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First Detection of *Listeria ivanovii* in Aborted Sheep Fetuses in The Iraqi Nineveh Governorate

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ABORTION causes significant financial losses for the sheep trade. It's essential to determine the etiology in order to successfully deal with abortions. The current analysis identifies the role of *Listeria ivanovii* in ovine abortion in Iraq. During the months of November and December 2022, a total of 500 clinical samples (100 fetuses) were investigated for the isolation and identification of *L. ivanovii*. The API-Listeria system , *L. mono* confirmatory agar (chromogenic medium), and conventional polymerase chain reaction were used to confirm the isolates' diagnoses. On analysis, four isolates were identified as *Listeria ivanovii*. Two of the four isolates (LIVANOVII31 strain) were isolated from the brain tissue, while one was isolated from the placenta and the other (LIVANOVII53 strain) from the stomach contents of the aborted fetus. The two isolates were deposited in GenBank under accession numbers OQ983887.1 and OQ983888.1, respectively. An overall isolation rate of 0.8% was observed for *L. ivanovii*. All the bacterial isolates were positive for Act A, In1A and In1J virulence factors.In conclusion , *L. ivanovii* is one of the important causal agents of abortion in sheep flocks in Nineveh province , Iraq.

Keywords: Sheep, Abortion, *Listeria ivanovii* and virulence factors, Polymerase chain reaction.

Introduction

Abortion is one of the most important reasons for infertility, and it leads to significant financial losses in small ruminant livestock [1-2]. Listeric abortion caused by *L. ivanovii* (subsp. *ivanovii* and *londoniensis)* is a considerable issue for ruminants as it causes endemic abortion with placentitis in the last trimester (from 12 weeks on),and stillbirth in ruminants [3- 5].In addition to *L. monocytogenes*, there is *L. ivanovii*, previously named as *L.monocytogenes* serotype 5, which is the only other pathogen in *Listeria species* [6].Later DNA-DNA hybridization using the SI nucleus-trichloroacetic acid technique [7] validated the distinctness of the species *L. ivanovii* and *L. monocytogenes*, and this resulted in the nomination of the new species *L. ivanovii* in 1984 [8]. *L. ivanovii* has the same ability as *L. monocytogenes* to adhere to human amniotic cells, penetrate the cytoplasm, lyse the phagosome, generate actin tails, and spread to additional cells[9]. Rocha *et al.* [10] showed the trophoblasts are susceptible to *L. ivanovii*, which might explain the bovine listeric abortions and reproductive failures. The current method of diagnosing animal listeric illness, microbiological or histological studies, has the limitation of being difficult and time consuming. As a result, molecular approaches are rapidly being

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adopted as newer, quicker diagnostic tools with improved sensitivity and reproducibility[11-12].Several research have been conducted to identify the bacterium in ovine abortion [13 -15], caprine mastitic and abortion[16], and bovine mastitis[17].
L. monocytogenes was seriously studied in Iraq over the last few years due to their importance as a food-borne human pathogen [18-19], however, until the completion of this investigation, *L. ivanovii* was not isolated in Iraq. Based on selective plating media, biochemical characterization, and certain virulence associated genes, the current study aimed to shed light on *L. ivanovii* as one of the causal agents of abortion in sheep flocks in the Iraqi Nineveh province.

Material and Methods

Ethical Approval

The institutional care of animals and utilization authority of the Veterinary Medicine College, University of Mosul, acknowledged this research (authorization number UM.2022.032).

Sample collection

During November-December 2022, one hundred aborted fetuses from 50 flocks in the Iraqi Nineveh province were screened for the presence of *Listeria ivanovii* .500 samples were collected from the blood, stomach contents, placenta, brain, and gall bladder of the aborted fetus in the last stage of gestation. All obtained samples were quickly transferred to the laboratory under refrigerated (4 ^{o}C) conditions and processed.

