

Surveillance of *Listeria monocytogenes* as an emerging public health threat in Domestic Animals and Human in Egypt

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1. Abstract

The present study will involve conducting surveillance for a zoonotic bacterial agent, *Listeria monocytogenes* in domestic animals and human in Egypt. The aim was to help in recording much more data concerning such disease and determining the most likely sources of Listeriosis infecting humans and the distribution of the various serotypes in the region. This information is important in order to optimize and determine appropriate intervention strategies and management of Listeriosis cases in animals especially ruminants and rabbits livestock. The results revealed that the occurrence of *L. monocytogenes* in the examined animal samples was 1.5 % (9/620) with high incidence in cows 1.3% (4/300) and in human acute blood samples was 0.8% (2/250). Most of the recovered isolates (72.7 %) showed high incidence of *L. monocytogenes* serotype 4 (8/11). Antibiogram was done for the recovered isolates and showed high resistance patterns for phenicols, polypeptides, cephalosporines and lincosamides. Genotypic analysis for the recovered isolates was carried out by using PCR with specific nucleotides sequence for *prfA* genes followed by sequencing and phylogenetic analysis. This analysis revealed that cows act as natural niches for that emerging pathogen.

Key words: *L. monocytogenes*, surveillance, *prfA* gene, sequencing, Phylogenetic analysis

2. Introduction

Listeria monocytogenes is a significant food-borne pathogen with widespread distribution in nature, and ability to survive in a wide range of environmental conditions, although human listeriosis has a low rate of incidence, *L. monocytogenes* causes severe illness and mortality in susceptible individuals. Neonates, the elderly, and the

immunosuppressed are particularly at risk. It may occur as a mild febrile gastroenteritis or as a more severe invasive disease such as meningo-encephalitis or sepsis [1]. *L. monocytogenes* has been reported as the most heat resistant non-spore forming foodborne pathogen [2]. It is the agent of listeriosis, a serious zoonosis caused by eating foods contaminated with such bacteria. A wide variety of animal species can be infected with *Listeria*

monocytogenes, but clinical listeriosis is mainly a ruminant disease, with occasional sporadic cases in other species. The main clinical manifestations of animal listeriosis are encephalitis, septicemia and abortion, and the disease is often associated with stored forages, usually silage. Although the morbidity of listeriosis is relatively low, the mortality of the systemic/encephalitic disease can be very high, with values in the vicinity of 20–30% [3]. Phenotypic morphological and biochemical resemblances of *L. monocytogenes* to other *Listeria* species, and the non-specific clinical manifestations of listeriosis [1], the availability of rapid, specific and sensitive diagnostic tests capable of distinguishing *L. monocytogenes* from other *Listeria* species is essential for the effective control of the disease. In addition, with *L. monocytogenes* comprising a diversity of strains [4], the development of subtyping procedures is critical to the epidemiologic investigation of listeriosis outbreaks. Furthermore, since *L. monocytogenes* demonstrates strain variations in virulence and pathogenicity [5], the ability to determine accurately and rapidly the pathogenic potential of *L. monocytogenes* isolates is essential to limit the spread of listeriosis and reduce unnecessary recalls of food products. The application of molecular techniques has facilitated the identification and characterization of major virulence-associated genes and proteins in *L. monocytogenes* [4].

Besides the differentiation of *Listeria* species, serotyping methods are also potentially useful for defining subtypes and clonal groups of *L. monocytogenes*. Indeed, it has been observed that *L. monocytogenes* serotypes 1/2a, 1/2b and 4b are responsible for 98 % of documented human listeriosis cases, whereas serotypes 4a and 4c are rarely associated with outbreaks of the disease [6]. Furthermore, while *L. monocytogenes* serotype 4b strains are isolated mostly from epidemic outbreaks of listeriosis, serotypes 1/2a and 1/2b are linked to sporadic *L. monocytogenes*

infection [7]. On a note of veterinary relevance, *L. monocytogenes* isolates from sheep encephalitis are usually of serotypes 1/2b or 4b, and those from septicaemia and abortion cases are predominantly of serotype 1/2a [8].

In vitro amplification of nucleic acid is a more recent addition to the genetic detection methods for pathogen identification and diagnosis. Among several elegant approaches to nucleic acid amplification, PCR was the first and remains the most widely applied technique in both research and clinical laboratories. As PCR has the ability to selectively amplify specific targets present in low concentrations, it offers exquisite specificity, unsurpassed sensitivity, rapid turnover, and ease of automation for laboratory detection of *L. monocytogenes* from clinical specimens, in addition to its value for identifying both cultured and non-cultivable organisms. By exploiting molecular differences within 16S and 23S rRNA genes, intergenic spacer regions, *hly*, *inlA*, *inlB*, *iap* and *prfA* [9]. *L. monocytogenes* develops acquired resistance to antibiotics rarely. However, some studies have recently reported an increased rate of resistance to one or several clinically relevant antibiotics in environmental isolates and increasing MICs of antimicrobials underline the need for active and continuous surveillance of the susceptibility to antibiotics [10].

Control of the pathogen in animals can cease the zoonotic circle and decrease the public health hazard. Therefore, the current study was planned to conduct surveillance through isolation and identification (phenotypic and genotypic) of local endemic strains of *Listeria monocytogenes* from livestock and human.

3. Materials and Methods

3.1. Sampling:

Animal samples: 620 different samples were collected from aborted and/or mastitic cows, sheep, goats, rabbits will be delivered

from different farms by the aid of co-investigators from Animal Reproductive Research Institute (Table 1). 400 blood samples was drawn from different animals species under surveillance (100 samples from each species), whenever possible to be subjected for serological tests as Listeria Lateral flow

Human samples: 250 blood samples will be drawn from feverish patients (Temp $\geq 38^{\circ}\text{C}$ for 2-3 days) in the fever hospitals as an acute sample. A second sample (convalescent) will be collected whenever possible 1–3 weeks after the acute sample or on discharge from the hospital. Both acute and convalescent samples will be subjected for serological tests, while culture will be applied on acute whole blood (Table 2).

3.2. Phenotypic identification:

For the isolation and identification of *Listeria monocytogenes*, the techniques recommended by [11] will be implemented as follow; the 1st selective enrichment step involved a selective liquid media (Half Fraser Enrichment Broth With Antibiotic supplement), while the 2nd selective enrichment: 0.1 ml from half Fraser broth is transferred into 10 ml of Fraser broth and incubated at 35°C for 48 hours. From the culture obtained in Fraser broth, a loopful is streaked onto PALCAM agar plates and incubated at 37°C for 24 to 48 hours. The plates are examined for the presence of characteristic colonies presumed to be *Listeria*; Also API Listeria could be used and Serotyping of the recovered isolates by using standard *Listeria monocytogenes antisera* (BD, USA) was carried out according [12]. Human serum samples subjected to serological tests as Listeria lateral flow kits and indirect haemagglutination according to [13].

