tissue DNA purification Kit (Vivantis, Malaysia). The template DNAs were stored at -80 °C until the process of amplification.

The *Salmonella*-specific primer pairs were used in PCR for amplification of the IS200 element gene of Salmonella spp. [19]. The sequence of forward primer was 5'-CGATGAAAGCGTAAATAAGG-3' and reverse primer was 5'-TCTCTTGTCAGTCTCAAAC-3'. Specific primers showed a single amplicon which size was 200 bp.

The PCR mixture was prepared in a total volume of 50 µL containing 5 µL of 10 X PCR buffer, 0.2 µM each of the four dNTPs (Vivantis, Malaysia), 1.5 mM MgCl2, 0.5 µM of each primer (IDT, USA), 1.25 U of Taq DNA polymerase (Vivantis, Malaysia), and 5 µL of template DNA. The amplification was performed in a thermal cycler (Biorad gradient, T100) with the following steps: 1 × 3 min at 94 ºC, 30 × 30 s at 94 ºC, 30 s at 51.6 ºC, 60 s at 72 ºC, and a final extension at 72 ºC for 5 min. S. enterica subsp. enterica serovar Typhimurium (ATCC 13311) was used as the positive control, and nuclease-free water was used as the negative control.

The PCR products (10 µL) were then analyzed by electrophoresis on 2% agarose gel (Applichem, A2114), and the gel was stained with ethidium bromide (1.5 µg/mL) and photographed.

ELISA. Salmonella spp. were detected using an ELISA kit (Ridascreen Salmonella, R-Biopharm, GmbH, Darmstadt, Germany). The test was performed according to the manufacturer's instructions. A 25 g of raw chicken meat sample was transferred to sterile polyethylene bags containing 225 mL of buffered peptone water (Oxoid, Hampshire, UK) and homogenized using a stomacher (Stomacher400, France) for 5 min. It was incubated at 37 °C for 24 h for pre-enrichment, and this homogenate was then used in the ELISA test. The positive and negative kit control process and a known positive meat sample were run in each assay. Optical density values were read with an ELISA reader spectrophotometer (ELX800, Bio-Tek Inst Inc USA).

RESULTS

During this work, 80 whole raw chicken, 60 raw chicken drumstick, and 60 raw chicken wings samples were analyzed. A total of 34 (17%) Salmonella spp. were isolated from 200 raw chicken meat samples. All *Salmonella* isolates were confirmed as Salmonella spp. by PCR assay. 166 (83%) of all samples were found negative. Salmonella spp. were isolated from 12 of 80 raw whole chicken samples, 12 of 60 raw chicken legs samples, and 10 of 60 raw chicken wings samples by culture method (Table 1).

Salmonella spp. DNA was directly detected from 32 of 200 raw chicken meat samples by PCR. The PCR products were obtained by agarose gel electrophoresis (Figure 1). All PCR-positive samples were found to be culture-positive. In addition, 12 of 80 raw whole chicken samples, 10 of 60 raw chicken legs samples, and 10 of 60 raw chicken wings samples were found to be PCR positive (Table 1).

Salmonella spp. antigen was detected in 26 (13%) out of 200 raw chicken meat samples by ELISA technique. Of the 26 positive samples, ten were from the whole raw chicken, eight were from raw chicken legs, and eight were from raw chicken wings. The remaining 174 (87%) raw chicken meat samples were negative for Salmonella spp. antigen by ELISA assay (Table 1).

According to our results, the presence of *Salmonella* was detected at 34 %, 32 %, and 26 % by classic culture, PCR, and ELISA, respectively. In this direction, the classic culture technique was found the most selective method in the present study.

