The Haryana Veterinarian (December, 2024) **63(2),** 244-250 ISSN 0033-4359 (P) **Research Article**

ISOLATION AND CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA* SPP. IN ANIMALS AND HUMANS

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ABSTRACT

Listeria spp. including *L. monocytogenes* and *L. ivanovii* is an important species of food-borne pathogen responsible for multiple outbreaks and a high case fatality rate in human and animal populations worldwide. In the present study, a total of 462 animal samples including 136 samples each of faeces, blood, vaginal swabs, and 54 urine samples from 78 goats and 58 sheep, whereas 107 human samples comprising 47 blood, 52 urine and 8 vaginal swabs were analysed for isolation and identification of *Listeria* spp., followed by biochemical profiling and molecular confirmation by conventional PCR. Out of 78 blood, faeces, vaginal swabs, and 32 goat urine samples analysed, *Listeria* spp. were isolated from 1.88% of each of the faecal and vaginal samples, respectively. Out of 58 blood, faeces, vaginal swabs and 22 sheep urine samples analysed, 2.55% and 0.51% of the faecal and vaginal swabs, respectively were positive for the *Listeria* species. However, no isolate was obtained from the blood and urine samples of both goats and sheep. Out of the 52 urine samples, 8 vaginal samples, and 47 blood samples from humans, 1.86% and 0.93% of urine and vaginal samples, respectively showed the presence of *L. monocytogenes*. Whereas, none of the blood samples were positive for *Listeria* spp. Among 462 samples from sheep and goats, 16 (3.46%) samples showed *Listeria* spp., of which 12 isolates were of *L. monocytogenes*. From a total of 107 samples from humans, 2.80% samples were positive for *L. monocytogenes*. CAMP and HSA tests revealed haemolytic activity in 17 out of the 19 isolates. Multiplex PCR serotyping of the *L. monocytogenes* isolates revealed that in sheep and goats, 66% belonged to the 4b strain, and the remaining 34.44% to the 1/2b strain. Out of the 3 human isolates, 66.67% belonged to the 1/2b strain and 33.33% to the 4b strain. Multiplex PCR for the detection of virulence genes in animal samples revealed a positivity of 100% for *hly*A, 75% each for *act*A and *plc*A, whereas the *iap* gene was absent. Among the 3 isolates from human samples, 100% and 33.33% were positive for *hly*A and *act*A genes, respectively and the *plc*A and *iap* genes were absent. The study observes that small ruminants provide strong evidence of the circulation of 4b strains of *L. monocytogenes*. The presence of pathogenic strains of *Listeria* spp. in farm workers in the current study highlights the importance of Listeriosis as an occupational zoonosis.

Keywords: *Listeria monocytogenes*, Blood, Sheep, Goat, Multiplex PCR, Virulence genes

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Listeriosis is an important emerging foodborne illness in India, having 21 species in the genus, of which *L. monocytogenes* and *L. ivanovii* are potentially pathogenic to humans and animals. The disease expression varies significantly in severity ranging from no apparent infection in healthy adults to severe symptoms like meningoencephalitis, stillbirth, abortions and gastroenteritis in immuno-compromised, geriatric, and pregnant patients (Barbuddhe *et al.*, 2022; CDC, 2022). *Listeria* is ubiquitous, and commonly found in the intestine of many domestic small ruminants, particularly sheep. Therefore, animals and foods of animal origin (unpasteurized milk and undercooked or uncooked meat) can be a source of human infection (Singh *et al.*, 2019; Farber *et al.*, 2007).

With rising demand for mutton and chevon in Maharashtra, ensuring microbial safety is crucial. Low-income, small-scale farmers often hesitate to follow food safety norms thus increasing infection risks. This highlights the need for awareness among livestock farmers, stricter food safety enforcement and assessing risks from *Listeria* spp.

