The Haryana Veterinarian (June, 2025) **64(1),** 81-85 ISSN 0033-4359 (P) **Research Article**

PREVALENCE OF *LISTERIA* SPP. IN CHICKEN MEAT IN PUNJAB

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Received: 12.09.24; Accepted: 28.11.24

ABSTRACT

Listeria spp. is ubiquitous bacteria present worldwide. It is associated with wide variety of foods including meat and is used as a hygiene indicator in all stages of the food processing chain. Meat being a highly susceptible and perishable food commodity needs to be monitored for the presence of various food-borne pathogens including *Listeria* spp. The current study aimed to estimate the prevalence and molecularly characterize *Listeria* spp. from chicken meat samples (n = 698), collected from retail outlets and butcher shops located in different districts of Punjab. A total of 14 (2.0%) samples were positive for *Listeria* spp. and were characterized into three different *Listeria* species *viz*. *L. innocua* (1.15%), *L. grayi* (0.72%) and *L. welshimeri* (0.14%). The presence of *L. innocua* in the study is not of direct pathogenic significance but indicates prevailing unhygienic conditions and potential risk of contamination with pathogenic *Listeria* spp., like *Listeria* monocytogenes. This study emphasizes strict hygiene practices in meat processing and thorough cooking before consumption.

Keywords: Chicken, *Listeria*, Punjab

How to cite: Singh, K., Kaur, S., Singh, R. and Aulakh, R.S. (2025). Prevalence of *Listeria* spp. in chicken meat in Punjab. *The Haryana Veterinarian* **64(1)**: 81-85.

Listeria monocytogenes is a food-borne pathogen present worldwide with a high case-fatality rate (CFR) of approximately 20% to 30% (Choi *et al.*, 2018). It is reported to cause highest hospitalization rate (90.5%) of all the foodborne pathogens (CDC 2000), ranking second among the most frequent causes of death due to foodborne illnesses after salmonellosis (Rossi *et al.*, 2008). Presence of *Listeria* spp. other than *L. monocytogenes* in food and other associated environments is also an indicator of unhygienic conditions of food storage and processing (Siqueira *et al.*, 2017).

Poultry sector in India is growing at a fast pace. Majority of the poultry sector in the country is of the unorganized type in which the birds are slaughtered in retail meat shops without any inspection or sanitary measures and such meat is also not properly stored, hence contributing towards serious health challenges. Additionally, Indian consumers prefer buying freshly slaughtered chicken from the wet markets where chances of cross contamination of chicken carcasses is very high. Keeping in mind the growing poultry sector, increased demand and availability of the chicken meat and the unhygienic conditions prevailing in the food processing environment, the present study was undertaken to assess the prevalence and molecular characterization of *Listeria* spp. in chicken meat samples collected from Punjab state of North India.

MATERIALS AND METHODS

Study design and Estimation of sample size

The study was conducted in all the 22 districts of Punjab, a state located in the north-western region of India. The laboratory work was carried out in the Centre for One Health, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana during May 2019 to August 2020.The sample size was estimated according to simple random sampling method using the formula mentioned below (Thrusfield, 2007).

$$n = \frac{1.962\ P_{exp}\ (1-P_{exp})}{d^2}$$

where, n = sample size, P_{exp}= expected prevalence and d = desired absolute precision.

The expected prevalence value (P) used in the current study for calculation of sample size of chicken was 1.7%, with precision (d) as 1% or 0.01(Kaur *et al.*, 2018). The sample size was estimated with a confidence interval of 95%. For the population size (N), the total population of broilers used in the study was 6,417,614.

Based on the sample size calculation an estimated sample size of 642 for chicken was calculated for the entire state of Punjab. These samples were then further divided over 22 districts of Punjab state, proportionately to the broiler population of respective district (n) using formula mentioned below.

$$\text{Sample Size of Chicken}_{\text{District}} = \frac{\text{Total Broiler Population of District(n)} \times 642}{\text{Total Broiler Population of the State (N)}}$$

The total broiler population of Punjab and broiler population of each district, used in the study was as per the population estimates of poultry documented in 19th Indian Livestock Census- 2012, Department of Animal

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Husbandry, Punjab (Available at:http://www.husbandry punjab.org/pages/livestock.htm).

Collection of samples

A total of 698 chicken samples, with each of them weighing about 50 gm were collected from retail outlets and butcher shops located in different districts of Punjab from the period of May 2019 to March 2020 (Table 1).

