DETECTION OF *LISTERIA MONOCYTOGENES* IN RAW MILK AND ABORTED COW CASES AT SALAHUDEEN PROVINCE

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ABSTRACT

***Listeria monocytogenes* is a pathogen that causes infectious diseases in animals. It is one of the causal agents causing abortion in infected cows. This study was conducted to detect *listeria monocytogenes* in aborted cows. Also to estimate the role of the milk in the distribution of the pathogen by detecting the bacteria in milk of aborted cows as well as in raw milk from the market of Salahudeen province. The study includes 46 aborted cows from which 46 milk samples were taken to detect the causative agents. Also 38 vaginal swabs were taken from the same aborted cows and 8 samples from fetuses. 30 raw milk samples were also taken from market at Salahudeen province. The results showed that *Listeria monocytogenes* were detected in 5 (13.1%) of vaginal swabs, 2 (25%) of aborted fetuses, 13 (28.26%) in milks from aborted cows, and 9 (30%) of raw milks. The isolated pathogens were screened for the presence of 3 virulence factors; InlJ, InlA, and HIY. The results showed that theses virulence genes were found in the majority of the isolates and the isolation rate ranged between 75%-100%. The study concluded that milk is one of the main sources for the pathogen spreads to other animals.**

Keyword: *Listeria monocytogenes* bacteria, abortion in infected cows. animals diseases.

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الكشف عن LISTERIA MONOCYTOGENES في الحليب الخام وحالات الأبقار المجهضة في محافظة صلاح الدين

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المستخلص

يعد بكتريا *Listeria monocytogenes* أحد انواع البكتريا المسببة للأمراض المعدية في الحيوانات. وهو أحد العوامل المسببة للإجهاض في الأبقار المصابة به. أجريت هذه الدراسة للكشف عن *Listeria monocytogenes* في الأبقار المجهضة. كما تم تقدير دور الحليب في انتشار العامل الممرض من خلال الكشف عن البكتيريا الموجودة في لبن الأبقار المجهضة وكذلك في الحليب الخام من سوق محافظة صلاح الدين. تضمنت الدراسة 46 بقرة مجهضة وتم أخذ 46 عينة لبن منها للكشف عن العوامل المسببة للاجهاض. كما تم أخذ 38 مسحة مهبلية من نفس الأبقار المجهضة و 8 عينات من الأجنة. تم أخذ 30 عينة لبن خام من سوق محافظة صلاح الدين. أظهرت النتائج وجود بكتريا Listeria monocytogenes في 5 (13.1٪) من مسحات مهبلية، وفي 2 (25٪) من الأجنة المجهضة ، وفي13 (28.26٪) من حليب الأبقار المجهضة، و 9 (30٪) من الحليب الخام. بعد ذلك تم فحص مسببات الأمراض المعزولة للكشف عن وجود ثلاث من عوامل ضراوة هي InlJ و InlA و HIY. أظهرت النتائج وجود الجينات عوامل الضراوة في غالبية العزلاتن وتراوحت معدل العزلة بين 75٪ –100٪. وخلصت الدراسة إلى أن اللبن هو أحد المصادر الرئيسية لانتشار العوامل الممرضة إلى الحيوانات الأخرى.

الكلمات المفتاحية: بكتيريا *Listeria monocytogenes* ، الإجهاض في الأبقار المصابة. أمراض الحيوانات.

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INTRUDUCTION

Listeria monocytogenes is an important foodborne pathogen in human and veterinary health (Blanchard et al., 2020). It is intracellular gram positive rode or Coccobacilli bacteria. It is non-spore forming, non-capsulated, motile as tumbling and rotatory motility by peritrichus flagella at room temperature. Grow with smooth and rough colony and narrow zone of blood hemolysis (Collee *et al*., 1996). *Listeria monocytogenes* distributed in all over the world, its able to infect more than 40 animal types and more than 20 bird types, and also many types of arthropods conceder mechanical vehicle (Cocolin *et al*.,2002, Dhama *et al*., 2015, Owusu et al., 2018). It has many virulence factors like Listeriolysin O, Phospholipase-C, Act A protein and Internalines, (Bierne and Cossart,2002, Matle *et al*., 2020). The main method of infection is oral way and then the bacteria reach blood stream by enterocytes and peyer's patches then engulfed by macrophage and reach to mesentery lymph node and reticulo endothelial system (Cebra *et al*.,2001. Main disease in animal are Encephalitis (circuling disease) which occur due to transmission of bacteria through trigeminal nerve and characterized by fever, Unilateral fascial paralysis, dysphagia, circling. Listeria is of great veterinary importance in livestock due to its negative impact on animal health leading to premature death or reproductive failure responsible for economic losses. (Radostitis *et al*.,2007, Blanchard *et al*., 2020).