Isolation of *Listeria* spp. remains the gold standard for diagnosis (Rocourt *et al.*, 1983), with serovars 1/2a, 1/2b and 4b linked to most listeriosis cases globally and 4b being predominant in India (Bracegirdle *et al.*, 1994, Barbuddhe *et al.*, 2016). Molecular methods like PCR offer higher specificity and sensitivity compared to culture -based techniques, even in low-pathogen samples (Schoonbroodt *et al.*, 2023). Traditional serodiagnostic methods are less reliable due to cross-reactivity with other bacteria like *Enterococci*, *Staphylococci* and *Bacillus* spp. (Singaravelu *et al.*, 2018). According to Dudhe *et al.* (2014), the PI-PLC assay, which evaluates the pathogenicity of *Listeria* spp., reliably differentiates pathogenic strains.

Listeriosis caused by *Listeria monocytogenes* has a high case-fatality rate of 20-30% (Choi *et al.*, 2018), primarily due to the development of septicemia and encephalitis in humans and animals (Barman *et al.*, 2020). Its sturdy persistence is enhanced by biofilm formation on various surfaces (Osek *et al.*, 2022). Diseased animals shed the pathogen through faeces, urine, milk and nasal secretions, contaminating the environment. Asymptomatic faecal shedding by farm animals further spreads *L. monocytogenes* to handlers, posing food safety risks (Schoder *et al.*, 2022). Additionally, asymptomatic carriers are found to be

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widespread among mammals (Povolyaeva *et al.*, 2020).

The western region of Maharashtra characterized by its advanced agricultural practices, with farmers in this area owning a substantial number of dairy animals, contribute significantly to the state's agricultural output and economic growth. Moreover, 25.56 percent of farmers have animal husbandry as their main occupation in the Western Region of Maharashtra (Desai *et al.*, 2012). According to a study by Darade *et al.* (2023), a significant number of dairy farmers in the Western Region of Maharashtra had only medium extension contacts, social participation and source of information regarding biosecurity measures and practices adopted at farm levels.

Considering the above points, the current study was undertaken focusing on the prevalence estimation of *Listeria* spp. in sheep and goats and their handlers from clinical samples (urine, blood, and vaginal swabs). The study also aimed at the isolation, molecular confirmation, and virulence potential of the different serotypes.

MATERIALS AND METHODS

Study Design: The present study targeted the western region of Maharashtra mainly covering the areas-Pune, Satara, Ahmednagar, Sangli and Mumbai districts. One farm from each district was selected namely- NARI farm from Satara, MPKV animal farm- from Rahuri area of Ahmednagar district, Mumbai Veterinary College Farm in Mumbai and one migratory flock from Bhor region of district Pune. Random selection and sample collection of animals and their handlers were done after obtaining the voluntary consent provided by the participants.

Samples: The research study sample set was segregated into 2 portions with one sample set collected from sheep and goats and another human set. A total of 462 samples were collected from sheep (196) and goats (266). A total of 107 samples which consist of 47 blood, 52 urine and 8 vaginal swabs were collected from humans. The faecal samples and vaginal swabs from sheep, goats and humans were collected in sterile Brain Heart Infusion (BHI) broth, whereas, blood and urine were collected in sterile bottles and transported to the laboratory under refrigeration conditions at 4º C.

Isolation: The isolation of the organism was done as per USDA protocol (Gasanov *et al.*, 2005). Samples were subjected to a two-step enrichment process with UVM-1 (University of Vermont Medium-1) and UVM-2 (University of Vermont Medium-2) under strict sterile conditions. Following the enrichment, plating of the inoculum was done on Polymyxin Acriflavine Lithium Chloride Ceftazidime Aesculin Mannitol Agar (PALCAM). For vaginal swabs and faecal samples, 1 ml of inoculum from the sample in BHI broth was transferred to 10 ml of UVM-1. After overnight incubation at 30º C, 0.1 ml inoculum from UVM-1 was transferred to UVM-2 and incubated overnight.

For blood and urine, 5 ml of the collected sample was mixed with 50 ml of UVM-1 and incubated overnight at 30º C, followed by secondary enrichment in UVM-2. A loopful of inoculum after secondary enrichment from each of the sample categories was plated on PALCAM agar and incubated for 48 hours at 25º C. For phenotypic characterization, Gram staining of presumptive *Listeria* isolates was done followed by biochemical tests. Also, sugar fermentation tests were done to further identify and confirmthepatternofcarbohydratefermentation demonstrated by the presumptive bacterial culture (Nayak *et al.*, 2015).

