

How to Cite:

Abdzed, H. S., Saleh, M. A., & Kadhim, K. S. (2022). Molecular detection of *Listeria monocytogenes* in raw milk in Kerbala Province. *International Journal of Health Sciences*, 6(S4), 9115–9122. <https://doi.org/10.53730/ijhs.v6nS4.11902>

Molecular detection of *Listeria monocytogenes* in raw milk in Kerbala Province

Hind Salim Abdzed

Department of public health /College of Veterinary Medicine, University of Karbala, Iraq

Mohammed Assad Saleh

Prof. Dr., Department of Internal Medicine/College of Veterinary Medicine, University of Karbala, Iraq

Corresponding author email: mohammed.asaad@uokerbala.edu.iq

Kadhim Saleh Kadhim

Prof. Dr., Department of public health /College of Veterinary Medicine, University of Karbala, Iraq

Email: Kadhim.Salih@uokerbala.edu.iq

Abstract---*Listeria monocytogenes* was discovered as a source of contamination during sampling of raw milk and its products from local cows at dairy farms, markets, and shipping points. The goal of the current study was to define the accuracy of *Listeria monocytogenes* in raw milk contamination. This study was conducted by randomly collecting samples from various areas in the Karbala province and its surroundings. From January to March 2022, 250 samples of milk and dairy products (yogurt, cheese, and butter) were randomly selected, processed in accordance with *Listeria monocytogenes* standard procedures, and sent to a lab from individual cows for analysis. Some biochemical experiments were conducted to ascertain the physical and biochemical characteristics of milk and handled in accordance with *Listeria monocytogenes* standard procedures before being delivered from the individual cows for analysis to the lab, the study includes the isolation and molecular detection of *Listeria monocytogenes* from animal sources by employing selective media for this bacteria (oxford agar) for growing, as well as the molecular analysis of *L. monocytogenes* performed by conventional PCR technique using particular 4 primers (Hyl-1-f, Hyl-2-R, List-F, List-R).

Keywords---raw milk, *Listeria monocytogenes*, molecular detection, Kerbela province, conventional PCR.

Introduction

Listeria monocytogenes is a pandemic facultatively intracellular organism, non-spore forming hazardous pathogen that causes serious and dangerous disease throughout the world (Baer et al.,2013). Due to its distinctive appearance, *Listeria monocytogenes* is frequently referred to as a coryneform organism. It is a tiny, coccoid to rod-shaped, positive organism in gram stain, demonstrating a peculiar motility at normal room temperature (tumbling) (Pirie,1940). By examining the cell structure, colony morphology, and hemolytic reaction—which show up as blue to green color colonies on sheep blood agar when studied microscopically under direct lighting with triptose agar—many isolates from the bacteria can be found. (Seeliger et al.,1986). Positive catalase and negative oxidase are found in the microbe, and fermentative glucose metabolism indicates the yield of lactic acid. A large number of other sugars produce acid but no gas. (Griffiths,1989). There are ten species in it, including *Listeria monocytogenes* and *Listeria seeligeri*. *Listeria weihenstephanensis*, *Listeria ivanvi*, *Listeria innocua*, *Listeria Grayi*, *Listeria Welshimer*, *Listeria marthi*, *Listeria rocourtia*, and *Listeria rocourtia* (Halter,2013). *Listeria. Monocytogenes* is a zoonotic disease that spreads through food and causes devastating infections in immunocompromised people and pregnant women (Portnoy et al.,1992). Numerous variables, including farm size, animal density, hygienic and sterilization standards, farm management procedures, variety in sample types and types of samples evaluated, use of different detection methodologies, geographic location, and weather conditions, all have an impact on the incidence of pathogens in milk. In spite of their differences, all of these studies unmistakably show that milk can be a significant source of foodborne illness with regard to human health importance (Oliver et al2005).

Materials and Methods

The animal

The case history of animal was taken, animals with previous abortion cases is preferred for taken samples of raw milk also general examination of the udder and teats and detection of the infected part. collection of samples from raw milk: Collection of raw milk samples: Over the course of four months, 250 samples of raw milk were gathered from various market places in Karbala City. These samples were then tested for the persistence of *L. monocytogenes*.