MATERIALS AND METHODS

Samples: In the current study, 46 samples were collected from aborted cow from December 2018 – June 2019 in Salahudeen province. Also 30 raw milk samples were taken from the market. These samples are as follows:

1- Aborted cow samples

a- Vaginal swabs: 38 vaginal swab were collected from aborted cow in period not more than 7days after abortion

b- Fetus samples: 8 cow fetus sample were taken, an

2- Milk sample

a- Milk from aborted cow: 46 milk sample were collected from aborted cow.

b- Raw milk: 30 cow milk samples were collected from markets at Salahudeen province.

1ml of milk sample were taken and mixed with 0.1 ml of sodium citrate. All sample kept at 4°C for three days then cultured on trypton soya broth at 30° C for 48hours. (VanNetten *et al*.,1989).

DNA extraction: DNA was extracted from broth media by using extraction kit (ABIOpureTM Total DNA- ABIOpure, USA) according to instructions of the manufacturer's. =In table (1) showed PCR reaction mixture and in table (2) showed thermocyclar program

Table 1.Compounds used in the preparation of PCR reaction mixture

Compounds		Volume	Out product size
Master Mix- Promega- USA		10 µl	
Listeria monocytogenes	5`-GCCGCCAAGAAAAGGTTACA-3`	1 µl	208bp
	5`-GCTGAGTGTTAATGAATCACG-3`	1 µl	
Nuclease Free Water		3 µl	
DNA template		10 µl	
Total		25 µl	

Table 2. Timer program of the Thermocycler used for detection *Listeria monocytogenes*

Stage	Temperature (c°)	Time (mints)	Cycles (nombers)
First Denaturation step	95	5 mints	1
Denaturation step	95	30 second	
Primer-annealing step	54	1 min	30
DNA extension step	72	1 mint	
Final DNA extension step	72	7 mints	1
End Temperature	10	10 mints	————

Primers used for detection of virulence factors: in the current study three types of primer used as in table (3).

Table 3. Types, sequence, and product size of primers used for detection of virulence factors

Gene	Target	Sequence (5–3)		Size	Reference
InlJ	Internalin J	F	TGT AAC CCC CGC TTA CAC AGT T	238	Liu et al. (2007)
		R	AGC GGC TTG GCA GTC TAA TA		
InlA	Internalin A	F	ACG AGT AAC GGG ACA AAT GC	800bp	Liu et al. (2007)
		R	CCC GAC AGT GGT GCT AGA TT		
HIY	Listeriolysin O	F	GCCTGCAAGTCCTAAGACGCCAATC	706bp	Hudson et al., 2001
		R	CTTGCAACTGCTCTTTAGTAACAGC		

Tables (4,5,6) showed thermocyclar program used

Table 4. Timer program of the Thermocycler used for detection of InlA gene of *Listeria monocytogenes*

Stage	Temperature (c°)	Time (mints)	Cycles (nombers)
First Denaturation step	94	4 mints	1
Denaturation step	94	30 second	
Primer-annealing step	52	1MINT	30
DNA extension step	72	2.5 mint	
Final DNA extension step	72	7 mints	1
End Temperature	10	10 mints	———

Table 5. Timer program of the Thermocycler used for detection of InlJ gene of *Listeria monocytogenes*

Stage	Temperature (c°)	Time (mints)	Cycles (nombers)
First Denaturation step	94	4 mints	1
Denaturation step	94	30 second	
Primer-annealing step	58	1mint	30
DNA extension step	72	2.5 mint	
Final DNA extension step	72	7 mints	1
End Temperature	10	10 mints	———

Table 6.Timer program of the Thermocycler used for detection of HLY gene of *Listeria monocytogenes*

Stage	Temperature (c°)	Time (mints)	Cycles (nombers)
First Denaturation step	94	5 mints	1
Denaturation step	94	30 second	
Primer-annealing step	50	45second	30
DNA extension step	72	1 mint	
Final DNA extension step	72	7 mints	1
End Temperature	10	10 mints	———

RESULTS AND DISCUSSIONS

The result of this study showed that milk was the best sample for the isolation of listeria in aborted cows, since the isolation rate was 28.26% from milk samples followed by 25 % from fetuses and 13.1% from vaginal swabs. On the other hand, listeria was isolated from 30% of raw milk samples as shown in table (7). Figure (1) shows the positive result of PCR test.

