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Prevalence of *Salmonella* Enterica Serovar *Gallinarum* in Broiler Chickens from District Sanghar

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RB conceived and designed the study. SB analyzed the data. RB drafted the manuscript. SGM participated in overall helped during the study period.

Key words
***Salmonella gallinarum*, Prevalence, Poultry, PCR, Ceca, Sanghar district**

ABSTRACT

Salmonella gallinarum is a significant etiological pathogen of fowl typhoid, which results in huge losses in poultry production especially in the developing countries. The current study was conducted in the year 2025 to monitor the prevalence rate of *S. gallinarum* at various talukas of district Sanghar. Five broiler farms of each talukas (i.e. Tando Adam, Shahdadpur, Sinjhoro, Sanghar, Jam Nawaz Ali and Khipro) were visited to examine the suspected birds to *S. gallinarum,* A total of 120 cecal samples were collected from clinically suspected birds and identified using conventional bacteriological methods followed by PCR targeting *rfbS* gene for confirmation. The overall prevalence rate of *S. gallinarum* was recorded as 36.6% (44/120). The highest prevalence rate was found in Tando Adam (55%) followed by Shahdadpur (45%), Sinjhoro (35%) and the lowest prevalence were recorded in Khipro (25%). Statistical analysis revealed non-significant difference among the talukas ($p>0.05$). The current study concluded that *S. gallinarum* is widely present in poultry farms of district Sanghar with non-significant variations among the different talukas, indicating the local factors such as farm management practices, biosecurity measures and environment causes. The relatively higher prevalence in Sanghar highlights the need for improved disease control strategies. Therefore strengthening biosecurity practices, implementing regular health monitoring and increasing farm awareness are essential steps to reduce the spread of fowl typhoid and minimize economic losses in poultry production.

INTRODUCTION

The poultry industry of Pakistan is a major livestock sub-sector, playing a pivotal role in economic growth and rural development. Rearing chickens has been a widespread human activity for thousands of years. The historical timeline of chicken domestication is still a controversial subject of research efforts (Peters *et al*., 2022). Salmonellosis is an important disease causing serious impediment to the development of livestock and become a serious problem in recent decades. It is one of the main public health concerns responsible for managing a wide range of water and food born disorder affecting poultry, humans, and other animals. The highest number of *Salmonella typhimurium* serotypes was found in isolates from oviduct samples of layers, followed by isolates from cecal content samples (Al-Khayat, 2017). *Salmonella* spp. is a kind of zero-tolerance foodborne pathogens, which poses a great threat to quality of food products and public health (Wang *et al*., 2018). It has been recognized as intestinal pathogen in both humans and animals (Newell *et al*., 2010). Many studies have reported the importance of these bacteria as a pathogen by having multidrug resistant bacteria due to the use of non-specific antibiotic and random administration of antibiotic and this will make persistent strains have ability to resist a large number of antibiotics (Hasan *et al*., 2018).

Salmonella enterica serovar *Gallinarum* (*Salmonella gallinarum*) is a host-adapted, Gram-negative pathogenic bacterium, which is obligately associated with avian species, especially domestic chicken and turkey. This organism consists of two related biovars, *Gallinarum* and *Pullorum* which are the etiological agents of fowl typhoid and Pullorum disease, respectively both of which