Isolation procedure

Types of *Listeria* are isolated using Oxford agar (OXA) as a selective culturing media for *Listeria* isolates, inoculated (OXA), and incubated in 35C for 24-48 hours (Warburton et al.,2003). Samples of both raw milk and its products are taken within 24 and 48 hours of incubation and are then inoculated on Oxford *listeria* selective agar and incubated in 37C. A catalase test is performed, samples are stained with gram stain, and *listerial* colonies in plates from both enrichment media cultures (primary and secondary) are studied at 24 to 48 hours for morphology and ascoline hydrolysis. Four perfect colonies are transferred from the dish to sheep blood agar and cultured for 24 hours at 37°C to detect

hemolysis when the catalase test is positive and a Gram positive tiny coccobacilli are discovered.

Genomic DNA extraction

A 0.7 percent agarose gel electrophoresis followed by ethidium bromide coloring is used to verify the presence of genomic DNA in fully prepared samples (Dmitry et al.,2006). *Listeria monocytogenes* isolates are identified using a PCR assay with specific primers, whole samples are repeatedly multiplied in a fraser broth, and special medium, such as Oxford, are used for culture (Oxoid , Hampshir, U.K.). *Listeria* spp. suspect isolates are verified using PCR identification of the bacteria, which uses 4 primers based on the invasive proteins genes of *L. monocytogenes*. Thermocyclic reaction is used to carry out DNA amplification., the cycling condition to PCR mentioned in table 1.

Table 1 Thermal cycling protocols for detection of *L. monocytogenes*.

species	Initial denaturation	Denaturation	Annealing	Extension	Final Extension
Listeria monocytogenes	95C 5 Minutes	95C 45 seconds	65C 1 minute	72C 1 minutes	72C 10 minute
Species	Target gene	Primer sequence (5'to3')	Size	Reference	
L.Monocytogenes	List-f	5'GGACCGGGG CTAATACCGAAT GATAA-3' (26mer)	1100pb	(Jacobsen et al.,2011)	
	List-R	5'TTCATGTAGG CGAGTTGCAGC CTA-3' (24mer)	250pb		
	Hly-1-f	5'ATTTTCCCTTC ACTGATTGC-3' (20mer)			
	Hly-2-R	5'CACTCAGCATT GATTGCCA-3'(20mer)			

Identification of listeria monocytogenes by conventional PCR

The polymerase chain reaction (PCR) is a test tube method for replicating DNA that enables the "targeting" DNA Sequence to be selectively amplified millions of times in a short period of time. The PCR establishes amplification to a predefined DNA fragment (the target), which may, for example, range in length from 100 to 1000 bases.

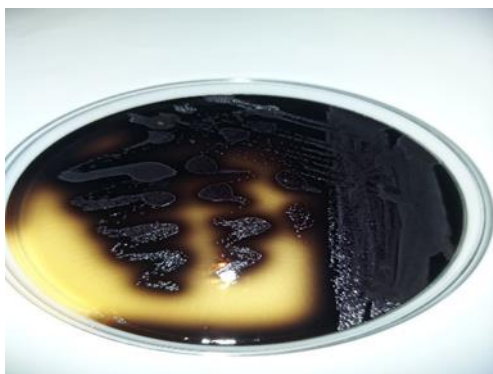
DNA sequence method

The DNA sequencing method is used in the study of genetic variation among local *listeria monocytogenes* isolations.

Results and Discussions

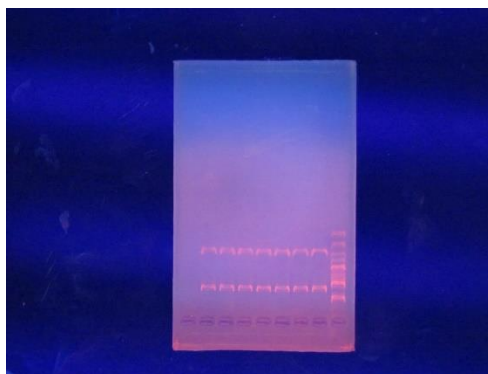
Results

Result of growth on culture media: after acquiring colonies suspected of being bacteria *L. monocytogenes* growing on blood agar medium, which was characterized as small white to gray colonies B-hemolysis. These colonies were vaccinated in the culturing media to observe the shape and characteristics of the colonies, as they appeared on: Oxford agar: Colonies of *L. monocytogenes* appeared on this medium black with the color of the medium changing from yellow to black as shown in fig1.