Microbiological analysis and biochemical characterization

Isolation of *Listeria* spp. from the chicken samples was attempted as per the United States Department of Agriculture (USDA) method described by McClain and Lee, (1988) after making suitable modifications. About 25 gm of chicken sample was triturated with sterile pestle mortar after which the entire mixture was transferred to a sterile flask and 225 ml of University of Vermont Medium (UVM)-I was added to it. After incubating the flask at 30º C for 24 hrs, 1 ml of UVM-I was transferred to 9 ml of UVM-II which was further incubated at 30º C for 24 hrs. A loopful of the inoculum was then streaked onto Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM) agar plates, which is a selective media for isolation of *Listeria* spp. The plates with typical greenish gray color colonies with black sunken centres showing diffuse black zones of aesculin hydrolysis were considered as presumptive *Listeria* spp. The presumptive colonies that revealed Gram-positive coccobacillary morphology on gram staining were further subjected to subculture in brain heart infusion (BHI) broth and incubated at 37º C for 12-18 hrs. The biochemical characterization was then carried out as per the method described by Bergey's Manual of systematic bacteriology (1984) for characterization into different *Listeria* spp.

Molecular detection of the genus *Listeria* by Real time PCR (qPCR)

After performing biochemical and sugar fermentation tests for detection of *Listeria* spp., confirmation was carried out by qPCR. All presumptive isolates that were subcultured in BHI broth were subjected to DNA extraction by QIAamp DNA kit (Qiagen Pvt. Ltd., New Delhi, India) as per the manufacturer's protocol.

For optimization of qPCR for detection of *Listeria* genus and *L. monocytogenes*, primers targeting *prs* gene and *hly*A gene were used, respectively, as described by Barbau-Piednoir *et al.* (2013). The qPCR amplification was carried out in LightCycler® 96 Roche (Germany). A total reaction volume of 25ul containing 12.5 µl Power SYBR® Green PCR master mix (Thermo Fisher Scientific, UK), 1.0 µl of 10pmol/µl of primer set containing forward and reverse primer each, 2 µl DNA template and sterilized nuclease free water to make up the reaction volume was used.

The standard strain of *L. monocytogenes* (ATCC 19115) was used as positive control, while nuclease free water as negative control. The cycling conditions for qPCR included a single cycle of initial denaturation for 5 min at 94°C followed by 30 amplification cycles of 30s at 94°C (denaturing step) and 30s at 60°C (annealing and extension step). The final result of each reaction was expressed in threshold cycle (Ct). The qPCR software (LightCycler® 96 SW 1.1) was used for data analysis.

Sequencing of prs gene and phylogenetic analysis

Representative amplicon of putative phosphoribosyl pyrophosphate synthetase (*prs*) gene were purified using PCR purification kit (Qiagen) and outsourced for sequencing in both directions from Eurofins Genomics India Pvt Ltd. Each gene sequence fragment was assembled by BioEdit and the assembled sequences were blasted using online BLASTn software. The Mega 6.0 software was used to construct phylogenetic trees using the maximum likelihood algorithm.

RESULTS AND DISCUSSION

Out of 698 chicken meat samples collected during the study, 14 chicken meat samples were found to be positive for *Listeria* spp. as confirmed by isolation, biochemical analysis, and real time PCR. In the real time PCR targeting genus specific *prs* gene, the standard strain of *Listeria* showed amplification with Ct value of 17.92 and melt curve in the range of 72-73° C. Melt curve of all the 14 *Listeria* spp. isolated in the present study also lied in the range 71-73° C and with Ct value in the range of 19-28 (Fig. 1a and 1b).

In the species specific real time PCR with *hly*A gene, the standard strain of *L. monocytogenes* showed amplification with Ct value of 21.84 and melt curve in the range of 75-76° C. However, none of the isolates were positive for *L. monocytogenes* by species specific qPCR (Fig 2a, 2b).

Based on the sugar fermentation results, 14 isolates from 698 chicken samples were characterized into three different *Listeria* species i.e. 1.15% (8/698) as *L. innocua*, 0.72% (5/698) as *L. grayi* and 0.14% (1/698) as *L. welshimeri*. None of the isolates were identified as *L. monocytogenes* on the basis of sugar fermentation tests as well as molecular detection.