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are systemic septicemic diseases with high morbidity and mortality and large financial losses in poultry production globally. In contrast to the majority of the *Salmonella* strains, *S. gallinarum* and *S. pullorum* are not motile, and are extremely adapted to their birds, and symptoms of the disease depend on the age and immunity state of birds (Farhat *et al*., 2023). *S. gallinarum biovar Gallinarum* causes fowl typhoid and is able to affect birds of any age, which normally prolapses as a severe, systemic infection of major organs including liver and spleen. Clinical symptoms can consist of anorexia, fatigue, anemia, and organs swelling, which can end in death without treatment (Wales and Lawes, 2023; Jia *et al*., 2024). The economic burden of *S. gallinarum* is especially significant in the developing world, where control activities, biosecurity and vaccination programs are less strictly followed than in the developed world where the pathogen has been almost eliminated through systematic poultry health interventions (Farhat *et al*., 2023; Jia *et al*., 2024). Moreover, the existing data show that *S. gallinarum* carries a diverse range of virulence factors, such as *Salmonella* pathogenicity islands, which facilitate systemic infection, and becomes more and more multi-drug-resistant, which makes treatment and control of poultry farms more difficult (Farhat *et al*., 2023; Hu *et al*., 2024). Since fowl typhoid continues global issue, to create additional useful diagnostic tools, vaccines, and antimicrobial stewardship measures, there is need to learn more about the biology, epidemiology, and molecular peculiarities of *S. gallinarum* to reduce the impact of the disease in commercial and backyard poultry flocks. Therefor the current study was aimed to determine the prevalence and molecular confirmation of *S. gallinarum* in broiler chickens and compare its distribution across different talukas of district Sanghar.

MATERIALS AND METHODS

Study area and samples collection

A cross sectional study was conducted during the year 2025 in district Sanghar. The district is divided into six talukas viz: Tando Adam, Sanghar, Shahdadpur, Sinjhoro, Khipro, and Jam Nawaz Ali. A total of 5 different broiler poultry farms of each talukas were visited to examine the suspected birds to *Salmonella Gallinarum*. Where total 120 numbers of birds were identified by clinical findings like reduced appetite, depression, labored breathing and diarrhea causing feces to stick to the vent. After slaughter of these birds a total of 120 samples of ceca were collected in polythene bags which contain normal saline and was brought to Central Veterinary Diagnostic Laboratory Tando Jam for further process.

Isolation and identification of S. gallinarum

Salmonella Shigella agar (S.S agar) was prepared for culture of *Salmonella* and then ceca contents was diluted with normal saline, after that sample was streaked with the help of wire loop on already prepared media then it was incubated at 37°C for 24 h to obtain primary culture. Then suspected colonies of *Salmonella* from each plate were collected for presumptive identification by their morphological characteristics and biochemical tests. The primary tests included Gram's stain, catalase, oxidase, motility, Triple Sugar Iron agar (TSI agar), indole, methyl red, Voges-Proskauer, citrate utilisation test and sugar fermentation tests.

DNA extraction

A heat-boiling technique was used to get genomic DNA. Single *Salmonella colonies* were inoculated into 1 mL of brain heart infusion (BHI) broth, and allowed to incubate overnight at 37 °C. The bacterial suspension was centrifuged the following day at 4,000 × g in 10 min. The pellet was resuspended again in freshly prepared TE buffer (10 mM Tris-HCl, 1 mM EDTA) and then a spin was made to resuspend the pellet. Eppendorf tubes were put in water bath of 100 C temperatures and then put on a freezer of -80 C temperature, lasting 3 min. This freeze-thaw process was done thrice in order to be sure that the cells were fully lysed. The tubes were then centrifuged at 14,000 × g centrifugation at 4 °C for 5 min. Supernatant with extracted DNA was transferenced into clean tubes to be utilized further on molecular characterization. The Nanodrop spectrophotometer was used to measure the concentration and purity of DNA.

PCR amplification of rfbS *gene*

PCR amplification was performed in a thermal cycler under the following condition, initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, extension at 72 °C for 1 min and a final extension at 72 °C for 7 min.

S. gallinarum specific primers having the following nucleotide sequence based on the *rfbS* gene of *S. gallinarum* F-TCA CGA CTT ACA TCC TAC and R- CTG CTA TAT CAG CAC AAC was used in this study for PCR. The amplified product was analysed by electrophoresis on (2% w/v) agarose gels.

Statistical analysis

Data were entered and coded in a Microsoft Office Excel 2016 spread sheet. Analysis was performed using statistical software i.e., Statistix 8.1version, USA through Chi-square tests.