Isolation and identification of bacterial isolates

Septically collected 10-25 g or ml of each sample (depending on the amount of sample available) was minced into 225 ml of TSYEB broth (tryptic soya yeast extract, MERCK, Germany).Next, 1 ml of the mixture was inoculated into 9 ml TSYEB broth and incubated at 4^{o}C for five days (cold incubation method) in order to reduce other bacterial contamination because only *Listeria* can grow at low temperatures, overgrowing other organisms that grow more slowly if at all (20). A loop-ful of the incubated broth was streaked onto blood agar and incubated at 37 °C. Examine bacterial growth after 24 and 48 hours. Examine five colonies (or all if fewer are available) for cell shape, Gram response, and hemolytic activity on blood agar tumbling motility at 22°C. Then, the growth was initially streaked on Oxford agar (HiMedia, India) supplemented with Listeria selective supplement FD061 containing Polymyxin B sulfate, Ceftazidime, and Acriflavine hydrochloride [21]. The media was incubated at 37°C for 24 hours. After purification by sub culturing the bacteria, the pure isolates were examined microscopically using Gram stain and biochemical confirmation tests (Hi Listeria identification kit, HiMedia Labs, Mumbai, India) manually as described by the manufacturer.

For detection of the species of *Listeria*. All isolates were cultured on L. mono confirmatory agar base ,chromogenic media (HiCrome™ Listeria Agar Base / Modified / M1417 (Hi Media, India),and incubated overnight at 37°C. This medium was identified using chromogenic measurements of beta-glucosidase activity and sugar fermentation. Other organisms cannot use the chromogenic substrate and hence produce colorless colonies when *Listeria species* hydrolyze the pure chromogenic substrate in the medium forming bluish green colored colonies**.**The colonies of *Listeria monocytogenes* and *Listeria innocua* appear bluish green with a yellow halo (rhamnose positive and xylose negative) while the colonies of *Listeria ivanovii* appear blue without a yellow halo (xylose positive and rhamnose negative) .

Molecular identification

Conventional PCR technique was utilized to confirm four isolates suspected of being *L. ivanovii*. Bacterial genomic DNA was extracted from the bacterial cells grown at 35 °C overnight in TSB-YE using a genomic DNA extraction kit (Thermo Fisher Scientific), following the manufacturer's instructions. The DNA was stored at -20 °C. The primer pairs designated as Lis1A; *5'*-ATGAATATGAAAAAAGCAAC -3' and Lis1B; 5'- TTATACGCGACCGAAGCCAAC -3 [22] were used to amplify a 1600 bp region in the ape gene for the detection of *Listeria genus.*

In addition, primer pairs designated as 27F; 5'-GAGTTTGATCCTGGCTCAG -3'and R; 5'-GGTTACCTTGTTACGACTT -3'were used to detect *Listeria ivanovii* isolates harboring 16S rRNA that amplify a 1492bp fragment [23], (Table 1)

The DNA was extracted according to the manufacturer's instructions using a bacterial DNA extraction kit (Qiagen, Germany). The PCR technique reaction mixture for amplification consisted of 12.5 microliters of 2× PCR master mixtures (Thermo Fisher Scientific, USA), 1 microliter (10 pmol/µL) of each primer (ILS, Haryana, India), 2 microliters of DNA template and nuclease-free water (NFW) to make a final volume of 25 microliters. The primer sets used for PCR technique were listed in Table 1.

The cycling conditions for PCR technique consisted of initial denaturation for five minutes at 94°,thirty cycles each of denaturation for thirty seconds at 94°C, annealing for thirty seconds at

53°C, extension for thirty seconds at 72°C and a final extension for five minutes at 72°C. The PCR technique was performed in a thermal cycler (Eppendorf, Germany). The PCR technique program for the 16S RNA ribosomal region investigated was as follows: initial denaturation at 95°C for five minutes, followed by thirty cycles of denaturation at 94°C for 15seconds, annealing at 59°C for thirty seconds, and extension at 72°C for 45 seconds and a final extension at 72°C for five minutes. PCR technique amplification products were analyzed electrophoretically on a 1% horizontal agarose gel [22].

TABLE 1. Target genes and Primer sequences used.