***In vitro*-pathogenicity test:** The biochemically confirmed isolates were then subjected to *in vitro*-pathogenicity tests namely-haemolysis on sheep blood agar (HSA), Christie-Atkins-Munch-Peterson (CAMP) and Phosphatidylinositol-specific phospholipase C (PI-PLC) tests. Samples processed for haemolysis pattern were observed in sheep blood agar, CAMP test was used for the segregation of species based on their haemolysis pattern whereas PI-PLC testing helps in determining the pathogenicity potential of isolates (McKernan *et al.*, 2020).

DNA extraction and PCR amplification: DNA was extracted from the isolates obtained from PALCAM agar as per the already established methods for phenotypically confirmed *Listeria* spp. colonies (Doumith *et al.*, 2004). A multiplex PCR assay was standardized for the detection of three major serovars of *L. monocytogenes* namely 1/2a, 1/2b and 4b isolated from clinical samples of sheep and goats with a history of spontaneous abortion or reproductive disorders and from farm workers closely working on farms by following the methodology as described with suitable modifications (Pournajaf *et al.*, 2016). For amplification of *Imo*0737, ORF2819, ORF2110 and *prs* genes, primers were obtained from Sigma Aldrich (Table 2). Standardization of PCR was done using the standard strain of *L. monocytogenes* 4b (MTCC 1143), *L. monocytogenes* 4b (NCTC 11994), *L. monocytogenes* 1/2a (NCTC 7973) and *L. monocytogenes* 1/2b (NCTC 10887).

PCR amplification was performed in Master Cycler Gradient Thermocycler (Eppendorf, Germany) with a final volume of 25 µl containing 5.0 µl of 10X PCR buffer (consisting of 100 mM Tris-HCl, pH 8.3; 500 mMKCl; 15 mM $MgCl_2$ and 0.01% gelatin), 1.5 µldNTP mix (10 mM, with a final concentration of 0.2 mM), 4 µl of 25 mM $MgCl_2$ (final concentration 2 mM) and 1 µl of forward and reverse primer of each set with final concentration 0.1 µM each and 2 U/µl of TaqDNA polymerase, 5 µl of DNA

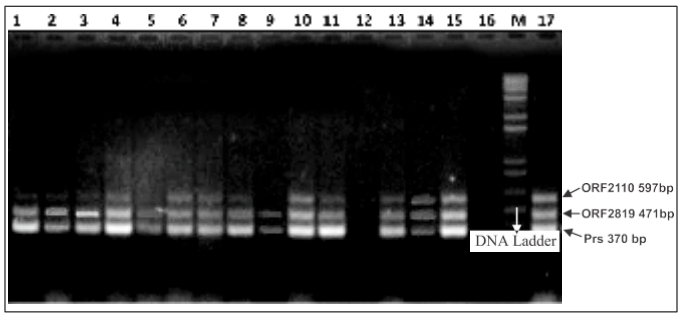

Fig. 1. PCR serotyping-based profiling of associated genes of *L. monocytogenes* - ORF2819-471bp, ORF2110-597bp, Prs-370bp. 1-NH5V (ORF2819 and prs), 2-SS16F (ORF2819 and prs), 3-NG3V (ORF2819 and prs), 4-BH2U (ORF2110, ORF2819 and prs), 5-SG8F (ORF2819 and prs), 6-NG2F (ORF2110, ORF2819 and prs), 7-SS19V (ORF2110, ORF2819 and prs), 8-NH5U (ORF2819 and prs), 9-GG2V (ORF2819 and prs), 10-RG23V (ORF2110, ORF2819 and prs), 11-NS18F (ORF2110, ORF2819 and prs), 12- Negative Control 13-RG6V (ORF2110, ORF2819 and prs), 14-SG2F (ORF2110, ORF2819 and prs), 15-NS9F (ORF2110, ORF2819 and prs), 16-Negative Control, 17- RS4F (ORF2110, ORF2819 and prs), M- DNA Ladder 100-1000bp