3.3. Antimicrobial profile:

Susceptibility of the recovered isolates to various chemotherapeutic agents was investigated as follow; subcultures from the isolates were prepared and the test was applied. Four or five colonies of similar morphology were transferred using sterile loop into 5 ml of Muller Hinton Broth. The inoculated broth was incubated at 37°C for 28 h. The turbidity of inoculated broth was then adjusted to match a McFarland 0.5 barium sulphate standard tube, the selected antibacterial discs were placed on the inoculated plate, after incubation of the plates for 18 h at 37°C the degree of sensitivity was determined by measuring the easily visible and clear zones of inhibition of growth produced by diffusion of the antibacterial agent from the discs into the surrounding medium. The results were interpreted as in (Table 3) according to Laboratory Standards Institute [14].

3.4. Genotypic identification:

Freshly grown typical *Listeria*-like colonies are boiled in 400 μl of 1 X Tris-EDTA buffer (pH 8.0) (approximately 10^8 cells/ml) boiled for 10 min and centrifuged at 14,000 rpm for 10 min to remove denatured proteins and bacterial membranes. *Listeria monocytogenes* was distinguished on the basis of the *prfA* gene [15] that can be revealed by PCR (forward primer 5-CCCC-AAG-TAG-CAG-GA C-ATG-CTA-A-3 and reverse primer 5-GGT-AT C-ACA-AAG-CTC-ACG-AG-3) to amplify the 571-bp fragment. Amplification was performed with a volume of 50 μl containing 10 μl (200 ng) of extracted DNA template from bacterial cultures, 5 μl 10X PCR buffer, 0.375 μl MgCl₂ (1.5 mM), 1.25 μl dNTPs (250 μM), 0.25 μl (1.25 Unit) Ampli Taq DNA polymerase, 0.25 μl (0.5 μM) from each primer pairs. The volume of the reaction mixture was completed to 50 μl using DDW. The reaction mixture was overlaid

with mineral oil, and the tubes are placed in a DNA thermal cycler. The samples were subjected to an initial denaturation step of 94°C for 4 min, followed by 25 amplification cycles of 1 min at 94°C (denaturation), 1 min at 50°C (primer annealing), and 1 min at 72°C (primer extension) followed by a final extension step of 72°C for 5 min. PCR reaction products were separated on 1.5% agarose gels, stained with ethidium bromide and visualized. *L. monocytogenes* strain (ATCC 7494) was included as positive control. After purification, the nucleotide sequence of *L. monocytogenes* was sequenced and alignment was carried out. After then, phylogenetic relationship was performed.

3.5. Ethical approval:

The current investigation was carried out according to recommendations published by the (NRC, 2010) in the updated Guide for the Care and Use of Laboratory Animals and the procedures were approved in accordance with the United Kingdom (UK) Animals (scientific procedures) Act of 1986 prior to experimentation by the Cairo University Ethical Committee.

4. Results

The current studies quantify the frequency of the *L. monocytogenes* in domestic animals and human. The results recorded in table 4 disclose the prevalence of this pathogens distributions in Egyptian farms and hospitals as the isolation rates of *L. monocytogenes* in the examined animals and humans were 1.1% (11/1000), while in animal species alone, the rate was 1.5 % (9/620) with high incidence in cows 1.3% (4/300) and in human acute blood samples was 0.8% (2/250). Most of the recovered isolates (72.7 %) showed high incidence of *L. monocytogenes* serotype 4 (8/11) while the other isolates (27.3 %) were belonging to serotype 1 (3/11). Serological examination of human acute sera showed

22 % reactivity by using *Listeria* lateral flow kits (55/250). The same technique was applied on the convalescent sera. Only 30/130 showed reactive results (23%). Good to mention that all the 30 convalescent reactive sera were also reactive in previous acute phase, while all the reactive animal serum samples by rapid test were subjected to IHA test. In the 55 acute reactive samples, only 15 showed positive results (Titers ranged from 1:40 – 1:80). Those 15 samples showed also positive results in convalescent phase with rising titers (1:160 – 1:320) (table 5). The Antimicrobial sensitivity patterns for the recovered isolated in (table 6) showed high resistance patterns for phenicols, polypeptides, cephalosporines and licosamides (0/11), while moderate resistance for penicillins, fluroquinolones tetracyclines and macrolides(2/11), while the isolates were sensitive for rifampicin(5/11), aminoglycosides(6/11) and vancomycin (11/11). Molecular confirmation for the recovered isolates carried out by using specific nucleotides sequence for *prfA* genes at 571 bp in(fig 1) followed by sequencing and alignment the consecutive sequence to compare the identity percent with the Genbank sequence and followed with phylogenetic analysis by using by Maximum Likelihood method *Mega 7* software which revealed that there are common ancestor for our recovered isolates which come from cows clinical samples which may be act as a natural niches and reservoir for this emergence pathogen and also the analysis described that there are much more similarity between human, sheep and cows isolates.

5. Discussion

Listeriosis is a major public health problem throughout the world. It is a worldwide zoonosis caused by bacteria from the genus *Listeria* and considered as an occupational hazard for agricultural and dairy farmers, sewer workers, butchers and veterinarians. The natural reservoirs and

carriers of *Listeria monocytogenes* are domestic and wild animals especially ruminant, rabbits and birds. Humans can become infected through contact with infected animals or animal byproducts [16]. *L. monocytogenes* is ubiquitous in diverse environments and is endowed with robust physiological resistance mechanisms allowing it to survive under tough conditions such as low pH and temperatures, high salt concentration, osmotic stress, oxidative stress, and carbon starvation [17]. The current investigation described the occurrence rate of *L. monocytogenes* in domestic animals and human as the isolation rate in the examined samples was 1.1% (11/1000), while in animal species alone, the rate was 1.5 % (9/620) with high incidence in cows 1.3% (4/300) and in human acute blood samples was 0.8% (2/250).

As occurs in Chile, Colombia, Ireland, Japan, and other countries, serotypes recovered in the present study are responsible for human listeriosis cases [18-19]. In Uruguay severe cases of listeriosis in people have been attributed to serotypes 1/2a, 1/2b and 4b isolates. Unlike what occurs in some northern hemisphere countries, *L. monocytogenes* belonging to serotype 4b appears to be prevalent in foods from several South American countries including Uruguay [20]. Therefore, it would be interesting to perform other studies to test for a possible hemispheric correlation with *L. monocytogenes* serotypes. Especially taking into account the ability of this serotype to produce severe illnesses and also considering that some investigators suggest that 4b serotype strains may have a higher tendency to cause outbreaks when they are present in foods [21], our surveillance find that most of the recovered *L. monocytogenes* isolates (72.7 %) showed high incidence of *L. monocytogenes* serotype 4 (8/11) while the other isolates (27.3 %) were belonging to serotype 1 (3/11) which consider an urgent and surprise finding as now we can absolutely

suggested that there are more chance for listeriosis infection in Egypt and consider *L. monocytogenes* as an emergency public health enemy in our community.