Based on these results we can state that, the overall prevalence of *Listeria* in chicken meat is 2.0% (14/698) and the species detected were *L. innocua*, *L. grayi* and *L.*

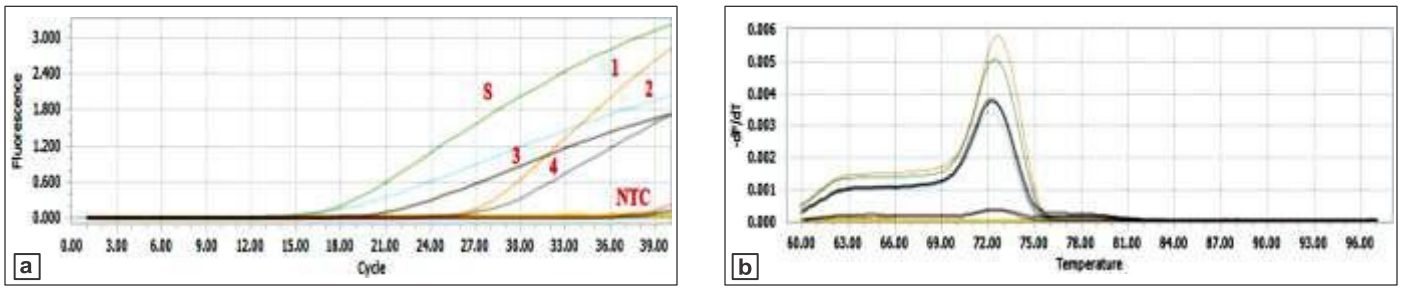

Fig. 1. **(a)** Amplification plots (qPCR) for detection of *Listeria* genus, S- Standard (ATCC 19115), 1-4- Field isolates positive for *prs* gene, NTC- Non template control **(b)** Melting curve (qPCR) for detection of *Listeria* genus

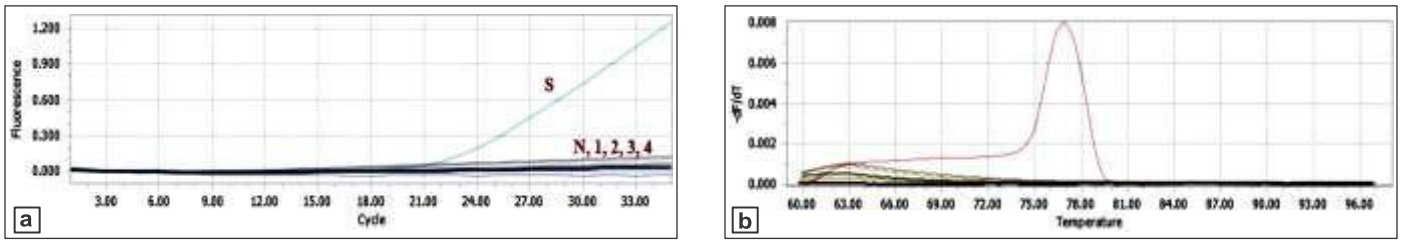

Fig. 2. **(a)** Amplification plot (qPCR) for detection of *L. monocytogenes*, S- Standard (ATCC 19115), 1-4- Field isolates negative for *hly*A gene, N- Non template control **(b)** Melting curve (qPCR) for detection of *L. monocytogenes*