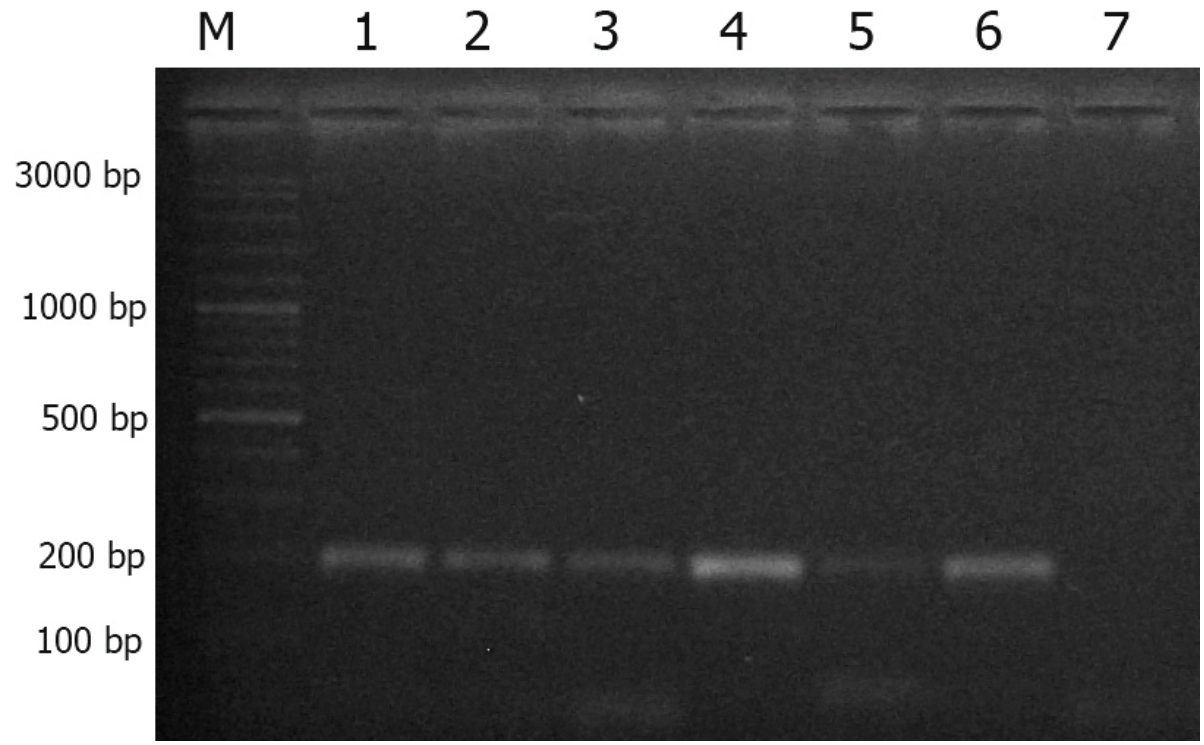

FIGURE 1
Detection of *Salmonella* spp. from samples by PCR. Line M, molecular weight marker (Gene RulerTM 100 bp DNA Ladder Plus, Fermentas); Line 1–5: *Salmonella* spp. positive samples, line 6: positive control for *Salmonella* spp. (ATCC 13311, 200 bp), line 7: negative control.

TABLE 1
The distributions of *Salmonella* spp. in raw chicken meat samples.

Samples	Number of sample	ELISA		Culture		PCR	
		Positive	Negative	Positive	Negative	Positive	Negative
Raw whole chicken	80	10	70	12	68	12	68
Raw chicken legs	60	8	52	12	48	10	50
Raw chicken wings	60	8	52	10	50	10	50
Total	200	26	174	34	166	32	168

DISCUSSION

In terms of human nutrition, chicken meat contains more protein and essential amino acids than red meat. Poultry meat is widely consumed globally due to its high digestibility and biological value [20]. Poultry carries a high risk of contamination due to many different processes in the slaughterhouse process. In addition, poultry carcasses provide a suitable environment for the growth of many bacteria due to their rich content [21]. Microorganisms found in internal poultry organs, intestines, skin, and feathers can easily contaminate poultry meat and pose a serious threat to consumer health by causing poultry meat to deteriorate [22]. In the present study, raw chicken samples collected from commercial products offered for sale in supermarkets, butchers, and restaurants were analyzed in the laboratory. The presence of Salmonella spp. was investigated by culture, ELISA, and PCR methods.