Several virulence factors play important roles in *L. monocytogenes* pathogenesis [22]. Those virulence factors include listeriolysin O encoded by *hly* gene, internalin which is encoded by *inlA*, *inlB*, *inlC*, and *inlJ* genes, phosphatidylinositol phospholipase C encoded by *plcA*, actin polymerization protein encoded by *actA*, and invasive associated protein (*iap*) [23]. All the virulence factors regulated by PrfA gene in *Listeria* pathogenicity island LPI [24], so in our study the molecular confirmation depend on using *prfA* gene specific sequence as showed in (fig 1) which give product at 571 bp and followed with neucleotides sequence as explained in (figures 2-12) followed by phylogenetic analysis in (fig 13) and the result analysis showed that there are much more similarity between human, sheep and cows isolates and also the phylogenetic tree is unrooted but showed that the main ancestor seeds come from cow clinical samples.

Antimicrobial exploring study is considered that *L. monocytogenes* sensitive to most of clinically relevant antibiotics with some exception [25], and also record that the antibiotic of choice for treatment of invasive listeriosis comprises the use of an aminopenicillin (e.g., ampicillin) in conjunction with an aminoglycoside such as gentamicin, [26]. However the heavy use of antibiotics as growth promoters in domestic animals and selective pressure exerted by over-prescription of antibiotics in human has accelerated the evolution of *L. monocytogenes* toward resistance [27]. and this explain our resistogram finding among the recovered isolates which showed high resistance patterns for phenicols, polypeptides, cephalosporines and lincosamides (0/11), while moderate resistance for penicillins, fluroquinolones, tetracyclines and macrolides (2/11), while the isolates were sensitive for rifampicin

(5/11), aminoglycosides (6/11) and vancomycin (11/11). The increase in antibiotic resistance of this pathogen is in line with a worldwide pattern of an increasing prevalence of antibiotic resistance among foodborne pathogens [28].

6. Conclusion

Our findings showed a significant occurrence of *L. monocytogenes* in domestic animals and human with increasing antibiotic resistance, also our survey is one of the little studies to report multiple-drug resistance in *L. monocytogenes* strains and detect the genetic relationship between strains from human and animals which will help in design proper control strategies .

7. References

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Table (1): Types and numbers of animal samples

Animal spp. Type of samples	Cows	Sheep	Goats	Rabbits	Total
Aborted feti	50	25	25	50	150
Uterine discharge	50	25	25	50	150
Mastitic milk	200	50	50	20	320
Total	300	100	100	120	620

Table (2): Numbers and sites Of human samples

Egyptian Hospitals	Blood	
	Acute	Convalescent
Al Kasr Al Aini Hospital	90	50
Naser Institute Hospital	75	20
Abo Al Rish Pediatric Hospital	125	60
Total	250	130

Table (3): Antimicrobial agents used in the current study

Antimicrobial	Disk Content
Penicillins Ampicillin	20 µg
Penicillin G	10 IU
Amoxycillin-clavulinic acid	10 µg
Cloxacillin	5 µg
Oxacillin	10 µg
Amoxicillin	30 µg
Fluoroquinolones Ofloxacin	10 µg
Enrofloxacin	10 µg
Ciprofloxacin	10 µg
Pefloxacin	30 µg
flumequine	10 µg
Aminoglycosides Gentamicin	10 µg
Neomycin	10 µg
Kanamycin	30 µg
Amikacin	30 µg
Spiramicin	10 µg
Streptomycin	10 µg
Phenicol chloramphenicol	30 µg
Tetracyclines Tetracycline	30 µg
Sulphonamides Trimethoprim-sulfamathoxazole	25 µg
Cephalosporones Cephataxime	30 µg
Cephalotin	30 µg
Lincosamide lincomycin, clindamycin	20 µg 20 µg
Polypeptides Bacitracin	30 µg
Glucospeptides Vancomycin	30 µg
Macrolides Erythromycin	10 µg
Rifampicin Rifampicin	10 µg

Table (4): Epidemiological analysis of *L.monocytogenes* in Egyptian domestic animals and human

Samples	Cows	Sheep	Goats	Rabbits	Human	Total
Aborted feti	1/50	2/25	0/25	0/50	-	3/150
Uterine discharge	2/50	0/25	0/25	2/50	-	4/150
Mastitic milk	1/200	0/50	1/50	0/20	-	2/320
Human Blood Acute Convalescence	-	-	-	-	250 130	2/250 0/130
Total	300	100	100	120	380	11/1000

Table (5): IHA results on animal sera

Animals	n=examined sample	n=Positive sample	%	Titer
Cows	100	20	20	> 1:320
Sheep	100	40	40	> 1:320
Goats	100	45	45	> 1:320
Rabbits	100	10	10	> 1:320
Total	400	115/400		

Table (6): Result of Antimicrobial sensitivity for *L. monocytogenes* isolated from human and domestic animals

Antimicrobials	No. of sensitive isolates
<u>Penicillins</u>	
Ampicillin (25µg)	2/11
Penicillin G (10IU)	1/11
Amoxy-clavulinic acid (10µg)	2/11
Cloxacillin (5µg)	2/11
Oxacillin (1µg)	2/11
Amoxicillin (25µg)	2/11
<u>Fluorquinolones</u>	
Ofloxacin (10µg)	2/11
Enrofloxacin (10µg)	2/11
Ciprofloxacin (5µg)	2/11
Pefloxacin (30µg)	0/11
Flumequine (30µg)	0/11
<u>Aminoglycosides</u>	
Gentamicin (10µg)	6/11
Kanamycin (30µg)	6/11
Spiramicin (100µg)	3/11
Neomycin (10µg)	3/11
Amikacin (30µg)	6/11
Streptomycin (10µg)	6/11
<u>Phenicoles</u>	
Chloramphenicol (30µg)	0/11
<u>Tetracyclines</u>	
Tetracycline (30µg)	2/11
<u>Sulphonamides</u>	
Trimethoprim-sulfamathoxazol 1:19 (25µg)	1/11
<u>Cephlosporones</u>	
Cefotaxime (30µg)	0/11
Cephalotin (30µg)	0/11
<u>Polypeptides</u>	
Bacitracin (10 units)	0/11
<u>Macrolides</u>	
Erythromycin (15µg)	2/11
<u>Glucosopeptides</u>	
Vancomycin (30µg)	11/11
<u>Rifampicin</u>	
Rifampicin (5 µg)	5/11
<u>Lincosamide</u>	
Lincomycin (2µg)	0/11
Clindamycin (2µg)	0/11