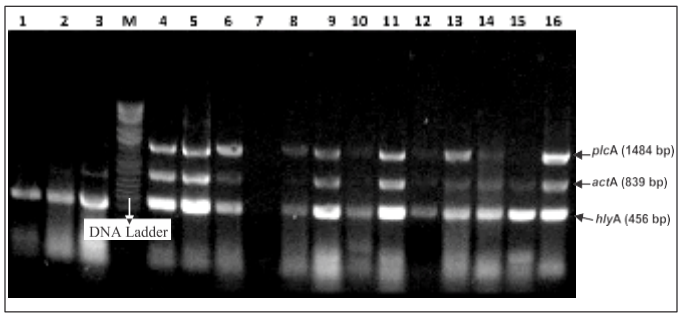

Fig. 2. PCR Results Based on virulence marker genes of *Listeria monocytogenes- plc*A, *act*A, *hly*A
1-NH5U (*hly*A), 2-NH5V (*hly*A), 3-BH2U (*hly*AandactA), 4-NS9F (*hly*A, *act*AandplcA), 5-NS18F (*hly*A, *act*AandplcA), 6-NG2F (*hly*A, *act*AandplcA), 7-Negative Control, 8-NG3V (*hly*AandplcA) 9-RS4F (*hly*A, *act*AandplcA), 10-RG6V (*hly*A), 11-RG23V (*hly*A, *act*AandplcA), 12-SS16F (*hly*A), 13-SS19V (*hly*A, *act*AandplcA), 14-SG2F (*hly*A, *act*AandplcA), 15-SG8F (*hly*AandactA), 16-GG2V (*hly*A, *act*AandplcA), M-DNALadder 100-3000bp

Table 1. *In-vitro* pathogenicity of *Listeria* spp. based on CAMPTest and Haemolysis Pattern

Sr. No.	Source of sample	Samplecode	*In-vitro* pathogenicity profile			
			Haemolysis on SBA	CAMP with *S. aureus* (S) Or *R. equi* (R)	PI-PLC	*Listeria* spp.
1	NARI farm, Faltan, Dist. Satara	NS9F	+	+S	+	*L. monocytogenes*
		NS18F	+	+S	+	*L. monocytogenes*
		NG2F	+	+S	+	*L. monocytogenes*
		NG3V	+	+S	+	*L. monocytogenes*
		NG17F	-	-	+without halo	*L. seeligeri*
2	MPKV animal farm, Rahuri,	RS4F	+	+S	+	*L. monocytogenes*
	Ahemadnagar (Rahuri farm)	RG6V	+	+S	+	*L. monocytogenes*
		RG10V	+	+R	+	*L. ivanovii*
		RG23V	+	+S	+	*L. monocytogenes*
3.	PSAD sheep and goat farm,	SS1F	+	+R	+	*L. ivanovii*
	Sangli (Sangli farm)	SS16F	+	+S	+	*L. monocytogenes*
		SS19V	+	+S	+	*L. monocytogenes*
		SG2F	+	+S	+	*L. monocytogenes*
		SG8F	+	+S	+	*L. monocytogenes*
4.	Goregaon farm MVC, Mumbai	GG2V	+	+S	+	*L. monocytogenes*
5.	Bhor Migratory herds, Pune	BG2F	-	-	+without halo	*L. seeligeri*
			Human Sample			
1.	NARI farm, Faltan, Dist. Satara	NH5U	+	+S	+	*L. monocytogenes*
		NH5V	+	+S	+	*L. monocytogenes*
2.	Bhor Migratory herds, Pune	BH2U	+	+S	+	*L. monocytogenes*

template and sterilized milliQ water to make up the reaction volume. In the initial stage of PCR, denaturation was performed at 94° C for 5 min followed by 35 cycles of 94° C for 30 sec, 54° C for 1.15 min,72° C for 1.15 min and a final extension cycle for 10 min. at 72° C.