Target Genes	Primer sequence	Product size (bp)	Reference
iap(Genus specific PCR)	Lis1A; 5'- ATGAATATGAAAAAAGCAAC -3'	1600	[22]
	Lis1B; 5'- TTATACGCGACCGAAGCCAAC -3		
16S rRNA	27F; 5'- AGAGTTTGATCCTGGCTCAG -3'	1492	[23]
(*L. ivanovii*)	1492R; 5'- GGTTACCTTGTTACGACTT -3'		
actA (actin polymerization protein)	(F) CGCCGCGGAAATTAAAAAAAGA	839	[24]
	(R) ACGAAGGAACCGGGCTGCTAG		
hlyA (Internalin A)	(F) ACG AGT AAC GGG ACA AAT GC	800	[25]
	(R) CCC GAC AGT GGT GCT AGA TT		
hlyJ (Internalin J)	(F) TGT AAC CCC CGC TTA CAC AGT T	238	[25]
	(R) AGC GGC TTG GCA GTC TAA TA		

Results

Five hundred clinical samples were analyzed over a 14-month period from November to December 2022. *L. ivanovii* was isolated from 4/500 samples (0.8%) based on *Listeria* colonies observed on Oxford agar (typical small, round, gray-black colonies, approximately 0.5 mm in diameter, bounded by diffuse black areas of aesculin hydrolysis, were considered to be Listeria spp.)(Figure 1) and (L. mono confirmatory agar)chromogenic agar plates (Figure 2). Of the four isolates, two were isolated from the brain tissue of the aborted fetus(LIVANOVII31 strain), while the other two were isolated from the placenta, and stomach content(LIVANOVII53 strain).The bacterial genome sequences were accessed at numbers OQ983887.1 and OQ983888.1 in the GenBank database (Table 2).The isolate was determined to be *L. ivanovii* based on beta hemolytic on sheep blood agar and microscopic findings (0.4-0.5 µm wide and 1-2 µm long, non-spore forming Gram-positive bacillus. All isolates exhibited typical biochemical features, including catalase production and formation of acids from d-xylose, as well as a negative response with mannitol, rhamnose, and alpha-methyl-d-mannoside from a diagnostic aspect. After culturing procedures and being determined to be Listeria spp. by genus specific PCR technique, it was identified as *L. ivanovii* as a result of species specific PCR technique (Figure 3). All the *L. ivanovii* isolates were found to be positive for targeted virulence associated genes, namely act A, in1A and in1J by PCR.

TABLE 2. Strains and accession numbers of the *Listeria ivanovii* isolated from aborted sheep fetuses in the Iraqi Nineveh governorate.

Strains	Gene name	Size (base pair)	Accession numbers	Sources
LIVANOVII31	16S ribosomal RNA gene	1410	OQ983887.1 https://www.ncbi.nlm.nih.gov/nuccore/OQ983887.1	Aborted fetus brain
LIVANOVII53	16S ribosomal RNA gene	1410	OQ983888.1 https://www.ncbi.nlm.nih.gov/nuccore/OQ983888.1	Aborted fetus stomach

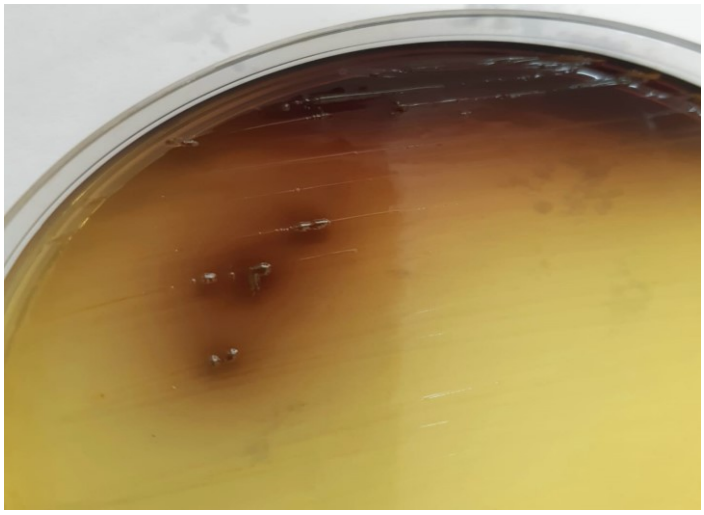

Figure 1. Colony of *Listeria spp.* on Oxford agar.