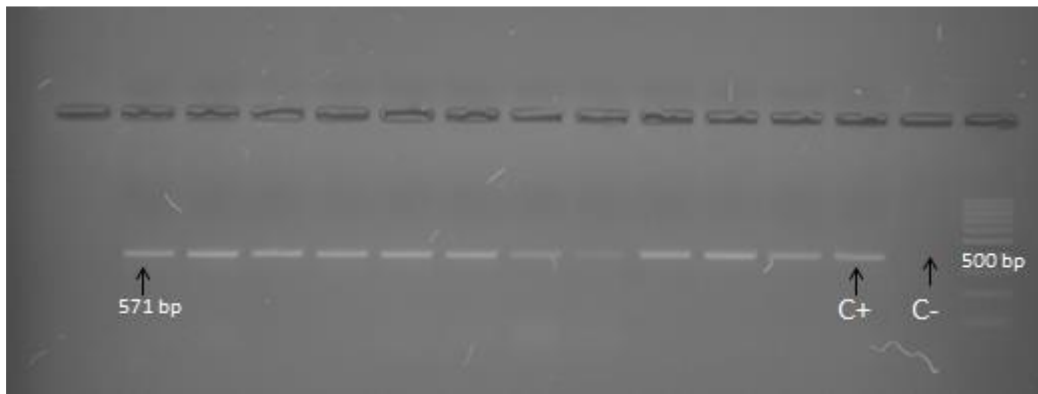


Fig 1: Agarose gel showing amplification at 572 bp for *prfA* gene in the examined 11 isolates (Lane 1 to 11) and positive control (Lane 12; *L. monocytogenes* ATCC 7494) and negative control (Lane 13)

ACCATTTCTCTTATTNACNGGGGCATATCTTTATGAGATAATCAAGATTTTGTACATAAAAGCATGAATTTTATACACGATAACTTTCTCTT
 GCTTTAATTTGAAATAATTCTGCTAACAGCTGGGCTATGTGCGATGCCACTGAATATCCTAACTCTGCATTGTTAAATTATCCAGTGTA
 ATCTTGATGCCATCAGGAGTTTCTTTACCATACACATAGGTCAGGATTAAGTTGACTGCAAATAGAGCCAAGCTCCCGTTAATCGAAA
 AATCATTAAATTTAGCTAGACTGTATGAACTTGTTTTGTAGGGTTTGGAAAACATAGAAAAGTGCCTAAAATTTTGTCTGGCGGATT
 TTTAATTCATTGTTGCCAACAAACCCCGTCCGTTCCACAAATTTTATTTAAATATAGGAAAGAGAACTATCGGGGGTTATAATCTCT
 TGTACTTAGATCCAAAACATCGAAACCCCTAAAAAGATAGCCCACTAAAGAAGGGGACGGTTTCTATTAAGCAGCGCGCGCTGGGG
 AAACAA.

Alignments						
Download GenBank Graphics Distance tree of results						
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/> Listeria monocytogenes strain FDAARGOS_555 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP033737.1
<input type="checkbox"/> Listeria monocytogenes strain FORC_057 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP021174.1
<input type="checkbox"/> Listeria monocytogenes strain NCTC7974 genome assembly, plasmid_5	606	606	64%	2e-169	97.21%	LR134402.1
<input type="checkbox"/> Listeria monocytogenes strain NCTC7973 genome assembly, chromosome_1	606	606	64%	2e-169	97.21%	LR134397.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00540 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025568.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00543 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025221.1
<input type="checkbox"/> Listeria monocytogenes strain ATCC 51775 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025222.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00545 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025560.1
<input type="checkbox"/> Listeria monocytogenes strain ATCC 51779 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025567.1

Fig 2: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the cows aborted feti with 15 of the aligned sequences from Genbank

ACCATTTCTCTATTNACNGGGGCATATCTTTATGAGATAATCAAGATTTGTACATAAAAGCATGAATTTTTATACACGATAACTTTCTCTT
 GCTTTAATTTGGAATAATTCTGCTAACAGCTGGGCTATGTGCGATGCCACTGAATATCCTAACTCTGCATTGTTAAATTATCCAGTGTA
 ATCTTGATGCCATCAGGAGTTTCTTTACCATACAATAGTTCAGGATTTAAAAGTTGACTGCAAATAGAGCCAAGCTTCCCGTTAATCGAAAA
 ATCATTAAATTTAGCTAGACTGTATGAAACTGTTTTTGTAGGGTTTGGAAAACATAGAAAAAGTGCCTAAAATTTTGTCTCGGGGATT
 TTAATTTCTATTGTTGCCAACAAACCCGTCCTCCACAAATTTTATTAATAATAGGAAAGAAACTATCGGGGGTTATAAATCCTT
 GTACTTAGATCCAAAACATCGAAACCCCTAAAAAGATAGCCCACTAAAGAAGGGGACGGTTTCTATTAAGCAGCGCGCGCTGGGGG
 AAACAANN

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Listeria monocytogenes strain FDAARGOS_555 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP033737.1
Listeria monocytogenes strain FORC_057 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP021174.1
Listeria monocytogenes strain NCTC7974 genome assembly, plasmid_5	601	601	64%	1e-167	96.94%	LR134402.1
Listeria monocytogenes strain NCTC7973 genome assembly, chromosome_1	601	601	64%	1e-167	96.94%	LR134397.1
Listeria monocytogenes strain PIR00540 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP025568.1
Listeria monocytogenes strain PIR00543 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP025221.1
Listeria monocytogenes strain ATCC 51775 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP025222.1
Listeria monocytogenes strain PIR00545 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP025560.1
Listeria monocytogenes strain ATCC 51779 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP025567.1
Listeria monocytogenes strain CFSAN054109 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP028183.1
Listeria monocytogenes strain NH1 chromosome, complete genome	601	601	64%	1e-167	96.94%	CP021325.1
Listeria monocytogenes strain Lm16 chromosome	601	1202	64%	1e-167	96.94%	CP027029.1

Fig 3: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the cows uterine discharge with 15 of the aligned sequences from Genbank (isolate 1)

AACATTTTCATCTAATTTTAGGGGCTATCTTTGAGATATCAAGATTTGTACATAAAAGCATGAATTTTTATACACGATAACTTTCTCTTCTGCT
 TTAATTTGGAATAATTCTGCTAACAGCTGGGCTATGTGCGATGCCACTGAATATCCTAACTCTGCATTGTTAAATTATCCAGTGTAATCT
 TGATGCCATCAGGAGTTTCTTTACCATACAATAGTTCAGGATTTAAAAGTTGACTGCAAATAGAGCCAAGCTTCCCGTTAATCGAAAAATC
 ATTAATTTAGCTAGACTGTATGAAACTGTTTTTGTAGGGTTTGGAAAACATAGAAAAAGTGCCTAAGATTTTTGTCTCAGTAGTTCTTTTA
 GTTCTGTTATTTTATAACGATGCGGTAGCCTGCTCGTAATGACTTCTAAATTATAATAGCCAACCGATGTTTCTGTATCAATAAGCCA
 GACATTATAACGAAAGCCCTTTGTAGTATTGTAATTCATGATGGTCCCGTTCTCGCTAATACTCGTGACTTGTGGAAACCCAAAAAAA
 A