Table7. Showed the PCR test results for detection of *Listeria monocytogenes* from milk and aborted samples

Type of sample	No. of samples	No. of +ve sample	Percentage of +ve samples
Vaginal swabs	38	5	13.1
Aborted fetus	8	2	25
Milk from aborted cow	46	13	28.26
Raw milk	30	9	30

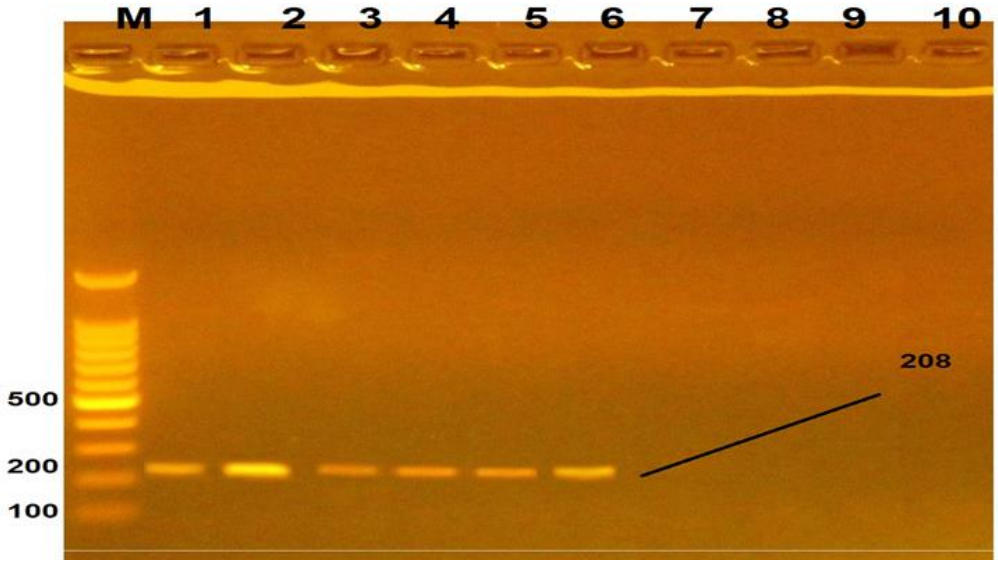

Figure 1. Agarose gel electrophoresis of PCR products. M: 100 bp DNA ladder, lines (1-6) positive result at 208 bp for *Listeria monocytogenes*

The results of study showed that listeria detected in rate of 15.2% from aborted case. The difference rate of listerial abortion depend on type of animal, strain of animal, age of animal, geographic location (Schlech, 1996). Listeria monocytogenes is ubiquitous in cattle production environments, including soil, feed, and occasionally water sources, and is a common enteric resident of cattle and other mammals. There are four genetically distinct lineages of L. monocytogenes (I-IV), with most lineage III and IV isolates obtained from ruminants. (Whitman *et a*l., 2020) The current study showed high rate of listeria detected in milk from aborted cows, that mean listeria either caused abortion and bacteremia occur, then bacteria reach to udder and release with milk or less commonly the presence of listeria in milk refer to mastitis or contamination during handing or mechanical milking, or may be the milk contaminated from infected milker (Radostitis *et al*.,2007; Dongyou Liu,2008, Ricchi et al., 2019).The result show that listeria detection from raw milk in rate of 16.6%, this percentage more than rate that recorded by (AL-Shammary, 2001) which is 12.5% in Baghdad that's due to difference in location of study, number of sample and seasonal variation of study. Winter is best season for listeria dissension. Type of diagnosis test predominating PCR is better than culture technique due to their ability to detect low number of bacteria and dead bacteria (AL-Shammary, 2001). Milk with high rate of water conceder ideal environmental for bacterial growing, also cow milk contain phospholipid which is suitable for listeria (Robinson, 2000) In figure (2,3,4) show positive result of PCR test for detection of virulence factors and in the table (8) showed appearance of virulence factors of *Listeria monocytogenes* isolated from different samples.

