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Molecular Investigation of *Salmonella SPP.* From Broilers

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ABSTRACT

Salmonella spp. are negative gram bacteria, rod-shaped; it belong to Enterobacteriaceae family. Salmonella is a wide variety of species that causes many diseases in humans and animals. This investigation was carried out to study the molecular investigation of Salmonella spp. from broilers. It was determined that approximately one hundred broilers were suffering from diarrhea, so feces and cloacal swabs were collected from them. These swabs were cultured on various media before sub-culturing on SS. agar and XLD. Following this, biochemical tests were performed to identify the bacteria. DNA extraction was carried out per the company's guidelines, and PCR was carried out to look for the invA gene, which is unique to Salmonella species. According to the findings, the percentage of individuals infected with Salmonella species was 17.39%. During this investigation, PCR was utilized to locate the in vA gene, and out of the eight tested samples, Salmonella species were identified in 8of them. Despite the severe and dangerous effects of the Salmonella pandemic on the agricultural and economic sectors as well as on human health, the findings showed that the PCR method had a high specificity in detecting Salmonella spp. when compared to other traditional methods of spotting the bacteria.

Introduction:

Salmonella is the most dangerous infectious disease that can rarely cause diarrhea in people worldwide (1). The transmission of *Salmonella* in livestock can be non-host-specific or host-specific. *Salmonella* is the most dangerous infectious disease. Chickens are the primary source of *Salmonella*, and contaminated food items like poultry flesh or eggs can cause human salmonellosis, Salmonellosis is an infectious disease that can be fatal (2).

After a period of quarantine, *Salmonella* enteritidis can be found in the flesh of Poultry, broiler chickens, and patients. Fever, abdominal cramping, diarrhea, nausea, and vomiting are all potential side effects of *salmonella* infection in humans (4). It is possible for the pathogens that are responsible for salmonellosis in humans to infect broilers and then move up the food chain to infect humans (5).

Traditional methods of diagnosis take a significant amount of time, whereas molecular detection methods take a great deal less time. This is due to the fact that the findings and reports generated by molecular techniques can be improved after a few days (6).

The multiplex PCR test is considered to be a monitoring tool due to the fact that it is able to link different strains from different sources back to their progenitor. It is a very difficult task to determine how different strains of Salmonella affect chicken and other Poultry. For instance, chickens can be affected by salmonella typhimurium lipopolysaccharides, which contain components of Gram-negative bacteria's cell walls and can cause immune and hormonal changes (7). Salmonella typhimurium lipopolysaccharides contain components of the cell walls of Gram-negative bacteria

Salmonella was found to be fully susceptible to some antibiotics, such as amoxicillin, but resistant to the majority of antibiotics (4). These findings were made in the area of medicines and antibiotics. *Salmonella* plasmid virulence in *Salmonella* spp. is essential in the process of replicating in the reticule-endothelial system cells in warm-blooded animals. In 1982, it was first suggested that virulence genes could be transported on a plasmid. In the pathogenicity island (SPI-1), which is essential for host epithelium cell penetration, there is a gene called *invA*. This gene has specific patterns that are unique to the Salmonella species. Because it is present in the vast majority of *Salmonella* strains, this gene is a primary target in identifying the bacterium known as *Salmonella* (8).

This study aimed to examine the molecular investigation of *Salmonella* species from broilers, so this investigation was carried out.

Materials and Methods:

Sample collection:

The diarrhea samples of the broilers are collected from Al- Diwanya /Al-Saniya directly from feces by using a sterile swap. The swap samples were placed in the plastic tube and then cooled by keeping at 4°C even reach to the microbiology laboratory to make the biochemists and molecular tests.

The selection procedure:

About (46) broilers experiencing diarrhea had their feces and cloacal swabs collected. The swabs were enriched by buffered peptone water After incubation for 18 hours at 37°C, 0.1 of the previously pre-enriched culture was inoculated into 10 milliliters of selenite f-broth, where it remained for 18-24 hours at 43 °C before being cultured onto S.S. Agarand XLD Agar and incubated at 37°C for about 24 hours.

The laboratory examination:

1. The biochemical examination:

Biochemical characteristics were evaluated using applicable tests for the detection of these bacteria.

2. Molecular examination:

DNA extraction was done according to the company's instructions (Geneaid Company, Taiwan).

Primers preparation:

Primers are targeting a particular region of the *invA* genome of *Salmonella spp.* (9) were purchased from the Alpha DNA business (Canada), as shown in table (1).

Table (1): the primers used in the present study with its sequence

Primer direction 16SrRNA	Sequence	Size
F	**GCTGCGCGCGAACGGCGAAG**	389bp
R	**TCCCGGCAGAGTTCCCATT**	

By dissolving each primer in one thousand liters of deionized and purified water, we were able to produce stockpiles of PCR primers with a concentration of 12 picomol / l as specified by the manufacturer's instructions. PCR identification of Salmonella species is required using particular gene primers known as *invA*.