In our research, 80 raw whole chicken carcasses, 60 raw chicken drumsticks, and 60 raw chicken wings samples were analyzed by culture technique. A total of 34 (17 %) Salmonella spp. was isolated from 200 raw chicken meat samples. The percentage of *Salmonella* contamination in various poultry meat samples was observed between 2% to 52% in previous studies in Turkey [23, 24, 25, 26, 27]. In a study conducted in the Bursa region of Turkey, 300 raw chicken meat samples were examined for the presence of Salmonella spp. by culture technique, and 17.3% of the samples were reported as positive [24]. In another study, a total of 150 samples, 75 of which were raw whole chicken carcasses and 75 of which were raw chicken meat pieces, were analyzed in the Samsun region of Turkey, and Salmonella spp. was isolated at a rate of 44.6% [26]. Two hundred raw chicken samples, consisting of 50 whole carcasses, 50 wings, 50 drumsticks, and 50 breast meat obtained from Afyonkarahisar, Turkey, were evaluated by culture technique. It was reported

that Salmonella spp. was isolated at 2%, 10%, 8%, and 6%, respectively [25]. Ceylan [23] reported that Salmonella spp. was isolated from 44% of 25 chicken breast meat and 52% of 25 chicken thigh meat samples collected from Tokat province. Cadirci et al. [27] found Salmonella spp. in Samsun region 34% (51 of 150) in poultry meat.

Salmonella spp. in raw chicken meat samples has been previously confirmed in several studies conducted worldwide. Among them, investigations with similar designs to our research highlighted different contamination levels in various countries. Myskova and Karpiskova [13] reported that 13.6% of Salmonella spp. were isolated from chicken meat samples in the Czech Republic. In another study, Fazal et al. [14] were investigated 400 samples and 27% of their samples were contaminated with Salmonella. Similar to our work they also confirmed their strains by PCR.

Schwaiger et al. [16] isolated Salmonella spp. in 17.0% of poultry meat samples in Germany. Additionally, Salmonella spp. was identified in 22.5% of poultry products in the Republic of China by Ren et al. [15] and Tirziu et al. [17] isolated Salmonella spp 9.1% of chicken meat samples in Romania. Comparing the results of the above studies using the classical culture method and the Salmonella spp. isolation findings obtained in our study show that the rates Tirziu et al. [17] and Telli [25] are meager. It is seen that the results reported by Myskova and Karpiskova [13], Schwaiger et al. [16], and Kurul [24] are compatible with our results. On the other hand, it is seen that the rates reported by Ren et al. [15], Ceylan [23], and Türk [25] are higher than the rates we found. The results obtained in the studies may be due to the differences in the regions where the studies were conducted, seasonal differences, storage conditions, or sampling methods.

Dümen et al. [28] examined 100 raw chicken carcasses collected from the Istanbul region of Turkey for the presence of Salmonella spp., using PCR and classical culture methods. In their research, the presence of Salmonella spp. in 15 (15%) of the samples was revealed by PCR and culture method, and these two methods were found to be 100% compatible. El-Aziz [29] investigated 100 raw chicken meat samples with PCR and culture methods in Egypt and reported 44% Salmonella spp. positivity was detected with both methods. In our study, Salmonella spp. DNA was detected in 16 of 17 raw chicken meat samples found positive with the culture method examined with PCR. The remaining 84 (84%) samples were negative for Salmonella spp. by PCR. It is seen that the results reported by Dümen et al. [28] and El-Aziz [29] are consistent with our results. The PCR method has the advantages of getting results within a day and detecting even the small number of Salmonella spp. DNA.

In the present study, Salmonella spp. antigen was detected in 26 (13%) of 200 raw chicken meat samples by ELISA assay. In a study conducted in Brazil, Schneid et al. [30] examined 154 chicken meat samples for Salmonella spp. with ELISA and PCR tests. They detected 26% positivity with the ELISA test and 23% with the PCR test. Croci et al. [31] examined 30 experimentally contaminated chicken meat samples with ELISA and PCR methods in Italy. They reported that Salmonella spp. in 19 samples after 5 hours of pre-enrichment in both methods was detected.

CONCLUSIONS

The outcomes of this research revealed that *Salmonella* species that cause high levels of food poisoning were found in raw chicken samples. The distinguished high contamination of Salmonella spp. in raw chicken meat may lead to a severe public health risk. Moreover, this risk also can become a potential source of transmission *Salmonella* to humans. In this sense, it can easily be said that actions such as taking the necessary precautions in the process from raising poultry to obtaining the final product, paying attention to cooling, cooking, and storage conditions, ensuring tool-equipment hygiene, and performing personnel training will be beneficial for increasing the quality of the final poultry product. In other respects, these situation reveals the importance that these actions should be mandatory for public health.

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