RESULTS AND DISCUSSION

Characteristics of Salmonella *isolates*

The results of all bacteriological tests to isolate and confirm *Salmonella gallinarum* in different media incubated at 37 °C for 24 h revealed that the bacteria appeared as smooth colonies and non-pigmented (grey-white) colonies, opaque on the nutrient agar, It appear as small, smooth, rounded with black center colonies on SS agar and small, smooth, rounded with black center colonies on XLD.

The results of biochemical diagnostic tests of *S. gallinarum* were demonstrated that catalase positive, On TSI the bacteria appeared as lactose non-fermenter, produce H2S, have the ability to gas production with alkaline slant and acid bottom Indol negative, Simmon's citrate were positive, in addition, Sulfate - Indol - Motility (SIM) test, founded that the bacteria was non motile positive with H2S production which seen as blackening in all the reaction tubes.

The isolates were Gram negative and characterised by rod bacillary in shape, non-spore forming aggregate as single or pairs bacterial cells (Fig. 1). The amplified DNA products from salmonella specific PCR was visible as 720bp band on 2% agarose gels (Fig. 1).

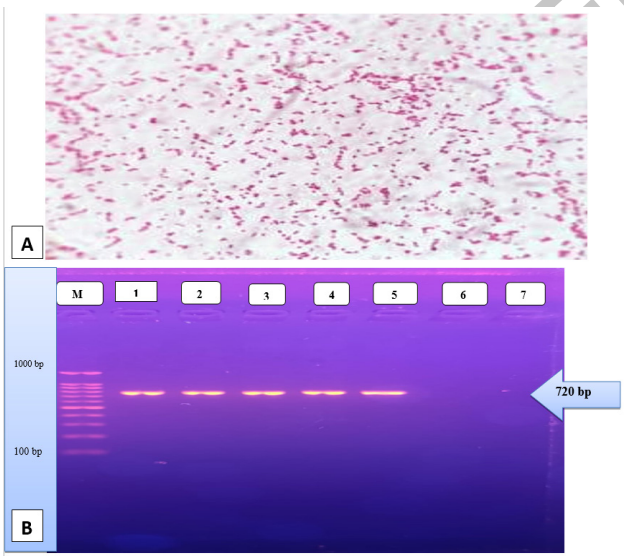

Fig. 1. *Salmonella gallinarum*: A, after staining by Gram stain. Magnification:100X. B, Lanes 1, 2, 3, 4 and 5 show positive PCR 720 bp produce with *rfbs* gene primers, Line M show PCR marker as ladder (100bp -1000bp).

Prevelance of S. gallinarum

A total of 120 samples of ceca were collected from district Sanghar and examined for the prevalence of salmonella gallinarum. Out of 120 samples 44 samples were found positive for Salmonella gallinarum while remaining 76 number of samples noted to be negative. The overall prevalence rate was found as 36.6% in distric Sanghar. This is only slightly lower than the 41.8% sero-prevalence reported by Shoaib *et al*. (2020) in Rawalpindi, underscoring that *S. gallinarum* remains a major concern in regional poultry. On broader scale global meta-analysis data shown an average prevalence of about 8.5% over many years (Zhou *et al*., 2022). Accordingly, *Salmonella* spp. was among the common bacteria found in the broiler's gut when they were isolated for the current investigation. In concurrence, *Salmonella* spp. have been identified as a prevalent bacterial illness affecting poultry birds digestive tracts (Castanheira and Garcia, 2017). At the same time, research has been conducted by Brenner *et al*. (2000) who noted that one of the primary sources of foodborne illnesses in the US is *Salmonella* spp., a kind of bacteria often present in the digestive tracts of diseased chickens. According to Castro-Vargas *et al*., (2020), broiler chickens have a 40.5% incidence of *Salmonella* spp. in their environment.

