www.ajlifesciences.com / ISSN: 2663-1040 *AJ Life Sci. 2021, 4 (2): 93-99*

Research Article

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.4.2.11

Targeting of FliC gene for the identification of *Salmonella Typhimurium* in Poultry chicken by PCR in District Swat

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Abstract

Salmonella is one of the prominent causes of food borne infection all over the world. The present study was performed to examine the existence of *Salmonella typhimurium* in poultry chicken samples and chicken wooden cutting boards. For this purpose, a total of 100 samples were collected to detect *S. typhimurium*. The samples were streaked on Salmonella Shigella agar media (SS media) for the growth of *Salmonella*. Out of total samples, 50% were appeared as positive on media plates. Total percentage of *Samonella* by sample wise was found to be higher in the liver (85%) followed by chicken wooden cutting boards (63%), food and proventriculus (50%), oesophagus and tissues (33%). Total percentage of *Salmonella* by area wise was found to be higher in Charbagh and Koza Bandai and Kanju (80%), Khwazakhela (55%), Mingora (55%), Sambat and Matta (30%). DNA was isolated from the positive samples and amplified with FliC gene-specific primer using optimized PCR protocol for the confirmation of *S. typhimurium*. In the screened samples, 28% of *S. typhimurium* were observed as positive samples by amplifying FliC gene sequence having a molecular band size of 620bp. Out of the total *S. typhimurium* positive samples, 6 were positive from Khwazakhela, 2 from Charbagh, 4 from Sambat and Matta and 2 from Mingora.

Article Info:
Received:
October 24, 2021
Received Revised:
December 28, 2021
Accepted:
December 28, 2021
Available online:
December 31, 2021

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1. INTRODUCTION

The genus *Salmonella* is recognized as a disease-causing agent in animals and humans[1]. It causes typhoid and enteric fever, diarrhea, food poisoning, and gastroenteritis. Salmonellosis is the most numerous food-borne illness which leads to cause of typhoid disease and enteric disease[2].

Poultry serves as a major source of energy; contains different vitamins, amino acids, fats, proteins, minerals, and energy[3]. Fowl is a quick and main resource of nourishment for the human population but poultry industries are facing different infections in Pakistan, e.g. Salmonellosis[4]. Poultry products are often polluted with *Salmonella* species, which is thought to be the main cause of the disease in humans[5]. *Salmonella typhimurium* is a gram-negative, rod-shaped, aerobic or facultatively anaerobic, motility through peritrichous flagella and is one of the causative agents of salmonellosis[6]. It infect chickens via water, feed, hatching eggs and through ecological factors including insects, birds, rodents and farm workers[7]. In poultry, it causes a range of chronic and acute diseases together with pullorum, paratyphoid, avian arizonosis, fowl typhoid, entero-toxigenic diarrhea and shigellosis plague[8]. Poultry houses provide a suitable environment for this typical zoonotic foodborne pathogen and the organism can survive for a long time in meat[9]. In Pakistan, the developing poultry industries are facing infections of *Salmonella* and which is the main source of food borne infections in human beings[10]. This organism enters the intestinal lumen deep into the small intestine epithelium where it causes infection and produces enterotoxin[11].

Infectious microorganism are identified from clinical specimens by different techniques[12]. The techniques which are rapid, easy, accurate and cost effective and easy are consider to be best choice in diagnosis of infectious microorganism[13].The conventional diagnostic techniques are laborious, now specificity and time-consuming[14,15]. In contrary to these techniques, the diagnostic results of polymerase chain reaction (PCR) are more authentic, specific and accurate[16, 17]. Therefore, the present study was designed to examine the existence of *S. typhimurium* in poultry using PCR assay.

2. MATERIALS AND METHODS

The study was conducted at the Veterinary Research and Disease Investigation Centre (VR&DIC) Balogram Swat. A total of 100 samples were collected from different regions of district Swat including Mingora, Manglor, Charbagh, Khwazakhela, Matta & Sambat, Koza Bandai and Kanju. A simple swab method was used for the collection of samples. The swab was dragged on different parts of chicken including liver, tissues, intestines, oesophagus, and proventriculus. The samples were also collected from the poultry cutting boards. The samples were maintained and characterized in the laboratory within 24 hours. *SS* media was prepared and samples were loaded on media plates. After loading, the plates were kept in the incubator for 24 hours at 37 °C (Fig 1). The process was done at the biosafety cabinet. After the growth of *Salmonella* (appeared as black dots), the colonies from the *SS* media were transferred into nutrient broth media by using the flame sterilized loop (Fig 2).