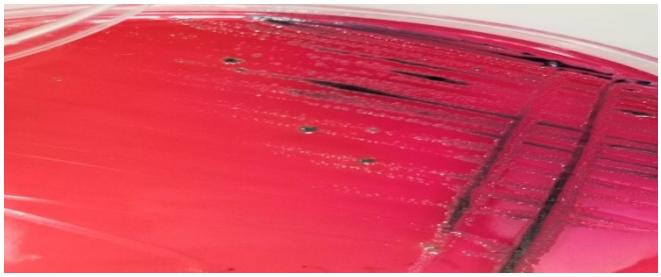

Figure 2. Colony of *L. ivanovii* in L. mono confirmatory agar.

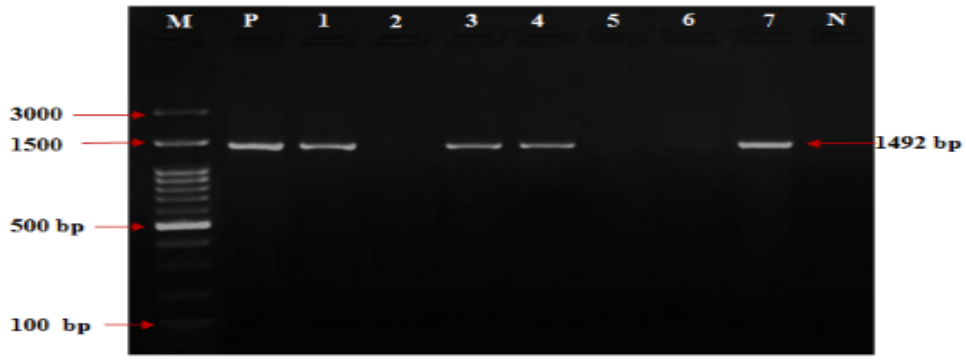

Figure 3. Gel electrophoresis of 16S rRNA (*L. ivanovii*) reaction products using polymerase chain reaction for detection of *L. ivanovii* from aborted sheep fetuses.M :DNA ladder (100-3000bp); Lane(1,3,4 ,7) positive samples 1492 bp; P: positive control ; N: negative control.

Discussion

Listeric abortion is a significant veterinary and public health concern globally,it is caused by either *L.monocytogenes or L. ivanovii. L. ivanovii* is a significant ruminant pathogen, causing fifteen percent of listeriosis in animals [26] and has been

associated with abortion occurrences in ruminants [14]. Listeric abortion caused by *L. ivanovii* in ruminants have not been reported in Iraq to date, based on our literature review, and thus this is probably the first report of *L. ivanovii*-induced abortion in ewes in Iraq. From this study the overall isolation rate of *L. ivanovii* was 0.8 %. This result is in agreement with the results reported by some published data that reveal low *L. ivanovii* prevalence in the range of 0-0.8%. Rahimi et al,[27] detected *Listeria spp.* in 12 of 85 bovine (14.1%) and 7 of 65 sheep (12.5%) raw milk specimens, with seven of them (2.7%) positive for *L. monocytogenes* and 2 (0.84%) positive for *L. ivanovii*.

In a Turkish study that included 538 examined samples, including 263 vaginal swabs,229 milk samples, and 46 stomach material of ovine aborted fetuses*, L. ivanovii* was successfully isolated from a single specimen using genus-specific PCR in conjunction with 16S rRNAgene sequencing[15]. In recent outbreaks from September 2018 to January 2019, a postmortem examination of 7 newborn lambs from 5 farms revealed a visceral *L. ivanovii* infection[28]. In Santa Fe, Argentina, *L. ivanovii* was also reported to have induced abortion in 10 Santa Inés ewes from a herd of 390 heads [14].As different from our findings, research in Turkey revealed 2.1% of *Listeria spp*., *L. ivanovii*, and *Listeria grayi* in 80 raw milk samples from Ankara (29). Yakuba et al. (2012)[30] found *Listeria monocytogenes*, *Listeria innocua*, *Listeria ivanovii*, *Listeria welshimeri*, and *Listeria seeligeri* in 8.9%, 20.3%, 7.3%, 2.1%, and 1% of raw milk samples from Spain.