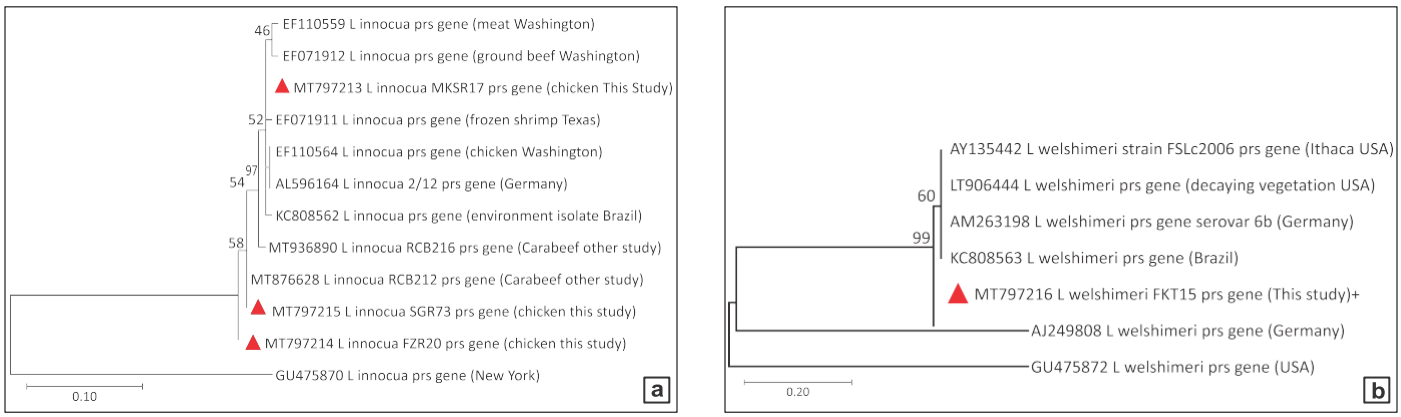

Fig. 3. **(a)** Phylogenetic tree of *L. innocua* isolates MKSR17, SGR73 and FZR20 **(b)** Phylogenetic tree of *L. welshimeri* isolate FKT15

welshimeri. The findings of the present study can be correlated with the findings of Ismaiel *et al.* (2014) who isolated only *L. innocua* and *L. grayi* from one sample each out of 30 frozen chicken samples in Egypt. Maharjan *et al.* (2019) reported absence of *Listeria* spp. from the chicken meat samples during slaughter process in Kathmandu, Nepal.

Similar finding of absence of *L. monocytogenes* in chicken meat samples were presented by Raja *et al.* (2016) in Chennai, Zade and Karpe (2010) in Vidarbha region of Maharashtra, Dhanashree *et al.* (2003) in Mangalore. Studies conducted by Kumar *et al.* (2014) in Hyderabad and Latha *et al.* (2017) in Kerala also reported absence of *Listeria* spp in chicken meat.The findings of our study can also be correlated with the few earlier studies conducted in the Punjab state *viz*., Kaur *et al.* (2018) and Dutta *et al.* (2020) who found only 1.7% and 2.0% of the samples to be contaminated with *L. monocytogenes*.

The current prevalence of *Listeria* spp. in chicken is on lower side as compared to some of the other studies which have stated a higher prevalence (Shakuntala et al. 2019, Panera-Martínez *et al.*, 2022). This large variation in the prevalence can be attributed to variation in microbiological analysis methods, geographical differences, environmental factors, sample size, sampling strategy, hygienic conditions, contamination by other secondary pathogens as well as seasonal variation during sample collection. The samples collected from internal or external part of carcass may also influence the prevalence as external region is more prone to contamination from surroundings. Most of the sampling done in the current study was from retail shops, wherein the chicken samples were collected randomly without distinguishing between the parts of the carcass being sampled. The obtained low prevalence in present study could also be due to actual lower prevalence of *L. monocytogenes* in Punjab. A detailed region wise

Table 1. Distribution of broiler population and the chicken sample size in various districts of Punjab

Sr. No.	District	District Broiler Population	Calculated Chicken Sample Size	Collected Chicken Samples
1.	Ludhiana	733,303	73	75
2	SAS Nagar	64,156	6	10
3	Jalandhar	355,851	36	38
4	Rupnagar	280,892	28	30
5	Mansa	123,229	12	14
6	Barnala	172,202	17	20
7	Patiala	141,001	14	20
8	Bathinda	118,983	12	14
9	Tarn Taran	100,982	10	14
10	Sangrur	950,416	95	100
11	Hoshiarpur	600,820	60	60
12	Gurdaspur	1,567,212	157	63
13	Pathankot			100
14	Amritsar	184,513	18	20
15	Fazilka	125,363	13	20
16	Ferozpur			
17	Faridkot	45,200	5	10
18	Sri Muktsar Sahib	91,368	9	10
19	Fatehgarh Sahib	430,965	43	45
20	Kapurthala	181,673	18	19
21	Moga	54,867	6	6
22	SBS Nagar	94,617	10	10
	Total	6,417,614	642	698

