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Short communication

# Prevalence and distribution of *Salmonella* serotypes in marketed broiler chickens and processing environment in Coimbatore City of southern India

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ABSTRACT

Broiler chicken is gaining popularity among the consumers of India. Since poultry is recognised as a leading food vehicle for *Salmonella* contamination, the prevalence and distribution of *Salmonella* serotypes in broiler chickens and processing environments of retail outlets has been studied. In the present study 214 samples of broiler chicken and 311 environmental samples from cage were analysed for the presence of *Salmonella*. Of the various body parts of live chicken analysed prevalence varied from 1.4% in cloacca to 6.9% in crop region. Environmental samples from the cage showed higher prevalence of *Salmonella* ranging from 0 to 16.67%. Apart from *Salmonella enteritidis*, which was the predominant *Salmonella* serotype in the chickens as well as in the environmental samples, other serotypes such as *S. bareilly*, *S. cerro*, *S. mbandaka* and *S. molade* were also encountered. The results of the research calls for strict hygiene standards for retail broiler chicken processing outlets.



## 1. Introduction

Salmonellosis has become an important food borne disease resulting in considerable burden to public health and economy. Poultry has been identified as an important vehicle for food borne salmonellosis (Van Asselt, Thissen, & van der Fels-Klerx, 2009). Persistent environmental contamination of housing is reported to be another important factor in increasing *Salmonella* infection in poultry. In India isolations of multidrug *S. typhi* and *S. typhimurium* has been reported previously (Sabherwal, Chaudhary, & Saini, 1992; Garg & Panigrahi, 1993) though reports from poultry are limited (Singh, Yadava, Singh, & Bhartia, 2010).

As in other parts of the world, increasing popularity of the chicken has resulted in the proliferation of small scale retail vendors of broiler chicken, who used to keep the chicken in small wire mesh cages in crowded conditions and process there itself. Most of the retail outlets do not follow standard operational procedures while processing the chicken and the chances of cross contamination is rather high in these outlets. The degree of cross contamination could be ascertained by tracing the distribution of *Salmonella* serotypes in the broiler chicken, processing tools and in the environment. Hence the present study has been taken up with an objective of identifying the prevalence level of *Salmonella* contamination and distribution of specific *Salmonella* serotypes in various body parts of the broiler chicken as well as in the environmental samples of the retail processing environment.

## 2. Materials and methods

The sampling sites for the study encompass 4 major broiler chicken retail outlets in the residential areas of Coimbatore City, South India. Using sterile cotton swabs, samples from various parts of live chicken such as body surface, mouth and cloaca were collected. Soon after swabbing the swabs were placed in sterile buffered peptone water (BPW) and brought to the laboratory. After excising the chicken, contents from caeca, crop and intestine were collected aseptically in sterile polyethylene bags. Environmental samples such as cage dust or litter, fresh faeces, feed and water were aseptically collected in sterile polyethylene bags and transported to the laboratory in a portable ice chest.

For isolation of *Salmonella* a modified method of Hatha and Lakshmanaperumalsamy (1997) was used. The samples were pre-enriched in BPW in a screw cap bottle at 37 °C for 24 h followed by selective enrichment in selenite cystine broth (SCB) and tetrathionate broth (TTB) 37 °C for 24 h. After 24 h of selective enrichment, a loopful of cultures from both SCB and TTB were streaked onto selective media such as Brilliant Green Agar (BGA), Hektoen Enteric Agar (HEA) and Xylose Lysine Deoxycholate Agar (XLD) plates and incubated at 37 °C for 24 to 48 h. The BGA, HEA and XLD plates were observed after 24 h and 48 h for typical *Salmonella* like colonies. Whenever present, 2 typical colonies were picked up from a positive plate, restreaked to ensure purity and maintained on Brain Heart Infusion Agar (BHI)

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slants at room temperature and characterised biochemically following standard methods (Buchanan & Gibbons, 1974). These isolates were further confirmed by slide agglutination test using polyvalent O sera (Wellcome Laboratories, Dartford, England) and serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh.

### 2.1. Statistical Analysis

The results were subjected to chi-square test for goodness of fit to determine the significant variation, if any, among the different outlets, or among the different *Salmonella* serotypes. The results of seasonal variation was subjected to Pearsons chi-square test of association (with $\alpha = 0.05$) to evaluate whether there is any association between the prevalence of *Salmonella* in sample sources and seasons.