The culture of *L. ivanovii* on blood agar in this study appeared as small transparent colonies with smooth borders and beta-hemolytic on sheep blood agar plates. Previous research indicates that only three *Listeria species*, *L. monocytogenes*, *L. ivanovii*, and *L. seeligeri*, frequently produce haemolysis on blood agar [31]. The hemolysing activity is most commonly shown using agar plates containing equine or ovine blood. *L. ivanovii* has a broad zone of hemolysis, possibly multiple zones [31].Haemolysis as well as acid production are key characteristics distinguishing the species [32].

β-haemolysis is induced by the Listeriolysin O (ivanolycin O) protein, which is encoded by the hly gene, which is situated within the virulence gene cluster, demonstrating their activity [33]. All virulent strains of Listeria ivanovii generate ivanolysin, a thiol-activated haemolysin, whereas non-ivanolysin producing strains are avirulent [34-35]. Considering that our four isolates showed distinct and broader areas of hemolysis, this means that they are pathogenic isolates.

All isolates exhibited typical biochemical features, including catalase production and production of acids from d-xylose, as well as a negative response to mannitol, rhamnose, and alpha-methyl-d-mannoside . Listeria species are distinguished by their ability to ferment rhamnose or xylose. *L. ivanovii* is distinguished from other Listeria species by its ability to ferment D-ribose [36].

By PCR technique, all of the *L. ivanovii* isolates in this study tested positive for targeted virulence related genes, namely act A, in1A, and in1J.Numerous publications on *L. monocytogenes* virulence factors have been published[18-37].The key virulence gene clusters of *L. monocytogenes* (prfA, plcA, hly, mpl, actA, plcB) had the same genomic structure and were located on the same chromosome as *L. ivanovii* [38-39].In *L. ivanovii*, the LIPI-1 cluster consists of six genes, including a pore-forming toxin (ivanolysin O) and two phospholipases (plcC and plcB), which work in conjunction to dissolve the membrane of the phagophore; responsible for intracellular bacterial Actin polymeric surface protein (ActA) for motility

and spreading; metalloprotease (mpl) involved in proPlcB processing; and transcriptional activator (PrfA) that directs LIPI-1 gene expression [40-41].

Conclusions

In this study, the authors isolated and molecularly identified *Listeria ivanovi* from sheep aborted fetuses for the first time, and also investigated its virulence determinants.

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Conflict of interest: There is no conflict of interest