Table I shows prevalence of *S. gallinarum* in different Talukas of Sanghar districts. The highest prevalence was recorded in Tando Adam (55%) followed by shahdadpur (45%) and Sinjhoro (35%). Moderate levels of infection were found in Sanghar and Jam Nawaz Ali (30% each), while the lowest prevalence (25%) was recorded in Khipro taluka. No statistically significant difference was observed (x^2=5.45, p=0.36) in prevalence rate among the talukas of district Sanghar. The observed non-significant difference in prevalence among talukas may be attributed to variation in environmental conditions, poultry density, biosecurity standards and management practices.

Table I. Number of positive ceca samples for *Salmonella gallinarum* from six talukas of district Sanghar.

Taluka	Sample	Positive (%)
Tando Adam	20	11(55.0)
Shahdadpur	20	9(45.0)
Sanghar	20	6(30.0)
Khipro	20	5(25.0)
Jam nawaz Ali	20	6(30.0)
Sinjhoro	20	7(35.0)
Total	120	44(36.6)

The present study shows an overall prevalence of 36.6% with significant spatial variation. The highest prevalence was observed in Tando Adam (55%), whereas

the lowest was noted in Khipro (25%), indicating heterogeneity in disease distribution within the district. These findings suggest that local environmental, managerial, and biosecurity factors may critically influence the epidemiology of fowl typhoid in this region. The overall prevalence of *S. gallinarum* detected in Sanghar (36.6%) is markedly higher than the global average prevalence reported for this pathogen (approximately 8.5%) in poultry worldwide (Zhou *et al*., 2022). This disparity likely reflects the endemic nature of fowl typhoid in South Asian poultry systems where control measures such as strict biosecurity, routine vaccination, and surveillance are often limited or inconsistently implemented. Indeed, in Pakistan and neighboring countries like Bangladesh, *S. gallinarum* prevalence has been reported to be relatively high under field conditions, particularly in small-scale or low-biosecurity flocks (Haque *et al*., 2021). The endemicity aligns with broader observations that developing country poultry sectors frequently experience high bacterial pathogen loads due to infrastructural and management constraint (Haque *et al*., 2021).

The non-significant ($p>0.05$) variation among talukas may be due to differential exposure risks driven by several interconnected factors. First, variation in biosecurity practices and farm management such as hygiene standards, control of visitor entry, and feed/water sanitation could result in uneven pathogen pressure across regions. Studies have documented that farms with low biosecurity and poor sanitation are more susceptible to fowl typhoid outbreaks (Rahman *et al*., 2019). Second, poultry density and flock movement patterns could differ between talukas; Tando Adam may have higher flock density or inter-farm connectivity, facilitating transmission via contaminated equipment, personnel, or vehicles. Third, environmental conditions such as humidity and temperature may influence pathogen survival in the environment and hence transmission likelihood (Julianingsih, 2023). Although local climate data were not collected in this study, microclimatic differences within the district could plausibly affect bacterial persistence in litter and water.

CONCLUSION

The current research shows that *S. gallinarum* is present in poultry cecal samples in Sanghar district in high prevalence rates (36.6%) with a non-significant ($p>0.05$) variation among the talukas. The prevalence is significantly higher than that in the rest of the world, indicating the endemicity of fowl typhoid in the area. The spatial heterogeneity also implies that variation in biosecurity, management practices, and local environmental conditions is very crucial in the distribution of diseases. Such results underline the necessity to enhance the level of biosecurity, regular monitoring, and specific control techniques aimed at the reduction of *S. gallinarum* among the poultry production systems in the district.

DECLARATIONS

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IRB approval

Institutional Review Board of Sindh Agriculture University, Tandojam approved the study (No. PHAR/274/2025 dated 09-04-2025).

Ethical statement

All animal experiments were performed in strict accordance with the guidelines for the Care and Use of Laboratory Animals as outlined in No. DAS/4281/2025.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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