Table 8. Appearance of virulence factors of *Listeria monocytogenes*

Type of sample	Number of positive sample	Virulence factors (number and ratio of positive samples)		
		InlJ	InlA	HIY
Vaginal swabs	5	5(100%)	5 (100%)	5 (100%)
Aborted fetus	2	2(100%)	2 (100%)	2 (100%)
Total of abortion case	7	7 (100%)	7 (100%)	7 (100%)
Milk from aborted cow	13	13 (100%)	13 (100%)	11 (84.6%)
Raw milk	9	8 (88.8)	8 (88.8)	6 (75%)

This study showed that appearance of InIJ and InIA in rate 100%. The result of the current study is approach with result of (Owusu-Kwarteng *et al.*,2018). *L. monocytogenes* expresses about 50 molecules, including the virulence genes listeriolysin O and two major internalins, InlA and InlB, to promote its cell infection cycle. InlA and InlB, known to

promote bacterial uptake by host cells, and the secreted pore-forming toxin listeriolysin O (LLO), which disrupts the phagosome to allow bacterial proliferation in the cytosol InlA facilitates both bacterial attachment and internalization in cells that express its receptor (Phelps *et al*., 2018). The similarity of virulence appearance in aborted sample and milk of aborted cow and raw milk may be refer to similarity of source and milk is main source to human infection (Camejo et al. 2011).

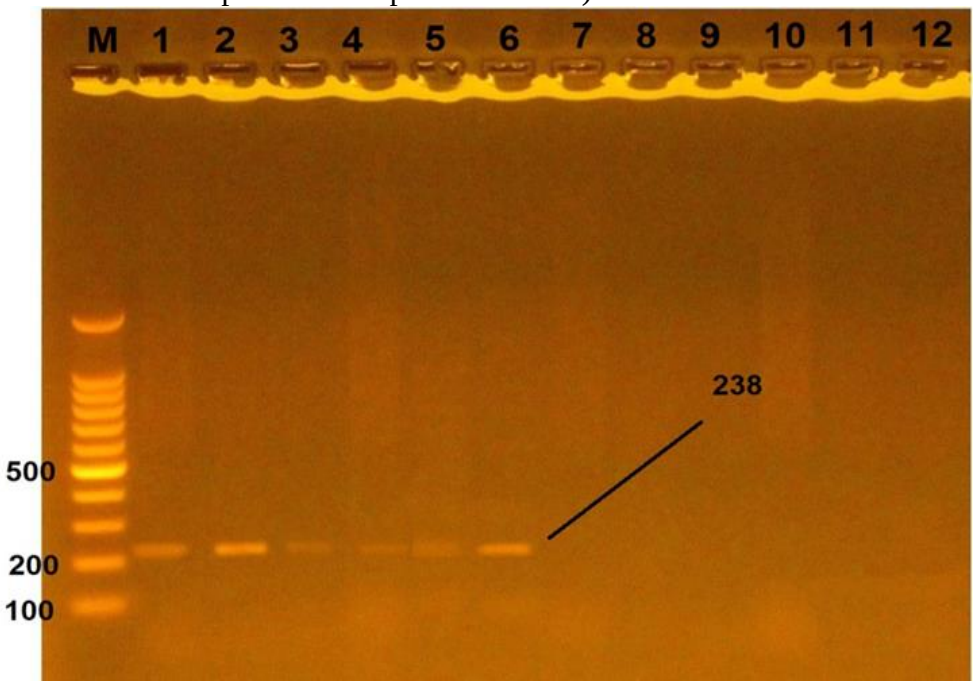

Figure 2. Agarose gel electrophoresis of PCR products. M: 100 bp DNA ladder, lines (1-6) positive result at 238 bp for internalin J (*InlJ*) gene of *Listeria monocytogenes*