Table 2. District wise prevalence of *Listeria* spp. in chicken meat samples

Sr. No.	District	No. of samples	No. of positive samples (%)			Total No. of positive samples
			L. innocua	*L. grayi*	*L. welshimeri*	
1.	SAS Nagar	10	0	0	0	0
2.	Fatehgarh Sahib	45	0	0	0	0
3.	Jalandhar	38	0	0	0	0
4.	Rupnagar	30	0	0	0	0
5.	Gurdaspur	63	0	0	0	0
6.	Patiala	20	0	0	0	0
7.	Bathinda	14	0	0	0	0
8.	Hoshiarpur	60	1 (1.66%)	2 (3.33%)	0	3(5%)
9.	Tarn Taran	14	0	0	0	0
10.	Fazilka	0	0	0	0	0
11.	Ferozpur	20	3 (15%)	2 (10%)	0	5 (25%)
12.	Sangrur	100	1 (1%)	0		1 (1%)
13.	Mansa	14	0	0	0	0
14.	Pathankot	100	0	0	0	0
15.	Kapurthala	19	0	0	0	0
16.	Moga	6	0	0	0	0
17.	Amritsar	20	0	0	0	0
18.	Sri Muktsar Sahib	10	1 (10%)	0	0	1 (10%)
19.	SBS Nagar	10	0	0	0	0
20.	Ludhiana	75	2 (2.66%)	1 (1.33%)	0	3 (4%)
21.	Barnala	20	0	0	0	0
22.	Faridkot	10	0	0	1 (10%)	1 (10%)
	Total	698	8(1.15%)	5(0.72%)	1(0.14%)	14(2.0%)

apparent prevalence of *Listeria* spp. in chicken meat from the 22 districts of Punjab state is shown in Table 2.

The ability to produce bacteriocin-like inhibitory compounds against *L. monocytogenes* leading to an underestimation of the prevalence of *L. monocytogenes* (Gwida *et al.*, 2020). Since two step enrichment broth procedure was used in this study, *L. innocua* might have outgrown *L. monocytogenes*. Carvalheira *et al.* (2010) reported that the presence of *L. innocua* in the samples analysed could inhibit the growth of *L. monocytogenes* initially present in the samples, resulting in false negative detection of *L. monocytogenes*. It has also been explored that *L. innocua* occupies the same ecological niche and its high incidence signifies potential contamination by *L. monocytogenes* (Walsh *et al.*, 1998). The existence in the similar ecological niches and comparatively high genomic similarity among *L. innocua* and *L. monocytogenes* also provides for the opportunity of transfer of virulent or resistant genes (Li *et al*., 2021).

Kaszoni-Rückerl (2020) also suggested that *L. innocua* is a good hygiene indicator and also a marker for unrecognized *L. monocytogenes* contamination events in the food processing environments. Presence of *Listeria* spp. other than *L. monocytogenes* in chicken samples may not be of direct pathogenic significance, however, these organisms can be used as indicators for pointing out towards deterioration in hygienic conditions and a potential risk of contamination with pathogenic *Listeria* spp. (Lakicevic *et al.*, 2010). Chickens are commonly identified as carriers of *Listeria* spp., often harboring these species in their intestines. The risk of *Listeria* spp. contamination extends to chicken meat during processing, particularly due to fecal contamination occurring during evisceration processes or to inadequate hygiene practices of the processing area (Aziz and Mohamed, 2020).

Four representative *Listeria* isolates MKSR17, SGR73, FZR20 and FKT15 were outsourced for sequencing for *prs* gene from Eurofins Genomics India Pvt Ltd. The obtained nucleotide sequences after assembling were subjected to BLASTn software on NCBI. BLAST results confirmed that MKSR17, SGR73, FZR20 were *L. innocua* and FKT 15 was *L. welshimeri*. All the four isolates' sequences were submitted in NCBI Genbank and the accession nos were obtained as MT797213, MT797214, MT797215 and MT797216. The phylogenetic analysis of the sequenced samples was carried out using Mega 6.0 software (Fig. 3a & 3b).

CONCLUSION

A total of 14 *Listeria* spp. were isolated from chicken with the prevalence of *Listeria* spp. as 2.0%. Out of the 14

Listeria isolates, 1.15% (8/698) were identified as *L. innocua*, 0.72% (5/698) as *L. grayi*, 0.14% (1/698) as *L. welshimeri*. Presence of *Listeria* spp. other than *L. monocytogenes* in chicken samples are not of direct pathogenic significance, however presence of *L. innocua* points to potential risk of contamination with pathogenic *L. monocytogenes* in poultry processing environment. Therefore, this study recommends strict compliance of hygiene practices at retail meat shops, butcher shops, slaughter houses and thorough cooking of meat before consumption. Additionally, shop owners and butchers should be made aware of zoonotic diseases like listeriosis and the importance of good hygiene practices. Furthermore, these butcher shops and retail outlets should be regulated by a governing body to ensure proper practices.

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