For virulence determination of *L. monocytogenes*, 4 genes were targeted namely *plc*A, *iapact*A and *hly*A. A multiplex PCR was standardized for primer sets namely

Table 2. Details of primers for amplification of target genes of Listeria monocytogenes employed in serotyping PCR

Gene target		Primer Sequence	Product Size (bp)	Serovar specificity
lmo0737	Forward	5'- AGG GCT TCAAGG ACT TAC CC-3'	691	*L. monocytogenes*
	Reverse	5'- ACG ATT TCT GCT TGC CAT TC -3'		1/2a
ORF2819	Forward	5'- AGC AAAATG CCAAAA CTC GT -3'	471	*L. monocytogenes*
	Reverse	5'- CAT CAC TAAAGC CTC CCATTG 3'		1/2b and 4b
ORF2110	Forward	5'- AGT GGA CAATTG ATT GGT GAA - 3'	597	*L. monocytogenes*
	Reverse	5'- CAT CCATCC CTTACT TTG GAC-3'		4b
prs	Forward	5'- GCT GAA GAG ATT GCG AAA GAA G- 3'	370	All *Listeria* species
	Reverse	5'- CAAAGAAAC CTT GGATTT GCG G -3'		

Table 3. Details of Primers used for amplification of virulence marker associated genes of *Listeria monocytogenes*

Gene target		Primer Sequence	Product Size (bp)
plcA	Forward	5'- CTG CTT GAG CGT TCATGT CTC ATC CCC C - 3'	1484
	Reverse	5'- CAT GGG TTT CAC TCT CCT TCTAC – 3'	
actA	Forward	5'- CGC CGC GGAAAT TAAAAAAAG A - 3'	839
	Reverse	5'- ACG AAG GAA CCG GGC TGC TAG - 3'	
hlyA	Forward	5'- GCA GTT GCAAGC GCT TGG AGT GAA - 3'	456
	Reverse	5'- GCAACG TAT CCT CCA GAG TGATCG - 3'	
Iap	Forward	5'- ACAAGC TGC ACC TGT TGC AG - 3'	131
	Reverse	5'- TGA CAG CGT GTG TAG TAG CA -3'	

Table 4. PCR-based profile of serotype-associated genes of *L. monocytogenes* obtained from animal and human clinical samples

Sr. No.	Source of sample	Sample code	Multiplex PCR amplification of Specific serotype associated genes for *L. monocytogenes*				Serovar of *L. monocytogenes* identified
			Lmo 0737 (691 bp)	ORF 2110 (597 bp)	ORF 2819 (471 bp)	Prs (370 bp)	
1.	NARI farm, Faltan, Dist. Satara	NS9F	-	+	+	+	4b
		NS18F	-	+	+	+	4b
		NG2F	-	+	+	+	4b
		NG3V	-	-	+	+	1/2b
2.	MPKV animal farm, Rahuri, Dist.	RS4F	-	+	+	+	4b
	Ahemadnagar (Rahuri farm)	RG6V	-	+	+	+	4b
		RG23V	-	+	+	+	4b
		SS16F	-	-	+	+	1/2b
		SS19V	-	+	+	+	4b
		SG2F	-	+	+	+	4b
		SG8F	-	-	+	+	1/2b
3.	Goregaon farm BVC, Mumbai	GG2V	-	-	+	+	1/2b
	Serotype-associated genes of *L. monocytogenes* obtained from human clinical samples						
	Vaginal swab samples						
1.	NARI farm, Faltan, Dist. Satara	NH5V	-	-	+	+	1/2b
	Urine sample						
1.	NARI farm, Faltan, Dist. Satara	NH5U	-	-	+	+	1/2b
2.	Bhor Migratory Herd	BH2U	-	+	+	+	4b

Table 5. PCR-based profile of virulence-associated genes in L. monocytogenes isolates Obtained from animal and human clinical samples