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Listeria monocytogenes strain FDAARGOS_555 chromosome, complete genome	946	946	95%	0.0	98.87%	CP033737.1
Listeria monocytogenes strain FORC_057 chromosome, complete genome	946	946	95%	0.0	98.87%	CP021174.1
Listeria monocytogenes strain NCTC7974 genome assembly, plasmid_5	946	946	95%	0.0	98.87%	LR134402.1
Listeria monocytogenes strain NCTC7973 genome assembly, chromosome_1	946	946	95%	0.0	98.87%	LR134397.1
Listeria monocytogenes strain PIR00540 chromosome, complete genome	946	946	95%	0.0	98.87%	CP025568.1
Listeria monocytogenes strain PIR00543 chromosome, complete genome	946	946	95%	0.0	98.87%	CP025221.1
Listeria monocytogenes strain ATCC 51775 chromosome, complete genome	946	946	95%	0.0	98.87%	CP025222.1
Listeria monocytogenes strain PIR00545 chromosome, complete genome	946	946	95%	0.0	98.87%	CP025560.1
Listeria monocytogenes strain ATCC 51779 chromosome, complete genome	946	946	95%	0.0	98.87%	CP025567.1
Listeria monocytogenes strain NH1 chromosome, complete genome	946	946	95%	0.0	98.87%	CP021325.1
Listeria monocytogenes strain Lm16 chromosome	946	1893	95%	0.0	98.87%	CP027029.1
Listeria monocytogenes isolate LMNC088 complete genome genome assembly, chromosome_1	946	946	95%	0.0	98.87%	LT985476.1
Listeria monocytogenes strain MF4545 chromosome, complete genome	946	946	95%	0.0	98.87%	CP025443.1

Fig 4: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the cows uterine discharge with 15 of the aligned sequences from Genbank (isolate 2)

TGATGGTCCCGTTCTCGTAATACTCGTGATTTTGGGAAAAACAAAAAAATTTTAGGGGGGGGAAACATTTCATCTATTTACGGG
 GCTACTTTTTGAGATATCAAGATTTTGTACATAAAAGCATGAATTTTATACACGATAACTTTCTCTTGAATTTGGAAATAATTCTGCT
 AACAGCTGAGCTATGTGCGATGCCACTGAATATCCTAAGTCTGCTGTTAAATATCCAGTGAATCTTGATGTCATCAGGAGTTCTTT
 ACCATACACATAGGTCAGGATTAAGTTGACTGCAAATAGAGCCAAGCTCCCGTTAATCGAAAAATCATAAATTTAGCTAGACTGTAT
 GAAACTGTTTTGTAGGGTTTGGAAAACATAGAAAAAGTGGCTAAGATTTTGTCTCAGTAGTTCTTTGATTCGTTATTTTGATAACGTA
 TCGGGTAGCCTGCTCGCTAATGACTTCTAAATTATAATAGCCAACCGATGTTTCTGTATCAATAAAGCCAGACATTATAACGAAAGCCCTT
 TGTAGTATTGTAATTCA

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Listeria monocytogenes strain FDAARGOS_555 chromosome .complete genome	885	942	93%	0.0	98.42%	CP033737.1
Listeria monocytogenes strain FORC_057 chromosome .complete genome	885	942	93%	0.0	98.42%	CP021174.1
Listeria monocytogenes strain NCTC7974 genome assembly .plasmid_5	885	942	93%	0.0	98.42%	LR134402.1
Listeria monocytogenes strain NCTC7973 genome assembly .chromosome_1	885	942	93%	0.0	98.42%	LR134397.1
Listeria monocytogenes strain PIR00540 chromosome .complete genome	885	942	93%	0.0	98.42%	CP025568.1
Listeria monocytogenes strain PIR00543 chromosome .complete genome	885	942	93%	0.0	98.42%	CP025221.1
Listeria monocytogenes strain ATCC 51775 chromosome .complete genome	885	942	93%	0.0	98.42%	CP025222.1
Listeria monocytogenes strain PIR00545 chromosome .complete genome	885	942	93%	0.0	98.42%	CP025560.1
Listeria monocytogenes strain ATCC 51779 chromosome .complete genome	885	942	93%	0.0	98.42%	CP025567.1
Listeria monocytogenes strain NH1 chromosome .complete genome	885	942	93%	0.0	98.42%	CP021325.1
Listeria monocytogenes strain Lm16 chromosome	885	1884	93%	0.0	98.42%	CP027029.1
Listeria monocytogenes isolate LMNC088 complete genome genome assembly .chromosome_1	885	942	93%	0.0	98.42%	LT985476.1

Fig 5: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the cows mastitic milk with 15 of the aligned sequences from Genbank

GCCATCAGGAGTTTCTTTACCATACACATAGGTCAGGATTAAGTTGACTGCAAATAGAGCCAAGCTCCCGTTAATCGAAAAATCATA
 AATATAGCTAGACTGTATGAAACTGTTTTGTAGGGTTTGGAAAACATAGAAAAAGTGCCTAAGATTTTGTCTCAGTAGTTCTTTAGTTC
 GTTTATTTGATAACGTATGCGGTAGCCTGCTCGCTAATGACTTCTAAATTATAATAGCCAACCGATGTTTCTGTATCAATAAAGCCAGACA
 TTATAACGAAAGCCCTTTGTAGTATTGTAATTCATGATGGTCCCGTCTCGCTAATACTCGTGCTTTGTGTTCCCAAAAAAATAAAG
 CCGTTCATTCCAATTCAGGGGGCAAATCTTTTGGAAAAATCAAGATTTTGGACATAAAGCATGAATTTTATACACGATAACTTTT
 CTCTCGCTTAATTTTCGAAAAATAATTCGCTAACAGCTGAGCTATGTGCGAAGCCACTCGAATATCCTAACTCTGCATCGTTAAATTATC
 CAGTGAATCTTGATAAAAAATTTATAA

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Listeria monocytogenes strain FDAARGOS_555 chromosome .complete genome	870	870	89%	0.0	97.11%	CP033737.1
Listeria monocytogenes strain FORC_057 chromosome .complete genome	870	870	92%	0.0	96.10%	CP021174.1
Listeria monocytogenes strain NCTC7974 genome assembly .plasmid_5	870	870	92%	0.0	96.10%	LR134402.1
Listeria monocytogenes strain NCTC7973 genome assembly .chromosome_1	870	870	92%	0.0	96.10%	LR134397.1
Listeria monocytogenes strain PIR00540 chromosome .complete genome	870	870	89%	0.0	97.11%	CP025568.1
Listeria monocytogenes strain PIR00543 chromosome .complete genome	870	870	92%	0.0	96.10%	CP025221.1
Listeria monocytogenes strain ATCC 51775 chromosome .complete genome	870	870	92%	0.0	96.10%	CP025222.1
Listeria monocytogenes strain PIR00545 chromosome .complete genome	870	870	92%	0.0	96.10%	CP025560.1
Listeria monocytogenes strain ATCC 51779 chromosome .complete genome	870	870	92%	0.0	96.10%	CP025567.1
Listeria monocytogenes strain NH1 chromosome .complete genome	870	870	92%	0.0	96.10%	CP021325.1
Listeria monocytogenes strain Lm16 chromosome	870	1741	92%	0.0	96.10%	CP027029.1
Listeria monocytogenes isolate LMNC088 complete genome genome assembly .chromosome_1	870	870	92%	0.0	96.10%	LT985476.1
Listeria monocytogenes strain MF4545 chromosome .complete genome	870	870	92%	0.0	96.10%	CP025443.1