listeria monocytogenes on oxford agar

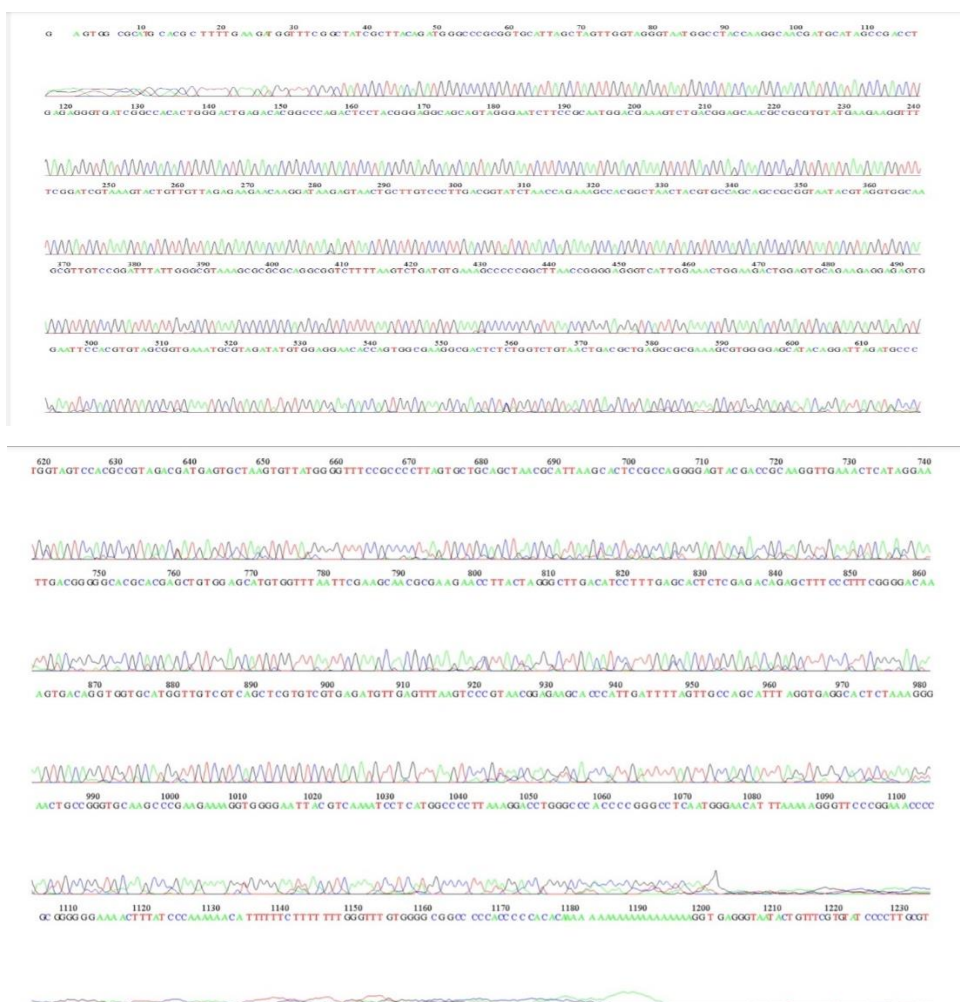
Result of molecular PCR: PCR technique showed that from 250 samples of raw milk and dairy products from cow susceptible infected with *listeria monocytogenes* by microscopically examination there is (48%) positive by conventional PCR and amplification use PCR and Sequencing of PCR (fig2).