References

1. Gebretensay, A., Alemayehu, G., Rekik, M., Alemu, B., Haile, A., Rischkowsky, B., Aklilu, F. and Widel, B. Risk factors for reproductive disorders and major infectious causes of abortion in sheep in the highlands of Ethiopia. *Small Ruminant Research*, **177** (1), 299 (2019). doi: 10.1016/j.smallrumres.2019.05.019.
2. Ali, S., Zhao, Z., Zhen, G., Kang, J.Z. and Yi, P. Z. Reproductive problems in small ruminants (Sheep and goats): A substantial economic loss in the world. *Large Animal Review*, **25**(6), 215-223(2019).
3. Boerlin, P., Rocourt, J., Grimont, F., Grimont, P.A.D., Jacquet, C. and Piffaretti. J.C. Listeria ivanovii subsp. londoniensis subsp. Nov. *International Journal of Systematic Bacteriology* , **42**, 69-73(1992). https://doi.org/10.1099/00207713-42-1-69.
4. Brugère-Picoux, J. Ovine listeriosis. *Small Ruminant Research*, **76**(1), 12-20(2008). DOI: 10.1016/j.smallrumres.2007.12.022
5. Constable, P.D., Hinchcliff, K.W., Done, S.H. and Grünberg, W. Veterinary Medicine: ATextbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. 11th ed., Amsterdam; Elsevier Ltd.,pp. 2278(2017).
6. Ivanov, I. Establishment of non-motile strains of Listeria monocytogenes type 5. In Problems of Listeriosis; Woodbine, M. (Ed.), Leicester University Press: Leicester, UK, pp. 18–26 (1975).
7. Seeliger, H. P. R. and Welshimer, H.J. Genus Listeria. In Bergey's Manual of Determinative Bacteriology, 8th ed.; Buchanan, R.E., Gibbons, N.E., Eds.; The Williams and Wilkins Co.: Baltimore, MD, USA, pp. 593–596 (1974).
8. Seeliger, H.P.R, Rocourt, J., Schrettenbrunner, A., Grimont, P.A.D. . and Jones, D. Notes: Listeria ivanovii sp. nov., *International Journal of systematic bacteriology*, **34**(3),33-6337(1984). https://doi.org/10.1099/00207713-34-3-336.
9. Ammendolia, M. G., Superti, F., Bertuccini, L., Chiarini, F., Conte, M. P., Cipriani, D., Seganti, L. . and Longhi, C. Invasive pathway of *Listeria ivanovii* in human amnion-derived WISH cells, *International Journal of Immunopathology and Pharmacology*, **20**(3), 509–518(2007). doi:10.1177/039463200702000309.
10. Rocha, C. E., Mol, J. P. S., Garcia, L. N. N., Costa, L. F., Santos, R. L. . and Paixao, T. A. Comparative experimental infection of Listeria monocytogenes and Listeria ivanovii in bovine trophoblasts, *PLoS ONE*, **12**(5), e0176911 (2017). https://doi.org/10.1371/journal.pone.0176911
11. Akça, D. and Mitat, S. Investigation of Listeria species isolated from milk and vaginal swab samples of cows in the province of Kars, Turkey. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, **17**(6), 987-993(2009). DOI:10.9775/kvfd.2011.4911.
12. Matto, C., Varela, G., Braga, V., Vico, V., Gianneechini, R.E. and Rivero ,R. Detection of Listeria spp. in cattle and environment of pasture-based dairy farms. *Pesquisa Veterinária Brasileira,* **38**(9), 1736-1741(2018). DOI: 10.1590/1678-5150-PVB-5663.
13. Sahin, M. and Beytut, E. Abortions in sheep due to Listeria ivanovii in the Kars Region, *Turkish Journal of Veterinary and Animal Sciences*, **30**(5), 503-506(2006) Available at: https://journals.tubitak.gov.tr/veterinary/vol30/iss5/12.
14. Della Rosa, P., Colque Caro, L. A., Cantón, G. J., Morrell, E. L., Hecker, Y. P., Paolicchi, F. A. . and Fiorentino, M. A. Aborto ovino asociado a Listeria ivanovii., In Proceedings of the XV Congreso Argentino de Microbiología, Buenos Aires, Argentina, 25–27 September (2019) Available online:

https://www.researchgate.net/publication/338404312_Aborto_ovino_asociado_a_Listeria_ivanovii.