Fig 6: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the goats mastitic milk with 15 of the aligned sequences from Genbank

CAGGAGTTTCTTTACCATACACATAGGTCAGGATTAAGTTGACCGCAATAGAGCCAAGCTTCCCCTTAATCGAAAAATCATAAATTT
 AGCTAGACTGTATGAAACTGTTTTTGTAGGGTTTGGAAAAACATAGAAAAAGTGCCTAAGATTTTTGCTCAGTAGTCTTTTAGTTCGTTA
 TTTTGATAACGTATGCGGTAGCCTGCTCGCTAATGACTTCTAAATTATAATAGCCAACCGATGTTTCTGTATCAATAAAGCCAGACATTATA
 ACGAAAGCCCTTTGTAGTATTGTAATTCATGATGGTCCCGTTCTCGCTAATACTCGTGACTTTTTGAAAACCAACCAAAAAGTTGGGT
 GGGTTAACTTTCATCCTATTGTAGGGGGCATATCCTTTTTGAGAAATCAAGATTTTGTACATAAAAAGCATGAATTTTTATACACGATAAC
 TTCTCTCGCTTAATTTTGGAAATAATTCAGCTAACAGCAGAGCTAAGTGCGAAGCCACTCGAATATCCTAACTCTGCATAGTAAATTA
 TCCAGTGAATCTTGATGCCAT

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Listeria monocytogenes strain FDAARGOS_555 chromosome .complete genome	894	894	94%	0.0	96.68%	CP033737.1
Listeria monocytogenes strain FORC_057 chromosome .complete genome	894	894	94%	0.0	96.68%	CP021174.1
Listeria monocytogenes strain PIR00540 chromosome .complete genome	894	894	94%	0.0	96.68%	CP025568.1
Listeria monocytogenes strain PIR00543 chromosome .complete genome	894	894	94%	0.0	96.68%	CP025221.1
Listeria monocytogenes strain ATCC 51775 chromosome .complete genome	894	894	94%	0.0	96.68%	CP025222.1
Listeria monocytogenes strain PIR00545 chromosome .complete genome	894	894	94%	0.0	96.68%	CP025560.1
Listeria monocytogenes strain ATCC 51779 chromosome .complete genome	894	894	94%	0.0	96.68%	CP025567.1
Listeria monocytogenes strain NH1 chromosome .complete genome	894	894	94%	0.0	96.68%	CP021325.1
Listeria monocytogenes strain Lm16 chromosome	894	1789	94%	0.0	96.68%	CP027029.1
Listeria monocytogenes isolate LMNC088 complete genome genome assembly .chromosome: 1	894	894	94%	0.0	96.68%	LT985476.1
Listeria monocytogenes strain MF4545 chromosome .complete genome	894	894	94%	0.0	96.68%	CP025443.1
Listeria monocytogenes strain MF4562 chromosome .complete genome	894	894	94%	0.0	96.68%	CP025442.1
Listeria monocytogenes strain MF6172 chromosome .complete genome	894	894	94%	0.0	96.68%	CP025440.1

Fig 7: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the sheep aborted feti with 15 of the aligned sequences from Genbank (isolate 1)

TTGATGCCATCAGGAGTTTCTTTACCATACACATAGGTCAGGATTAAGTTGACTGCAATAGAGCCAAGCTTCCCCTTAATCGAAAAAT
 CATAAATATAGCTAGACTGTATGAACTGTTTTTGTAGGGTTTGGAAAAACATAGAAAAAGTGCCTAAGATTTTTGCTCAGTAGTCTTTT
 AGTTCGTTTATTTTGATAACGTATGCGGTAGCCTGCTCGCTAATGACTTCTAAATTATAATAGCCAACCGATGTTTCTGTATCAATAAAGCC
 AGACATTATAACGAAAGCCCTTTGTAGTATTGTAATTCATGATGGTCCCGTTCTCGCTAATACTCGTGGGTTGGGATTAACAAAAA
 AAAAAAAGGGACCATTAACCTCAATTTACGGGGGCAATCTTTTTGAGAAAAATCAAAGATTTTGTACATAAAAAGCATGAATTTTTTA
 TACACGATAACTTTTCTCTCGCTTAATTTTGGAAATAATTTCTAGCTAACAGCTGAGCTATGTGCGATGCCACTGAATATCCTAACTCC
 TGCATCGTTAAATATCCAGTGAAT C

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Listeria monocytogenes strain FDAARGOS_555 chromosome .complete genome	880	880	96%	0.0	95.68%	CP033737.1
Listeria monocytogenes strain FORC_057 chromosome .complete genome	880	880	96%	0.0	95.68%	CP021174.1
Listeria monocytogenes strain NCTC7974 genome assembly .plasmid: 5	880	880	96%	0.0	95.68%	LR134402.1
Listeria monocytogenes strain NCTC7973 genome assembly .chromosome: 1	880	880	96%	0.0	95.68%	LR134397.1
Listeria monocytogenes strain PIR00540 chromosome .complete genome	880	880	96%	0.0	95.68%	CP025568.1
Listeria monocytogenes strain PIR00543 chromosome .complete genome	880	880	96%	0.0	95.68%	CP025221.1
Listeria monocytogenes strain ATCC 51775 chromosome .complete genome	880	880	96%	0.0	95.68%	CP025222.1
Listeria monocytogenes strain PIR00545 chromosome .complete genome	880	880	96%	0.0	95.68%	CP025560.1
Listeria monocytogenes strain ATCC 51779 chromosome .complete genome	880	880	96%	0.0	95.68%	CP025567.1
Listeria monocytogenes strain NH1 chromosome .complete genome	880	880	96%	0.0	95.68%	CP021325.1
Listeria monocytogenes strain Lm16 chromosome	880	1760	96%	0.0	95.68%	CP027029.1
Listeria monocytogenes isolate LMNC088 complete genome genome assembly .chromosome: 1	880	880	96%	0.0	95.68%	LT985476.1

Fig 8: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the sheep aborted feti with 15 of the aligned sequences from Genbank (isolate 2)

GCAAACATGTATTAATAATGTTTACTGACGGGGGGGACACACTAGATGATACAATCTATTGAGAGATCTAGATGTTGTACATGAAATCAT
 GAATTTTTAAACACAATAACTTTCTTGTCTATAATTTGGAAATAATTCAGCTAACAGCTGGGCTATGTGCGATGCCACTTGAATATCCTAA
 CTCCTGCATTGTTAAATATCCAGTGAATCTTGATGCCATCAGGAGTTTCTTTACCATACACATAGGTACAGGATTAAGTTGACTGCAA
 TAGAGCCAAGCTTCCGTTAATCGAAAAATCATTAAATTTAGCTAGACTGATGAAACTGTTTTGTAGGGTTTGGAAAACATAGAAAA
 GTGCGTAAGATTTGCTCAGTAGTTCTTTAGTTCGTTATTTGATAACGATGCGGTAGCCTGCTCGCTAATGACTCTAAATTATAATA
 GCCAACCGATGTTTCTGTATCAATAAAGCCAGACATTATAACGAAAGCCCTTTGTAGTATTGTAATTCATGATGGTCCCCTTCCCTAA
 TACTCGTAGCTTTGTATACCAAAGTTTTTCTTTTTTTTTTTTTTTTTTATTTTTATCCTTAAAAGGGAAAA