Figure 1: Growth of *Salmonella* on *SS media after inoculation*

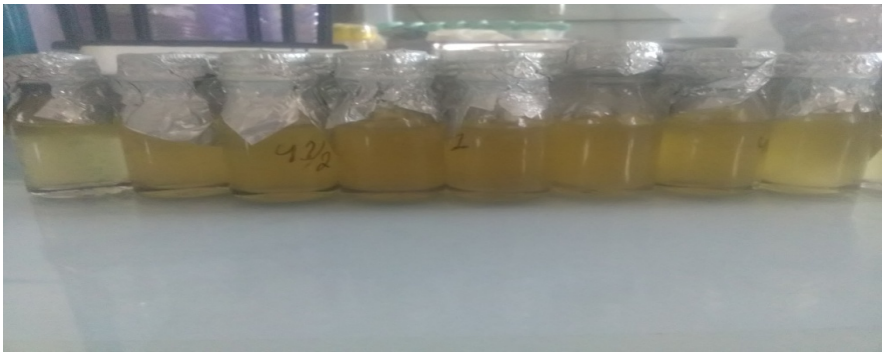

Figure 2: *Salmonella* Growth in nutrient broth media

2.1 DNA extraction

Under the aseptic condition, 1 ml broth sample was transferred to 1.5 micro centrifuge tubes by micropipette and then centrifuged for 10 minutes at 5000 x g. After centrifugation, the upper part supernatant was discarded and the lower part pellet was used for DNA extraction. The DNA extraction was performed by DNA Isolation kit (Genomic DNA Purification Kit Gene JET (Catalog number: K0721) following the manufacturer protocol. Genomic DNA was quantified by Nanodrop.

2.2 PCR Assay

The purified DNA was then subjected to PCR assay. Reaction mixture of PCR (25 µL) contained forward and reverse primers (0.5 µl of each), master mix (12.5 µL), DNA template (3.0 µL) and of nuclease-free water (8.5 µL). The PCR was performed by thermocycler (C-1000 Touch™ thermocycler). Optimized condition for thermocycler was; initial denaturation of DNA for 50 second followed by final denaturation at 94°C for 1 minute, annealing at 59 °C for 30 second, initial extension for 30 s at 72°C with final elongation step at 72°C for 7 minute. The PCR product was then run on agarose gel (1.5% w/v) stained with ethidium bromide. The amplified product on the gel was observed by gel documentation system (JY04S-3C). The sequences of forward and reverse primer is shown in Table 1.

Table 1. Fli15-TYP04, specific primer pair used in PCR assay for detection of *S. typhimurium.*

Primer	Target gene	Length	Primer sequence (5'—3')	Amplification product (BP)	References
Fli15 (2)	FliC (F)	22	CGGTGTTGCCCAGGTTGGTAAT	620	(Sdoliveria et al., 2002)
Typ04 (2)	FliC (R)	16	ACTGGTAAAGATGGCT		

3. RESULTS AND DISCUSSIONS

Salmonella is the common cause of food borne enteric diseases and their prevalence is quite higher in Pakistan as compared to other developing countries[8]. It is commonly found in contaminated chicken and water[18]. In this study, we investigated the different parts of chicken for the isolation and detection of *Salmonella.* Out of 100 samples, 50 were positive for *Salmonella*. The study conducted in Karachi reported

the prevalence of *Salmonella* of 48.75% [19]. Similarly high prevalence rate of 47.06% was reported in Egypt [20]. Both these reports are near to our findings. Furthermore, the data also revealed that among the selected areas the percentage of *Salmonella* was high in Charbagh and Koza Bandai and Kanju (80%) followed by Khwazakhela (55%), Mingora (55%), Sambat and Matta (30%) while total percentage by sample wise was found to be higher in the liver (85%) followed by chicken wooden cutting boards (63%), food and proventriculus (50%). The least percentage of *Salmonella* was found in oesophagus and tissues (Table 1&2). The study reported the high prevalence of *S. typhi* in liver, followed by Intestines and Proventriculus[6]. Total percentage *of S. typhimurium* by sample wise was found to be higher in liver followed by tissues, intestines and cutting board. These finding are similar to the previous study which reported the prevalence of *S. typhimurium* at rate of 44% in meat, 40% liver and 48% in tissues[7]. The difference in the reported data with our finding may be due to the sample size of the experimental units, geographic location and age of poultry. Identification of *Salmonella* is one the basic measure to control the spread of salmonellosis which needs rapid and modern techniques like PCR[21]. The positive samples of *Salmonella* were then screened for *S. typhimurium* by targeting the FliC gene using specific primer (Fig 3).