## 3. Results and discussion

Prevalence of *Salmonella* in various parts of broiler chicken and environmental samples is presented in Table 1. Crop had the highest prevalence (6.90%) followed by external surface (5.14%). While mouth region showed considerable prevalence, least incidence (1.40%) was recorded in cloacal samples. However there was no significant variation ($P = 0.574$) in the prevalence levels among different body parts. Of the 311 environmental samples collected from different sampling stations 8.04% of the samples were contaminated with *Salmonella*. Variation in the incidence of *Salmonella* among different environmental samples was found to be significant ($P < 0.001$). Cage floor made of wooden platform had the highest level of incidence of *Salmonella* (16.67%) followed by fresh faeces (13.89%) and litter samples (12.50%).

Serotyping of the *Salmonella* isolates showed that about 67% of the *Salmonella* strains encountered in various samples were *Salmonella enteritidis*. Incidence of different serotypes of *Salmonella* in various parts of broiler chicken and in environmental samples is shown in Table 2. Isolation of *S. enteritidis* from body surface, crop, caeca and small intestine were relatively more frequent when compared with other body parts. Prevalence of *S. enteritidis* was significantly higher ($P < 0.001$) when compared to other *Salmonella* serotypes in broiler chicken samples. *S. cerro* was the next frequently (12.96%) isolated serotype of *Salmonella* in broiler chicken samples. *S. mbandaka* and *S. molade* had equal level of incidence. Other serotype encountered was *S. bareilly*, which was encountered only in crop sample. While *S. mbandaka* was not detected in mouth and cloacal samples, *S. molade* was absent in samples from cloacae and caecae. Five serotypes such as *S. bareilly*, *S. cerro*, *S. enteritidis*, *S. mbandaka* and *S. molade* were encountered in environmental samples. *S. enteritidis* was the most frequently encountered (52%) serotype. Prevalence of *S. enteritidis* was significantly higher ($P = 0.001$) in environmental samples also.

**Table 1**
Prevalence of *Salmonella* in various body parts of marketed broiler chickens and environmental samples.

| Source | No. of samples analysed | No. of samples tested positive | Percentage of incidence |
|---|---|---|---|
| External surface | 214 | 11 | 5.14 |
| Cloaca | 214 | 3 | 1.40 |
| Crop | 203 | 14 | 6.90 |
| Caeca | 198 | 10 | 5.05 |
| Intestine | 198 | 8 | 4.04 |
| Mouth | 214 | 8 | 3.74 |
| Stock feed | 18 | 0 | 0 |
| Feed fed | 38 | 3 | 7.89 |
| Feeder tray | 38 | 2 | 5.26 |
| Tap water | 26 | 0 | 0 |
| Water from drinker | 32 | 2 | 6.25 |
| Drinker trough | 32 | 1 | 3.13 |
| Litter | 24 | 3 | 12.50 |
| Cage with wooden platform | 24 | 4 | 16.67 |
| Cage with metal mesh platform | 43 | 5 | 11.63 |
| Fresh faecal matter | 36 | 5 | 13.89 |

**Table 2**
Distribution of various *Salmonella* serotypes in different body parts of the broiler chickens.

| Source | *Salmonella* serotypes | | | | |
|---|---|---|---|---|---|
| | *S. bareilly* | *S. cerro* | *S. enteritidis* | *S. mbandaka* | *S. molade* |
| *Broiler chicken* | | | | | |
| Mouth | – | 1 | 5 | – | 2 |
| External surface | – | 2 | 7 | 1 | 1 |
| Cloaca | – | 1 | 2 | – | – |
| Crop | 1 | 1 | 9 | 2 | 1 |
| Caeca | – | 1 | 8 | 1 | – |
| Small intestine | – | 1 | 5 | 1 | 1 |
| Total | 1 (1.85)* | 7 (12.96) | 36 (66.67) | 5 (9.26) | 5 (9.26) |
| *Environmental samples* | | | | | |
| Feed | – | 1 | 2 | – | – |
| Feeder | 1 | – | 1 | – | – |
| Water | – | 1 | 1 | – | – |
| Drinker | – | 1 | – | – | – |
| Litter | 1 | – | 1 | 1 | – |
| Cage | 2 | 1 | 5 | – | 1 |
| Faeces | – | 1 | 3 | – | 1 |
| Total | 4 (16)** | 5 (20) | 13 (52) | 1 (4) | 2 (8) |

* Figures in parenthesis indicate percentage occurrence of each serotype in broiler chicken.
** Figures in parenthesis indicate percentage occurrence of each serotype in environmental samples.