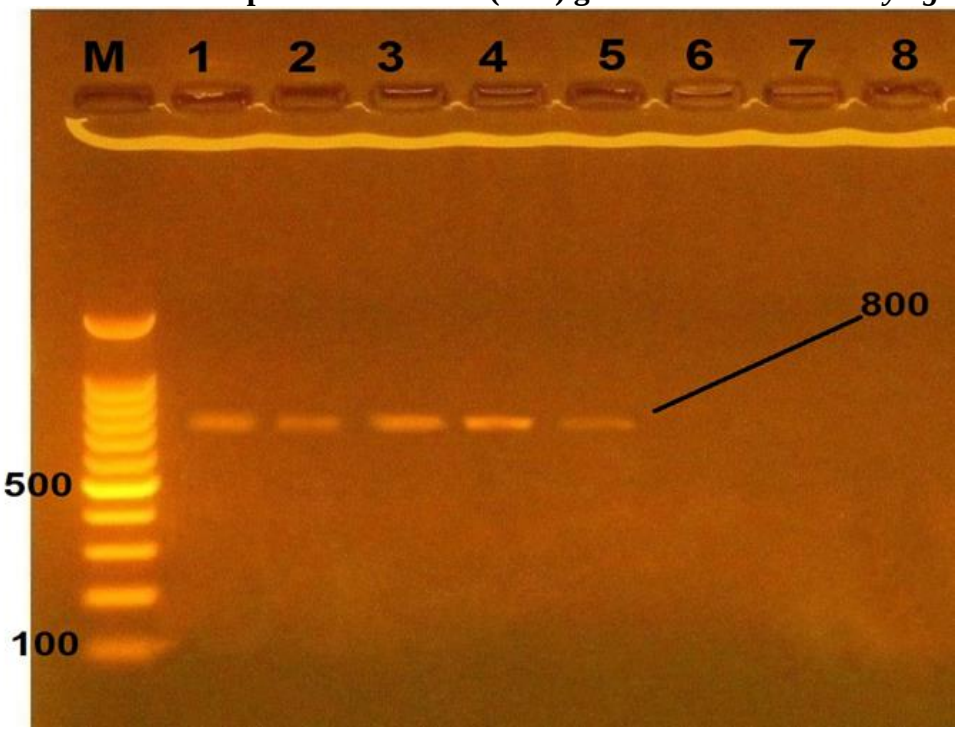

Figure 3. Agarose gel electrophoresis of PCR products. M: 100 bp DNA ladder, lines (1-5) positive result at 800 bp for inlAInternalin A (*InlA*) gene of *Listeria monocytogenes*

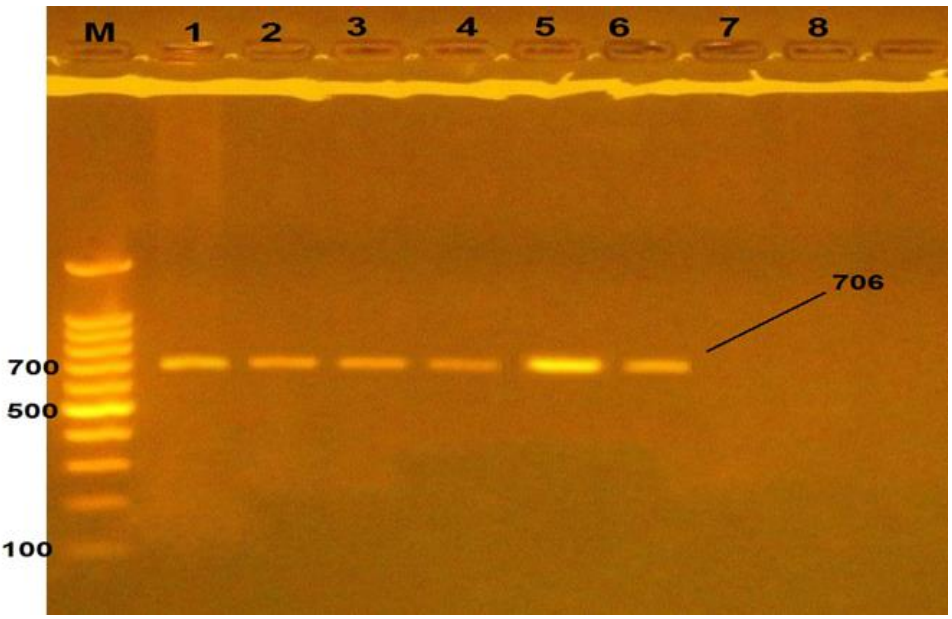

Figure 4. Agarose gel electrophoresis of PCR products. M: 100 bp DNA ladder, lines (1-6) positive result at 800 bp for Listerolysin O (*HIY*) gene of *Listeria monocytogenes*

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