Conventional PCR

Result of DNA sequence

Genetic variation between local listeria monocytogenes isolates which was carried out using the DNA sequencing process



Discussion

The results of our study showed that the isolation rates of *L. monocytogenes* from raw milk and milk products are 1.2 percent, and that the high rate of germ isolation from sail points samples compared to isolation rates of samples from farms, livestock fields, and supermarkets in our study is due to exposing milk samples to the contaminated open air. There are no scientific documents explaining why listeria monocytogenes was discovered in the Iraqi province of Karbala in cow's milk. However, *L. monocytogenes* can be isolated from raw milk, yoghurt, and ready-to-eat goods, many of which lack refrigerated transferring containers for transferring milk from farms and fields to local markets and shipping ports, Raw milk, yoghurt, and ready-to-eat foods can all be used to isolate *L. monocytogenes*, and many of those foods were popular in Iraqi

governorates. That makes this problem a risk to the public's health because the bacterium, which can spread and be transported through consumption of such tainted products, can cause a variety of diseases, culminating in human listeriosis (Safanaa et al.,2021). Due to differences in study location, sample size, and seasonal change, our results suggest that listeria detection from milk occurs at a rate of 1.2 percent, which is lower than the rate reported by (Alzubaidy,2013) which is 12.5% in Baghdad. The incidence of contamination in this study (1.2%) was greater than the levels of raw milk contamination in earlier studies conducted in Iraq, since these studies found that the rate of contamination in the north of Iraq [Erbil] was 3.4 percent (Al shammary,2001) in the western part of Iraq, 15.2 percent (Noomi et al.,2021). Additionally, a 2020 study of Baghdad (in the center of Iraq) found that 31.1 percent of the meat samples were contaminated with *L. monocytogenes*. However, less than 5% of raw milk samples have been found to have *L. monocytogenes* contamination (Vitas et al.,2004) (Okutani et al.,2004). The winter months are ideal for bacterial epidemics. The standard PCR method of diagnosis and testing is preferable to culturing from milk because milk contains a lot of water, which provides the ideal habitat for the growth of listeria. Moreover, cow milk contains phospholipids that are perfect for *Bactria* (Robinson et al.,2000). *Listeria monocytogenes* infection rates vary depending on a number of variables, including the type of animal, strain, age, and geographic region (Schlech et al.,1983). Additionally, *L. monocytogenes* are recovered from all raw milk samples with a variety of variations depending on the quantity of the germ in the oculums and the levels of microbotic; this phenomenon may be related to milk consumption (Besse,2002). Endemic macrobiotics play a significant role and serve as the primary interfering element, such as the many types of metabolites produced by macrobiotics in milk, which can create an uncomfortable environment and reduce bacterial growth or survival through competition (Jalali et al,2008). Our study's results also show significant differences from those of other studies carried out in other nations. For example, a study on milk products, frozen meat, and ready-to-eat food was probably conducted in Iran (Isfahan) and concluded that these products, as well as the frozen meat and ready-to-eat food industries, had contamination rates of 4% for *L. monocytogenes* (Goh et al.,2012). It is higher than what this investigation discovered. Additionally, compared to the results of the prior study, the incidence of *L. monocytogenes* in the present investigations is reduced (Hussein et al.,2015). Studies conducted in Iraq show that the occurrence of listeriosis disease occurred in extremely cold periods, which is why our investigations for the difference between the results of this study and those of the earlier work show that a wide variety of animal kinds, like mammals, all domesticated animals, can be infected with *L. monocytogenes*. (Bickley et al 1996). The varying amounts of contamination that have been reported from numerous local studies may have been caused by regional differences, variations in sample collection methods, or variations in molecular detection methodologies. Because calcium ions act as a PCR inhibitor in raw milk, more research must be done in farms utilizing a variety of samples in order to accurately establish the presence of *Listeria monocytogenes*. (Ho lappi et al.,2007) PCR can identify both live and dead bacteria, even in low concentrations. In our study, farms that continuously fed silage had a three to seven times higher risk of isolating *L. monocytogenes* than farms that did not. Because the prevalence of *L. monocytogenes* declines in the fall when silage is not used in food, the use of contaminated silage for animals most likely indicates the seasonality that can be

observed. For sheep and goats, contaminated silage is a source of listerial infection. It also significantly contaminates the environment on farms and in milk processing facilities (Ryser,2011). Compared to most other non-spore-forming foodborne bacteria, *Listeria monocytogenes* is more temperature tolerant. *L. monocytogenes* will be completely destroyed using the existing vat (63C for 30min) and high temperature, brief pasteurization (72C for 15s) techniques. Despite *L. monocytogenes*' potential to reach populations of 10⁶ cfu ml in commercial skim milk, all milk, and whipping cream after 8 days of storage at 8C (a temperature that home refrigerators frequently reach), this bacterium has only sometimes been found in pasteurized milk products (Robinson et al.,2000).