Seasonal variation in the prevalence of *Salmonella* in broiler chicken and environmental samples is given in Table 3. The results revealed a higher incidence of *Salmonella* during the monsoon season followed by post-monsoon and pre-monsoon in both broiler chicken and environmental samples. However, prevalence of *Salmonella* in broiler chicken and environmental samples did not vary significantly ($P = 0.375$) with seasons.

*Salmonella* contamination levels in broiler chicken encountered in the present study found to be comparable with reports from some countries, though it vary widely with reports from some other countries (Van Asselt et al., 2009; Yan et al., 2010) Considering the poor processing conditions in the retail outlets of the study area chances of cross contamination are rather high, even from a low level of prevalence. The results of our study showed more frequent isolation of *Salmonella*e from crop region followed by caecae. The shedding of *Salmonella* present in the crop, caeca and intestine would result in greatly amplified contamination, which may play an important role in the perpetuation of *Salmonella* in the environment.

*S. enteritidis* contamination was recorded most frequently both in the environment and in the broilers. *S. enteritidis* as the predominant *Salmonella* serotype isolated from humans and in poultry environment has been reported (Oliveira et al., 2010). The second most common serotype contaminating the samples was *S. cerro* followed by *S. molade* in chickens and *S. mbandaka* and *S. bareilly* in environmental samples. Prevalence of *S. enteritidis* was significantly higher ($P < 0.01$) when compared to other *Salmonella* serotypes in broiler chicken samples.

The results of *Salmonella* contamination in environmental samples revealed frequent isolation of salmonellae from cages with wooden

**Table 3**
Seasonal variation in the prevalence of *Salmonella* in broiler chicken and in environmental samples.

| Sample source | Percentage of incidence of *Salmonella* during | | |
|---|---|---|---|
| | Pre-monsoon | Monsoon | Post monsoon |
| Broiler chicken | 18.52 | 46.30 | 35.19 |
| Environmental samples | 12 | 52 | 36 |

platforms. In the retail outlets analysed in the present investigation, spillage of water from drinker and occasional rinsing with water kept the floor in moist condition, which could have provided ideal conditions for survival of salmonellae. The litter collected from the cages contained dry faeces, dust, and spilled feed. *Salmonella* could be isolated frequently from it. Singh et al. (2010) reported modest isolations of salmonellae from poultry farm environment in North India. Prevalence of *Salmonella* in litter is a good indication of flock infection and it is known that infected flocks introduced *Salmonella* to the processing plants. Removing the litter more frequently and cleaning the floors of cages with disinfectant is highly recommended, as such practises are not followed at present in the study area.

Fresh faecal samples recorded high frequency of *Salmonella* contamination. Contaminated faeces were the result of prevalence of *Salmonella* in the chick intestine. The survival capacity of organism outside the host in the contaminated environment and its prolonged survival will be an important factor in the horizontal transmission. In the retail outlets, the birds are exposed to their own excreta when kept on cages with wooden platforms resulting in increased contamination. High stocking density of the birds for slaughter at the retail outlets is also highly conducive for cross contamination. Since the recovery of *Salmonella* was high in cages with wooden platform, use of steel mesh platform in cages is recommended. Even though the stock feed and line water samples were negative for *Salmonella*, they could be recovered from feed and water samples kept in the cages for the birds suggesting cross contamination of feed and water in the cage environment.

From the results observed in the present investigation it is clear that seasons of the year are important factors influencing the prevalence of *Salmonella*. The highest risk for *Salmonella* prevalence was during the monsoon followed by post monsoon and pre monsoon. This result is in accordance with other investigations showing a higher probability of chickens getting infected during the wet and cold seasons (Annan & Jane, 1988). In the study area high humidity during monsoon months enhances the survival of *Salmonella*, as it would provide favourable growth conditions for *Salmonella*, and consequently increase the risk of persistence.

## 4. Conclusions

The present investigation revealed the prevalence of *Salmonella* in the broiler chickens and the environmental samples of the retail outlets. The worldwide increase in *S. enteritidis* in chickens is supported by the results of the present study. The best way to eradicate *Salmonella* is to control them in the primary production of food animals. Several strategies to reduce *Salmonella* colonisation in broiler farm level such as feed and drinking water acidification, immunity enhancement based on active and passive immunity, use of prebiotics, probiotics and symbiotics, modification of feed nutrient composition etc. are being evaluated in different countries of Europe (Vandeplas, Dubois Dauphin, Beckers, Thonart, & Thewis, 2010), to achieve this objective. Such techniques could be adopted in our country to avoid the spread of human salmonellosis through poultry, which is fast becoming a popular food item in India.

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