The 25 µl PCR amplification combination used to identify the *invA* gene contained12.5 µl master mix, 2.5 µl DNA, 1.25 µl of each forward and reverse primers,and7.5 µl of nuclease-free water, as shown in Table (2).

Table (2): The component of the master mix with its amount

The component	The amount
DNA templat	**2.5 µl**
F primers	**1.25 µl**
R primers	**1.25 µl**
nuclease free water	**7.5 µl**
Master mix	**12.5 µl**
Total	**25 µl**

The subsequent steps were carried out in the thermo cycler after the PCR containers containing the amplified mixture had been transferred there from the workstation: The DNA was first denatured by heating it to 95 degrees Celsius for 5 minutes, and then it was put through 35 cycles of denaturation for 90 seconds, annealing for 60 seconds, extension for 90 seconds, and elongation for 420 seconds at different temperatures (The final extension), as shown in Table (3).

Gel electrophoresis was employed in order to distinguish between the numerous PCR result patterns. After electrophoresis at 100V and 70 mA for 45-60 minutes, a total of 10 L of the reaction mixture was loaded onto an agarose gel containing 2%, and the process was repeated. Under ultraviolet light, the product could be seen clearly thanks to the use of tried-and-true techniques like agarose gel electrophoresis and ethidium bromide labeling.

Table (3): shows thermo cycler conditions, which are used in the current study

The step	Temperature	Time	Cycle
Initial denaturation Step	95 °C	5 minutes	1
The denaturation Step	95 °C	90 s	35 cycles
The annealing Step	57°C	60 s,	-
The extension Step	71 °C	90 s	-
The elongation Step	72°C	420 s	-

Statistical analysis:

SPSS software (V23) is used to analyze our study data by calculating the percentage and rates of the related study values and using the Chi-square test to determine the statistical differences.

The results:

The results showed that the percentage of positive infection isolated from broilers and caused by *Salmonella* spp. was(17.39) %, while the percentage of Negative samples of *Salmonella* spp. in the broilers was(82.61%), as illustrated in (Table 4, Figure 1).

Table (4): number and percentages of *Salmonella spp.* infection in broilers

Bacteria	Total samples	Positive samples n (%)	Negative samples n (%)
Salmonella spp.	**46**	**8 (17.39)**	**38 (82.61)**
X^2	**1.523**		
P value	**0.0351**		

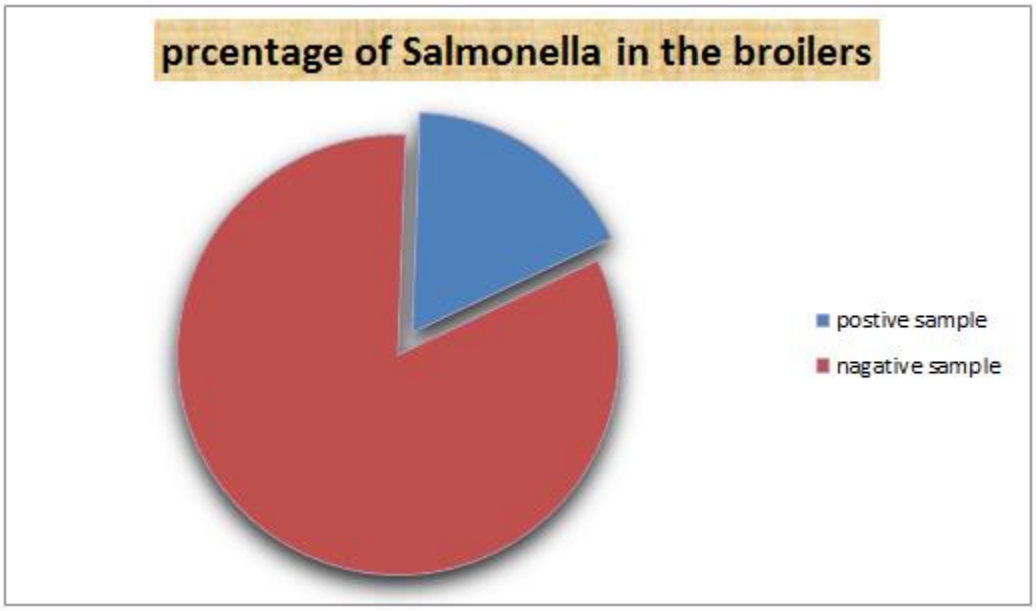

Figure (1):columns show of Salmonella isolation prevalence from infection in broilers

In this study, PCR was used to identify the *invA* gene in 12 samples, and 8 of those samples tested positive for the presence of Salmonella spp. (The results of the PCR are depicted in Figure 2, which can be found below.), as illustrated in figure (2).