The detection of *S. typhi* by targeting the FliC gene was also reported in previous studies[22,23,24]. The result of the PCR assay suggested that among the tested samples only in 14 samples the FliC gene sequence was amplified with a molecular band size of 620bp which confirm the presence of *S. typhimurium* in these samples. Among the *S. typhimurium* positive samples, 6 were positive from Khwazakhela, 2 from Charbagh, 4 from Sambat and Matta and 2 from Mingora. The findings of our study showed that 28% of samples were positive for *S. typhimurium* which is greater than the prevalence rate (14.5%) reported from Ecuador[25]. Another study in Quetta also reported the prevalence rate of 30.3% of *S. typhimurium*[26] which is close to our findings. The prevalence of *S. Typhimurium* in poultry was 5.5% among 108 Salmonella isolates in Republic of Srpska[27]. The difference in prevalence may be due to geographic location, unhygienic conditions, and differences in the age of poultry, infection dose etc.

Table 1. Total percentage of *Salmonella* by sample wise

Collected samples	Total	Overall positive	Overall Negative	Percentage
Liver	14	12	2	85%
Intestines	20	08	12	40%
Cutting Board	22	14	08	63%
Tissues	30	10	20	33%
Food	04	02	02	50%
Proventriculus	04	02	02	50%
Esophagus	06	02	04	33%
				Average percentage=50.4%

Table 2. Total percentage of *Salmonella* by area wise

Area Name	Samples collected	Positive samples	Negative samples	Overall positive percentage
Mingora	20	11	09	55%
Charbagh	20	16	4	80%
Khwazakhela	20	11	09	55%
Sambat & Matta	20	06	14	30%
Kuza Bandai &Kanju	20	16	04	80%
				Overall Percentage= 60%

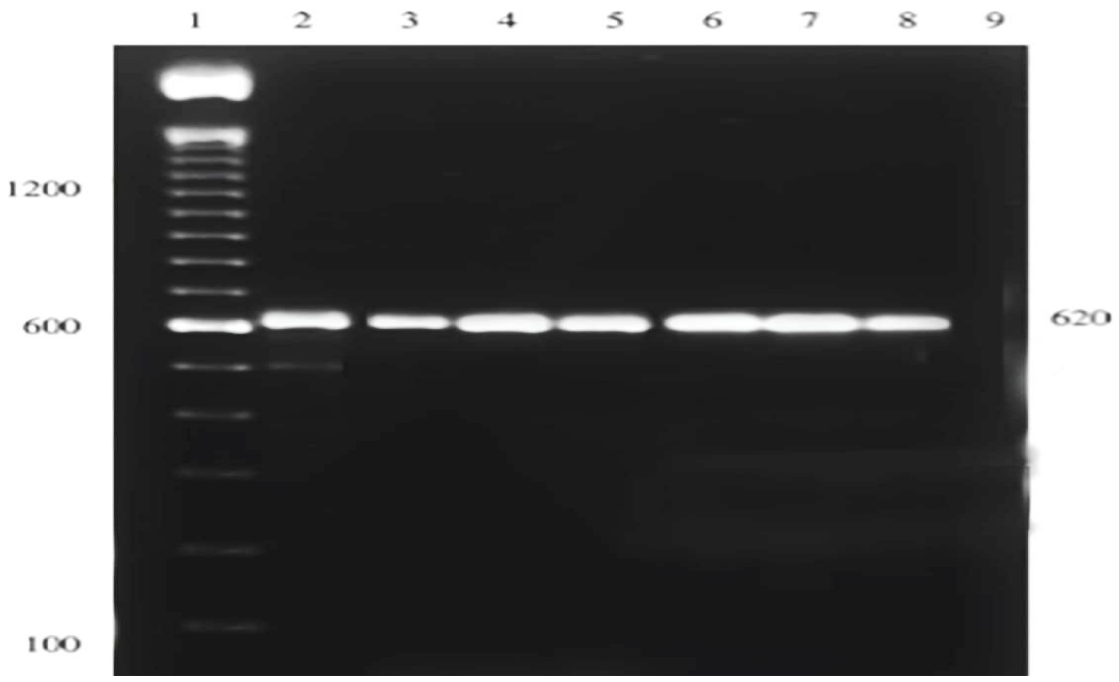

Figure 3: Gel image of PCR amplified products of FliC gene. Lanes: Lane 1, ladder (100 bp), lane 2-8 samples. Total samples (2-8) showing positive PCR product for FliC gene with an amplicon size of 620 bp.

4. CONCLUSIONS

It is concluded from the current study that *Salmonella* and *Salmonella typhimurium* are widely exists in poultry and is the main cause of Salmonellosis in district Swat. Furthermore, the PCR assay presented here provide a specific and rapid identification of *Salamonella* spp. in poultry flocks.

ACKNOWLEDGMENT

The authors acknowledge the Veterinary Research & Disease Investigation Center, Balogram for supporting the experimental work of the research study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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