15. Akca, D. , Buyuk, F. , Celik, E. , Saglam, A. G. , Otlu, S., Dag, S., Celebi, O., Coskun, M. R., Buyuk, E., Karakurt, E. and Sahin M. Phylogenetic positioning of Listeria ivanovii identified in aborted sheep in Kars Region (Turkey), *The Thai Journal of Veterinary Medicine*, **52**(1), 145–150(2022). Available online: https://he01.tci-thaijo.org/index.php/tjvm/article/view/255586.
16. Elezebeth, G., Malik, S., Chaudhari, S. P. and Barbuddhe, S.B. The occurrence of *Listeria* species and antibodies against listeriolysin-O in naturally infected goats, *Small Ruminant Research,* **67**(3),173–178(2007). doi: 10.1016/j.smallrumres.2005.09.029.
17. Rawool, D. B., Malik, S.V.S., Shakuntala, I., Sahare, A. M. and Barbuddhe, S.B. Detection of multiple virulence-associated genes in Listeria monocytogenes isolated from bovine mastitis cases, *International Journal Food Microbiology*, **113**(2), 201–207(2007). DOI: 10.1016/j.ijfoodmicro.2006.06.029.
18. Al-Ali, H. J., Al-Rodhan, M.A., Al-Hilali, S.A., Al-Charrakh, A.H., Al-Mohana, A.M. and Hadi, Z.J. Molecular detection of serotype groups of Listeria monocytogenes isolated from gallbladder of cattle and sheep in Iraq. *Veterinary World*, **11**(4), 431-436(2018). doi: 10.14202/vetworld.2018.431-436. Epub 2018 Apr 7.
19. Yassin, S. A., Nadhom, B. N. and Al-Gburi, N. M . Detection of Listeria monocytogenes in Several Types of Frozen Meat in Baghdad city. *Iraqi Journal ofSciences*, **62**(3), 742–750(2021). https://doi.org/10.24996/ijs.2021.62.3.4.
20. Geo, F., Janet, S. and Stephan A. Medial Microbiology Twenty. 2th ed., McGraw-Hill companies. pp. 192-193 (2001).
21. Cowan, S.T. In: Cowan and Steel's manual for identification of medical bacteria. Barrow, S.I. and Feltham, R.K. (Ed.), Cowan Cambridge University Press. pp. 317, (1993).
22. Bubert, A., Hein, I., Rauch, M., Lehner, A., Yoon ,B., Goebel, W. and Wagner, M. Detection and differentiation of Listeria spp. by a single reaction based multiplex PCR, *Applied and Environmental Microbiology*, **65**(10), 4688–4892(1999). doi: 10.1128/aem.65.10.4688-4692.1999
23. Lane, D.J. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, (editors). Nucleic acid techniques in bacterial systematics. John Wiley & Sons, New York, NY, USA. pp. 115-175, (1991).
24. Suárez, M., González-Zorn, B., Vega, Y., Chico-Calero, I. and Vázquez-Boland, J.A. A role for ActA in epithelial cell invasion by Listeria monocytogenes, *Cell Microbiol*ogy, **3**(12), 853-64(2001). doi: 10.1046/j.1462-5822.2001.00160.x. PMID: 11736996.
25. Liu, D., Lawrence, M. L., Austin, F. W. and Ainsworth, A. J. Amultiplex PCR for species-and virulence-specific determination of Listeria monocytogenes. *Journal of Microbiology Methods*, **71**(2), 133-140 *(*2007). doi: 10.1016/j.mimet.2007.08.007. Epub 2007 Aug 28..
26. McLauchlin, J. Listeria monocytogenes, recent advances in the taxonomy and epidemiology of listeriosis in humans. *Journal of Applied Bacterialogy*, **63**(1), 1-11(1987) DOI: 10.1111/j.1365-2672.1987.tb02411.x
27. Rahimi, E., Momtaz, H., Behzadnia, A. and Baghbadorani, Z.T. Incidence of Listeria species in bovine, ovine, caprine, camel and water buffalo milk using cultural method and the PCR assay, *Asian Pacific Journal of Tropical Disease*, **4**(1),50–53(2014). doi: 10.1016/S2222-1808(14)60313-3.
28. Dunnett, E., Florea, L., Thurston, L., Floyd, T., Collins, R. and Otter A. Deaths of weaned lambs with visceral *Listeria ivanovii* infections. *Veterinary Records Case. Reports*, **8**(4), 1679 (2020). doi: 10.1136/vetreccr-2020-001254.
29. Aygun, O. and Pehlivanlar, S., Listeria spp. in the raw milk and dairy products in Antakya, Turkey. *Food Control*, **17**(8), 676-679(2006). https://doi.org/10.1016/j.foodcont.2005.09.014
30. Yakubu, Y., Salihu, M.D., Faleke, O.O., Abubakar, M.B., Junaidu, A.U., Magaji, A.A., Gulumbe, M.L. and Aliyu, R.M. Prevalence and antibiotic susceptibility of in raw milk from cattle herds within Sokoto Metropolis, Nigeria**,** *Sokoto Journal of Veterinary Sciences*, **10**(2),13–17 (2012). DOI:10.4314/sokjvs.v10i2.3.