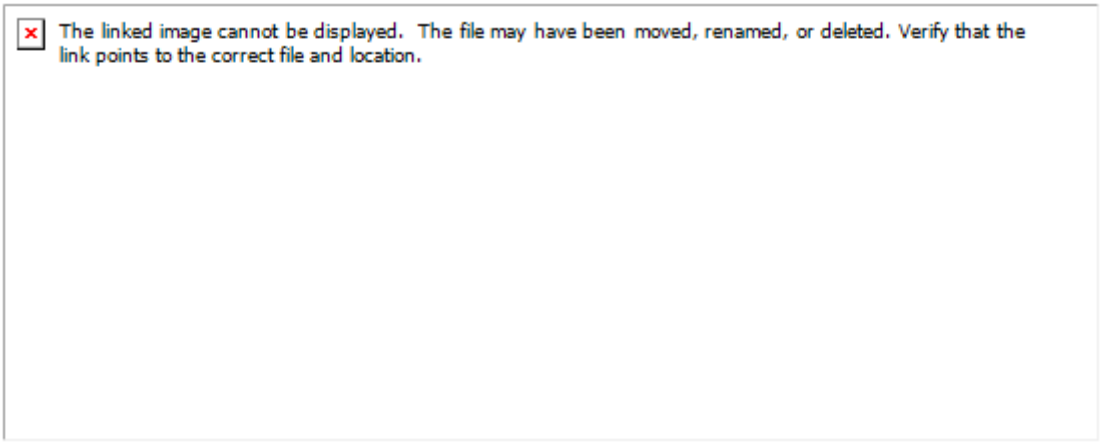

Figure (2): For particular samples, an agarose gel electrophoresis (1%), the figure is included bands with 389 bp fragment of the *InvA* gene

The discussion:

The findings of this study suggest that certain species of Salmonella could be responsible for the salmonellosis outbreak in broilers raised in the fields in and around the Baghdad region. Diseases that affect broilers of different ages and times of year include Salmonella spp., which is a zoonotic bacterial agent, Salmonella, S. Typhimurium, which is the most common samovar found in animals and humans, and S. Typhimurium, which infects the mucosal lining of the gastrointestinal tract. This could be because Salmonella spp. is a zoonotic bacterial agent which can spread from animals to humans. Both species have an increased risk of contracting the highly inflammatory diarrhea caused by these diseases (10).

During this research, the samples were cultured and subjected to biochemical tests. The results of these tests revealed that 8% of the samples contained *Salmonella* species. Molecular testing and

selective culture methods that rely on particular media are typically utilized to establish the presence of *Salmonella* when it is suspected. The overall prevalence of Salmonella species was 7.9% and 8.47%, respectively, in other research (11, 12). These figures were derived from the findings of other studies.

The identification of *Salmonella* starts with the fermentation of lactose, which is followed by the production of hydrogen sulfide when using traditional media such as S.S. agar. These kinds of media have an alarmingly high rate of producing false-positive findings, which requires additional testing, which is both time-consuming and costly (13).

Due to the fact that the traditional methods used to detect *Salmonella* spp. have a very low sensitivity, there have been a significant number of findings that have been incorrectly interpreted as positive (14).

Gel electrophoresis and ethidium bromide spotting were the methods that were utilized in order to locate a 389-bp segment that was derived from the particular PCR result. It has been demonstrated that the *invA* gene contains segments unique to *Salmonella* spp. isolates, demonstrating that this gene is an appropriate PCR target for these isolates. The results of the PCR were positive for each and every *Salmonella* isolate tested. Other research has shown that the gene *invA* is an effective PCR target for identifying *Salmonella* species (15, 16, 17).

This gene contains sequences that are specific to *Salmonella* isolates and can be found in the fimA, fimC, and hilA genes. The findings of the current study, which found that the *invA* gene found in all Salmonella samples had a length of 284 nucleotides, were consistent with the previous study's findings (18).

The results of this research showed that every single strain of Salmonella tested possessed the *invA* gene, which is in line with the findings of a previous investigation that came to the same conclusion (19). The findings of the current research supported the findings of a study conducted by (20) in Nasiriyah, which discovered that the prevalence of this locus was 100% in that region. The results of the present study were found to be in line with those obtained from a survey conducted in Egypt by (21).

Conclusion:

The findings showed that the PCR method was highly specific in detecting *Salmonella* spp. Despite the severe and dangerous effects of the *salmonella* pandemic on the agricultural and economic sectors and human health compared to other traditional spotting techniques.

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