Sr. No.	Source of sample	Sample code	Amplified PCR products of virulence-associated genes detected for *L. monocytogenes*			
			*plc*A (1484 bp)	*act*A (839 bp)	*hly*A (456 bp)	*iap* (131 bp)
1.	NARI farm, Faltan, Dist. Satara	NS9F	+	+	+	-
		NS18F	+	+	+	-
		NG2F	+	+	+	-
		NG3V	+	-	+	-
2.	MPKV animal farm, Rahuri, Dist. Ahemadnagar (Rahuri farm)	RS4F	+	+	+	-
		RG6V	-	-	+	-
		RG23V	+	+	+	-
		SS16F	-	-	+	-
		SS19V	+	+	+	-
		SG2F	+	+	+	-
		SG8F	-	+	+	-
3.	Goregaon farm, BVC, Mumbai	GG2V	+	+	+	-
	Vaginal swab samples					
1.	NARI farm, Faltan, Dist. Satara	NH5V	-	-	+	-
	Urine sample					
1.	NARI farm, Faltan, Dist. Satara	NH5U	-	-	+	-
2.	Bhor Migratory Herd	BH2U	-	+	+	-

*plc*A, *hly*A and *act*A as per the method described by Rahimi *et al.* (2014). For the amplification of the virulence genes, primers were obtained from Sigma Aldrich (Table 3). PCR reaction was run at 25 µl reaction volume containing 2.5 µl of 10X PCR buffer (consisting of 100 mMTris-HCl, pH 8.3; 500 mMKCl; 15 mM $MgCl_2$ and 0.01% gelatin), 2 µl dNTP mix (10 mM, with a final concentration of 1 mM), 6 µl of 25 mM $MgCl_2$ (final concentration 7.5 mM) and 0.25 µl of forward and reverse primer of each set (final concentration 0.1 µM each), 2 U/µl of TaqDNA polymerase and 2.5 µl of DNA template and sterilized Milli-Q water for volume makeup. The cycling conditions (Eppendorf, Germany) for PCR included an initial denaturation step at 95° C for 2 min followed by 35 cycles each of 15 seconds denaturation at 95° C, 30 seconds of annealing at 60° C and 90 seconds of extension at 72° C, followed by a final extension of 10 min. at 72° C and held at 4° C.

The PCR products from both reactions were then visualized by 2% agarose gel electrophoresis. The results were recorded using the Gel documentation system (Thermo Scientific, Waltham, USA). A 100-bp DNA ladder was used as a size marker (Sigma Aldrich, US).

RESULTS AND DISCUSSION

An overall positivity of 3.46% was recorded with 16/462 samples being positive for *Listeria* spp. in animals. Research conducted by Zhao *et al.* (2021) in China, showed twice the prevalence rate of up to 7.8% of *Listeria* spp. with *L. monocytogenes* around 11.41% (117/ 1025). In the current study, from animal samples, a prevalence of 4.86% (7/144) and 7.35% (10/136) of *Listeria* spp. was reported from vaginal and faecal samples, whereas none of the blood and urine samples showed the presence of *Listeria* spp. In a study conducted in Iran, *Listeria monocytogenes* was isolated from 11.3% of vaginal samples (Meghdadi *et al.*, 2019), higher than rates in the current study. However, the lower prevalence of bacteria in the current study is likely due to stricter biosecurity and increased farmer awareness in recent years.

The prevalence of *Listeria* spp. and *L. monocytogenes* amongst all the sheep cases (58 sheep) analyzed were found to be 10.34% and 8.62%, respectively, whereas for *L. ivanovii* prevalence rate of 1.72% was observed. *L. monocytogenes* was isolated from 23.5% of the ovine population studied by Hurtado *et al.* (2017) in Spain. Keelara *et al.* (2015) reported infection rates three times lower than the current study among organized and migratory flocks, underscoring the common issue of underreporting listeriosis (Ávalos-Flores *et al.*, 2022). A higher prevalence of *L. monocytogenes* was found in the NARI farm (3.27%), while Rahuri farm (2.94%) and Sangli farm (2.67%) showed almost similar prevalence.