31. Quinn, P.J., Markey, B.K., Leonard, F.C., Fitzpatrick, E.S., Fanning, S. and Hartigan, P.J. *Veterinary Microbiology and Microbial Disease*. 2nd ed., United Kingdom :Wiley-Blackwell. pp. 928 (2011) .
32. Bille, J., Rocourt, J. and Swaminathan, B. Listeria, Erysipelothrix, and Kurthia. In Murray P R, Baron E J, Pfaller M A, Tenover F C & Yolken R H. Manual of Clinical Microbiology Washington: ASM Press. pp. 346–356 (1999).
33. Liu, D. Handbook of Listeria Monocytogenes.1st edition. Boca Raton: CRC Press. pp.40,(2008). https://doi.org/10.1201/9781420051414.
34. Low, M. G. Degradation of glycosylphophatidy-linositol anchors by specific phospholipases. In Turner A.J., Molecular and cell biology of membrane proteins. Glycolipid anchors of cell surface proteins. England: E. howard Ltd., Chichester. pp. 35-63,(1990)
35. Kovassi, N. M. and Shelef, L. A. Listeriolysin O secretion by Listeria monocytogenes in the presence of cysteine and sorbate. Lett. *Applied Microbiology,* **20**(5), 295-299(1995) DOI: 10.1111/j.1472-765x.1995.tb00449.x
36. Orsi, R.H. and Wiedmann, M. Characteristics and distribution of Listeria spp., including Listeria species newly described since 2009. *Applied Microbiology and Biotechnology*, **100** (12), 5273–5287 (1995). doi:10.1007/s00253-016-7552-2. PMC 4875933. PMID 27129530
37. Alberti-Segui, C., Goeden, K.R. and Higgins, D.E. Differential function of Listeria monocytogenes listeriolysin O and phospholipases C in vacuolar dissolution following cell-to-cell spread. *Cell Microbiol*ogy, **9**(1), 179–195(2007). DOI: 10.1111/j.1462-5822.2006.00780.x
38. Gouin, E., Mengaud, J. and Cossart, P . The virulence gene cluster of Listeria monocytogenes is also present in Listeria ivanovii, an animal pathogen, and Listeria seeligeri, a nonpathogenic species. *Infection and Immunity,* **62**(8), 3550-3553(2007). DOI: 10.1128/iai.62.8.3550-3553.1994.
39. Chakraborty, T., Hain, T. and Domann, E. Genome organization and the evolution of the virulence gene locus in *Listeria* species . *International Journal of Medical Microbiology*, **290**(2), 167-174(2000). doi:10.1016/S1438-4221(00)80086-7 .
40. Frehel, C., Lety, M.A., Autret, N., Beretti, J.L., Berche, P. and Charbit, A. Capacity of ivanolysin O to replace listeriolysin O in phagosomal escape and in vivo survival of Listeria monocytogenes. *Microbiology (Reading*), **149** (Pt3), 611-620(2003).doi: 10.1099/mic.0.25986-0.
41. Dussurget, O., Pizarro-Cerda, J. . and Cossart, P. Molecular determinants of Listeria monocytogenes virulence. *Annual Review* of *Microbiology*, **58**, 587–610(2004).DOI: 10.1146/annurev.micro.57.030502.090934

أول اكتشاف لليستريا إيفانوفي في أجنة الأغنام المجهضة في محافظة نينوى العراقية

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يسبب الإجهاض خسائر مالية كبيرة لتجارة الأغنام. من الضروري تحديد المسببات من أجل التعامل بنجاح مع حالات الإجهاض. التحليل الحالي يحدد دور اللستريا ايفانوفي إجهاض الأغنام في العراق. خلال شهري نوفمبر وديسمبر 2022، تم فحص إجمالي 500عينة سريرية (100جنين) لعزل وتحديد بكتيريا اللستريا ايفانوفي. تم استعمال الابي الخاص بانواع اللستريا والأجار (الوسط اللوني) وتفاعل البلمرة المتسلسل لتأكيد تشخيص العزلات. عند التحليل تم تحديد أربع عزلات حيث تم عزل اثنتان من العزلات الأربع (LIVANOVII31) من أنسجة المخ بينما تم عزل واحدة من المشيمة والأخرى (LIVANOVII53) من محتويات معدة الجنين المجهض، على التوالي OQ983887.1 و OQ983888.1 وسجلت العزلتان في بنك الجينات العالمي. تحت أرقام الانضماموالحظ أن معدل العزل الكلي للبكتيريا كان 0.8%. وكانت جميع العزلات البكتيرية إيجابية لعوامل الضراوة.

الكلمات الدالة :الاغنام ، الاجهاض ، اللستريا ايفانوفي ، تفاعل البلمرة المتسلسل.