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Listeria monocytogenes strain FDAARGOS_555 chromosome, complete genome	883	883	82%	0.0	97.31%	CP033737.1
Listeria monocytogenes strain FORC_057 chromosome, complete genome	883	883	82%	0.0	97.31%	CP021174.1
Listeria monocytogenes strain NCTC7974 genome assembly, plasmid_5	883	883	82%	0.0	97.31%	LR134402.1
Listeria monocytogenes strain NCTC7973 genome assembly, chromosome_1	883	883	82%	0.0	97.31%	LR134397.1
Listeria monocytogenes strain PIR00540 chromosome, complete genome	883	883	82%	0.0	97.31%	CP025568.1
Listeria monocytogenes strain PIR00543 chromosome, complete genome	883	883	82%	0.0	97.31%	CP025221.1
Listeria monocytogenes strain ATCC 51775 chromosome, complete genome	883	883	82%	0.0	97.31%	CP025222.1
Listeria monocytogenes strain PIR00545 chromosome, complete genome	883	883	82%	0.0	97.31%	CP025560.1
Listeria monocytogenes strain ATCC 51779 chromosome, complete genome	883	883	82%	0.0	97.31%	CP025567.1
Listeria monocytogenes strain NH1 chromosome, complete genome	883	883	82%	0.0	97.31%	CP021325.1
Listeria monocytogenes strain Lm16 chromosome	883	1767	82%	0.0	97.31%	CP027029.1
Listeria monocytogenes isolate LMNC088 complete genome genome assembly, chromosome_1	883	883	82%	0.0	97.31%	LT985476.1
Listeria monocytogenes strain MF4545 chromosome, complete genome	883	883	82%	0.0	97.31%	CP025443.1

Fig 9: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the rabbit's uterine discharge with 15 of the aligned sequences from Genbank (isolate 1)

AGGAGTTTCTTTACCATACATAGGTCAGGATTAAGGTTGACTGCAATAGAGCCAAGCTTCCCGTTAATCGAAAAATCATTAAATTTA
 GCTAGACTGTATGAACTTGTTTTGTAGGGTTTGGAAAACATAGAAAAAGTGCCTAAGATTTTGTCTCAGTAGTTCTTTAGTTCGTTTAT
 TTTGATAACGTATGCGGTAGCCTGCTCGCTAATGACTTCTAATTTATAATAGCCAACCGATGTTTCTGTATCAATAAAGCCAGACATTATA
 CGAAAGCCCTTTGTAGTATTGTAATTCATGATGGTCCCGTTCGCTAATACTCTGCTTTTTGGAATAACGTTTCCATCTAATTTAGGGGG
 GCATATCTTTGAGATAATCAAGATTTGTACATAAAGCATGAATTTTATACAGATAACTTTCTTTGCTTTAATTTGGAAATAATCTG
 CTAACAGCTGGGCTATGTGCGATGCCACTTGAATATCCTAACTCCTGCATTGTTAAATATCCAGTGAATCTTGATGCCATCCCCAAAACC
 AAATTTAATGGGGG

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Listeria monocytogenes strain FDAARGOS_555 chromosome, complete genome	942	942	91%	0.0	99.24%	CP033737.1
Listeria monocytogenes strain FORC_057 chromosome, complete genome	942	942	91%	0.0	99.24%	CP021174.1
Listeria monocytogenes strain NCTC7974 genome assembly, plasmid_5	942	942	91%	0.0	99.24%	LR134402.1
Listeria monocytogenes strain NCTC7973 genome assembly, chromosome_1	942	942	91%	0.0	99.24%	LR134397.1
Listeria monocytogenes strain PIR00540 chromosome, complete genome	942	942	91%	0.0	99.24%	CP025568.1
Listeria monocytogenes strain PIR00543 chromosome, complete genome	942	942	91%	0.0	99.24%	CP025221.1
Listeria monocytogenes strain ATCC 51775 chromosome, complete genome	942	942	91%	0.0	99.24%	CP025222.1
Listeria monocytogenes strain PIR00545 chromosome, complete genome	942	942	91%	0.0	99.24%	CP025560.1
Listeria monocytogenes strain ATCC 51779 chromosome, complete genome	942	942	91%	0.0	99.24%	CP025567.1
Listeria monocytogenes strain NH1 chromosome, complete genome	942	942	91%	0.0	99.24%	CP021325.1
Listeria monocytogenes strain Lm16 chromosome	942	1885	91%	0.0	99.24%	CP027029.1
Listeria monocytogenes isolate LMNC088 complete genome genome assembly, chromosome_1	942	942	91%	0.0	99.24%	LT985476.1
Listeria monocytogenes strain MF4545 chromosome, complete genome	942	942	91%	0.0	99.24%	CP025443.1

Fig 10: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the rabbit's uterine discharge with 15 of the aligned sequences from Genbank (isolate 2)

TGATGCCATCAGGAGTTTCTTTACCATACACATAGGTCAGGATTAAGTTGACCGCAAATAGAGCCAAGCTCCCGTTAATCGAAAAATC
 ATTAAATTTAGCTAGACTGTATGAAACTGTTTTGTAGGGTTGGAAAACATAGAAAAAGTGCCTAAGATTTTTGCTCAGTAGTCTTTTA
 GTTCGTTATTTTGATAACGTATGCCGTAGCCTGCTCGTAATGACTTCTAAATTATAATAGCCAACCGATGTTTCTGTATCAATAAGCCA
 GACATTATAACGAAAGCCCTTTGTAGTATTGTAATTCATGATGGTCCCGTTCTCGTAATACTCTGACTTTTGGGTTAACAAAAA
 AAGGATAATAATTAATAAAACTATTGTAAGTGAAGGGGGGCATATTCTTTTGGAAAAATCAAAGATTTTGTACATAAAGCA
 TGAATTTTTATACACGATACTTTCTCTTCTTAATTTTGGAAAAATAATTCTGCTAACAGCTGAGCTATGTGCGAAGCCACTTGAATATC
 CTAACTCTGCATCGTTAAATATCCAGTGAATCT