The prevalence of *Listeria* spp. among goat samples

was 3.75%, whereas for *L. monocytogenes* it was 2.63%. From the vaginal swab samples, 1.50% were confirmed as *L. monocytogenes* and 0.38% as *L. ivanovii*. From fecal samples, 1.13% of isolates recovered were confirmed as *L. monocytogenes*, while 0.75% was positive for *L. seeligeri*. The highest prevalence (6.66%) was recorded in Goregaon farm, 5% observed in Bhor migratory goat herds, while the lowest was recorded with 2.97% in Rahurifarm. Osman *et al.* (2020) identified *L. monocytogenes* as the most reported species in sheep and goats, aligning with its prevalence in sheep with an abortion history or reproductive disorders in India (Ávalos-Flores *et al.*, 2022).

An occurrence of 2.80% was recorded in farm workers and animal handlers of the associated farms in the study. A total of one urine and vaginal sample each from NARI farm, Sangli and one urine sample from Bhor migratory herd were found to be positive, however, all the blood samples were negative for *Listeria* spp.

In the CAMP test, 12 isolates of animal origin showed enhanced haemolysis with *S. aureus* whereas, all 3 isolates from humans exhibited positive haemolysis, a key characteristic indicative of *Listeria monocytogenes*. CAMP and HSAidentified 12 isolates as *L. monocytogenes*, while 4 showed variability, identifying as *L. ivanovii* or *L. innocua*/ *L. welshimeri*.

For PI-PLC assay, 14 and 3 isolates from animals and humans, respectively showed characteristic of pathogenic strains whereas, the other 2 isolates had blue colonies without opaque halo observed in non-pathogenic strains. An interesting finding in the study was the presence of only pathogenic strains in affected humans, suggesting that non-pathogenic strains may be eliminated from the body, as they were absent in animal handlers (Table 1).

On PCR amplification of all 12 isolates of presumptive *L. monocytogenes* obtained from sheep and goats, 66.66% belonged to the 4b serovar, and the remaining 33.33% to the 1/2b serovar (Fig. 1, Table 4). Five isolates from faecal swabs and three from vaginal swabs belonged to serovar 4b, whereas the remaining 4 (2 each from faecal and vaginal swabs) were 1/2b strain. Among 3 isolates from humans, 2 isolates belonged to 1/2b serovar and 1 to 4b serovar (Table 4).

The virulence pattern on the isolates was determined by targeting 4 virulence genes *plc*A (1484 bp), *act*A (839 bp), *hly*A (456 bp) and *iap* (131 bp) (Fig. 2). Among the 12 isolates from animal samples, all the isolates were positive for *hly*A, 9 were positive for *act*A and *plc*A, whereas, *iap* gene was absent in the mentioned isolates (Table 5). A study conducted by Flores *et al.* (2022), also reported the presence of *hly*A gene from all their isolates. Among 8 isolates that were positive for a combination of *plc*A, *act*A and *hly*A, five isolates were recovered from faecal swab samples and three from a vaginal swab. One isolate each was positive for *act*A and *hly*A genes and *plc*A and *hly*A genes in combination, from faecal and vaginal swabs, respectively. Two isolates, one from vaginal swabs (RG6V) and another from faecal swabs (SS16F) contained only *hly*A gene (Table 5). Among the human *Listeria* isolates, all 3 were positive for *hly*A with 2 isolates demonstrating only *hly*A virulence genes while the 3rd isolate was positive for both *hly*A and *act*A associated genes (Table 5). Parihar *et al.* (2008) also observed three virulence-associated *hly*A, *act*A and *iap* genes in five isolates and *hly*A and *act*A genes in one of 144 human placental bits analysed.

CONCLUSION

The study provides strong evidence of the circulation of 4b strains of *L. monocytogenes* in small ruminants. Transmission is unidirectional, occurring from infected animals to humans, particularly affecting animal handlers, emphasizing its importance as an occupational zoonosis. Among *L. monocytogenes* isolates, virulence marker genes *viz*. *plc*A, *act*A and *hly*A all together or in various combinations were detected. PCR results obtained in the study confirm genetic variation in *L. monocytogenes* virulence markers, which is crucial for pathogenicity. Farm personnel in close contact with sheep and goats should regularly monitor for *L. monocytogenes* contamination in the farm environment.

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