Alignments Download GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/> Listeria monocytogenes strain FDAARGOS_555 chromosome, complete genome	893	893	89%	0.0	97.53%	CP033737.1
<input type="checkbox"/> Listeria monocytogenes strain FORC_057 chromosome, complete genome	893	893	89%	0.0	97.53%	CP021174.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00540 chromosome, complete genome	893	893	89%	0.0	97.53%	CP025568.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00543 chromosome, complete genome	893	893	89%	0.0	97.53%	CP025221.1
<input type="checkbox"/> Listeria monocytogenes strain ATCC 51775 chromosome, complete genome	893	893	89%	0.0	97.53%	CP025222.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00545 chromosome, complete genome	893	893	89%	0.0	97.53%	CP025560.1
<input type="checkbox"/> Listeria monocytogenes strain ATCC 51779 chromosome, complete genome	893	893	89%	0.0	97.53%	CP025567.1
<input type="checkbox"/> Listeria monocytogenes strain NH1 chromosome, complete genome	893	893	89%	0.0	97.53%	CP021325.1
<input type="checkbox"/> Listeria monocytogenes strain Lm16 chromosome	893	1786	89%	0.0	97.53%	CP027029.1
<input type="checkbox"/> Listeria monocytogenes isolate LMNC088 complete genome genome assembly, chromosome: 1	893	893	89%	0.0	97.53%	LT985476.1
<input type="checkbox"/> Listeria monocytogenes strain MF4545 chromosome, complete genome	893	893	89%	0.0	97.53%	CP025443.1
<input type="checkbox"/> Listeria monocytogenes strain MF4562 chromosome, complete genome	893	893	89%	0.0	97.53%	CP025442.1

Fig 11: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the human blood with 15 of the aligned sequences from Genbank (isolate 1)

ACCATTTCTTATTNACNGGGGCATATCTTTATGAGATAATCAAGATTTGTACATAAAAGCATGAATTTTATACACGATAACTTTCTCTT
 GCTTAAATTTGGAAATAATTCTGCTAACAGCTGGGCTATGTGCGATGCCACTTGAATATCCTAACTCTGCATTGTTAAATTATCCAGTGA
 ATCTTGATGCCATCAGGAGTTTCTTTACCATACACATAGGTCAGGATTAAGTTGACTGCAAATAGAGCCAAGCTCCCGTTAATCGAAA
 AATCATTAAATTTAGCTAGACTGTATGAAACTGTTTTGTAGGGTTGGAAAACATAGAAAAAGTGCCTAAAATTTTGTCTGGCGGATT
 TTTAATTTCTATTTGTTGCCAACAAACCCCTCCGTTCCACAAATTTTATTAATAATAGGAAAGAGAACTATCGGGGGTTATAATCTCT
 TGTACTTAGATCCAAAACATCGAAACCCCTAAAAGATAGCCCACTAAAGAAAGGGGACGGTTTCTATTAAGCAGCGCGCGTGGGG
 AAACA.

Alignments Download GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/> Listeria monocytogenes strain FDAARGOS_555 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP033737.1
<input type="checkbox"/> Listeria monocytogenes strain FORC_057 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP021174.1
<input type="checkbox"/> Listeria monocytogenes strain NCTC7974 genome assembly, plasmid: 5	606	606	64%	2e-169	97.21%	LR134402.1
<input type="checkbox"/> Listeria monocytogenes strain NCTC7973 genome assembly, chromosome: 1	606	606	64%	2e-169	97.21%	LR134397.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00540 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025568.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00543 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025221.1
<input type="checkbox"/> Listeria monocytogenes strain ATCC 51775 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025222.1
<input type="checkbox"/> Listeria monocytogenes strain PIR00545 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025560.1
<input type="checkbox"/> Listeria monocytogenes strain ATCC 51779 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP025567.1
<input type="checkbox"/> Listeria monocytogenes strain CFSAN054109 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP028183.1
<input type="checkbox"/> Listeria monocytogenes strain NH1 chromosome, complete genome	606	606	64%	2e-169	97.21%	CP021325.1
<input type="checkbox"/> Listeria monocytogenes strain Lm16 chromosome	606	1213	64%	2e-169	97.21%	CP027029.1

Fig 12: Alignment report between the sequenced *prfA* gene of *L. monocytogenes* isolated from the human blood with 15 of the aligned sequences from Geneank (isolate 2)

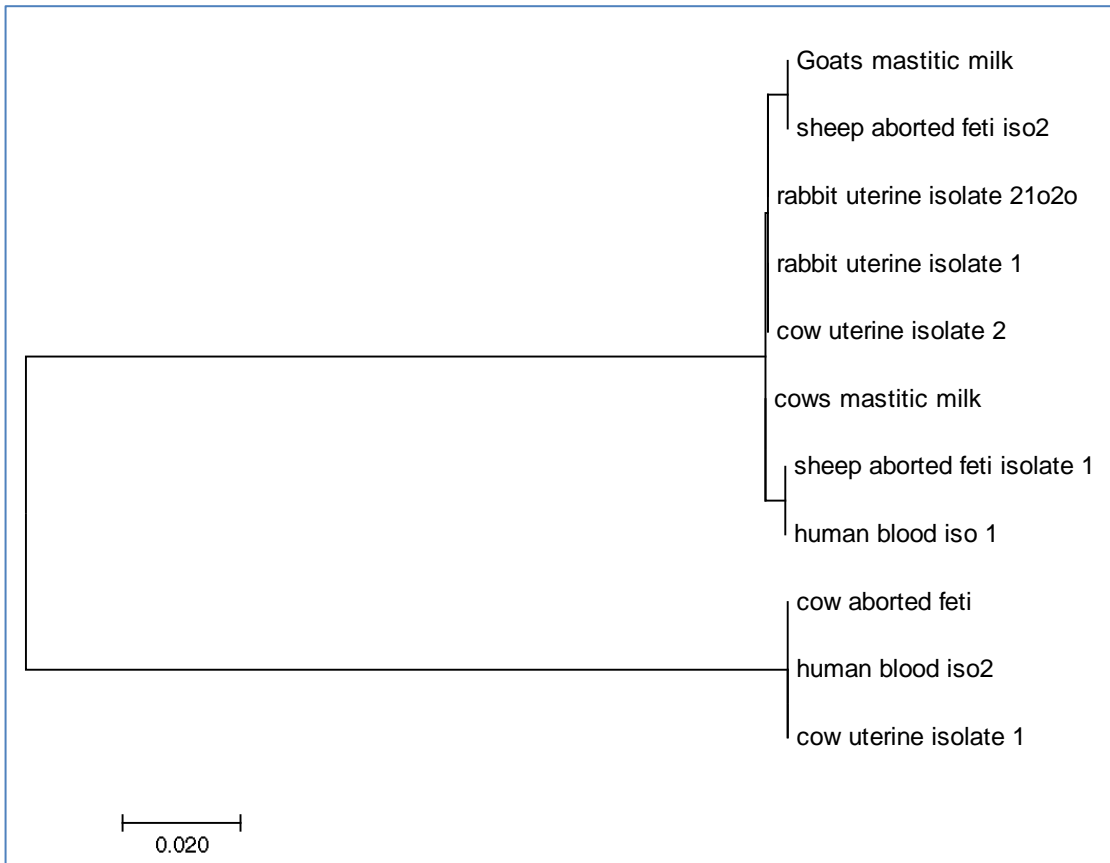


Fig 13: Molecular Phylogenetic analysis by Maximum Likelihood method for the recovered *L.monocytogenes* isolates recovered from domestic animals and human