Conclusion

Milk and dairy products have a major role in transmitting *L. monocytogenes* to humans and causing disease cases, Outbreaks of listeriosis in many countries, caused by consumption of milk and its derivatives which contaminated with *Listeria monocytogenes*, Refer the risk and danger to human health from such products that placed in markets.

Acknowledgments

We would like to thank everyone who contributed and volunteered, in particular the authors, commentators, and editors who made this matter possible.

References

- AL-Shammmary, A., H., A. (2001). The Incidence of *Listeria monocytogenes* in Milk and Some Dairy Products in Baghdad. PhD Thesis, college of vet. Medicine. Baghdad University.
- Alzubaidy, Z. (2013). Isolation and identification of *Listeria moncytogenes* by PCR from some food sources in Erbil city-5 (3): 14-26.
- Baer AA, Miller MJ, Dilger AC. (2013). Pathogens of interest to the pork industry: a review of research on interventions to assure food safety. *Compr Rev Food Sci Food Saf* ;12:183-217.
- Besse, G. (2002). Influence of various environmental parameters and of detection procedures on the recovery of stressed *L. monocytogenes*: a review. *J. Food Microbiol.* 19(2-3),221-34.
- Bickley, J., Short, J. K., McDowell, D. G., & Parkes, H. C. (1996). Polymerase chainmreaction (PCR) detection of *Listeria monocytogenes* in diluted milk and reversal of PCR inhibition caused by calcium ions. *Letters in Applied Microbiology*, 22(2), 153-158.
- Dmitriy V.; Joseph G.; Christine A. ; Robert E. D. and Anthony D. H. (2006):Discovery of Natural Atypical Non hemolytic *Listeria seeligeri* Isolates. *Applied and Environ. Microbiol.*, 72: 2439-2448.
- E T Ryser. (2011). *Listeria monocytogenes*, Michigan State University, East Lansing, MI, USA Elsevier Ltd. All rights reserved.
- Goh SG, Kuan CH, Loo YY, Chang WS, Lye YL. (2012) . *Listeria monocytogenes* in retailed raw chicken meat in Malaysia. *Poultry Science*, 91: 2686-2690.
- Griffiths, M. W. (1989). *Listeria monocytogenes*: its importance in the dairy industry. *Journal of the Science of Food and Agriculture*, 47(2), 133-158.

- Halter, E. L., Neuhaus, K., & Scherer, S. (2013). *Listeria weihenstephanensis* sp.nov., isolated from the water plant *Lemna trisulca* of a German fresh water pond. *International Journal of Systematic and Evolutionary Microbiology*, 63,641e647.
- Ho, A. J., V. R. Lappi, and M. Wiedmann. (2007). Longitudinal monitoring of *Listeria monocytogenes* contamination patterns in a farmstead dairy processing facility. *J. Dairy Sci.* 90:2517–2524.
- Hussien OA, Huda AA. (2015). Isolation and Identification of *Listeria monocytogenes* From Human and Animal in Al- Qadissiya Province. *Al-Qadissiya J. For pure science*, 12(1): 1122.
- Jalali M, Abedi D. (2008) . Prevalence of *Listeria* species in food products in Isfahan, Iran. *Int J Food Microbiol*, 122: 336-340.
- Noomi, B. S., Anwar, S. A., & Salih, S. M. (2021). Detection Of *Listeria Monocytogenes* In Raw Milk And Aborted Cow Cases At Salahudeen Province. *Iraqi Journal Of Agricultural Sciences*, 52(2), 315-321.
- Normatova, S. A. ., Botirov, M. T. ., Ruzmatova, K. K. ., & Mamarasulov, J. O. ugli. (2021). Hygienic basis for contamination of food products and production of dairy products until 2030. *International Journal of Health & Medical Sciences*, 4(1), 123-128. <https://doi.org/10.31295/ijhms.v4n1.1592>
- Okutani A, Okada Y, Yamamoto S, Igimi S. (2004). Overview of *Listeria monocytogenes* contamination in Japan. *Int J Food Microbiol.* 93: 131–140.
- Oliver, S. P., Jayarao, B. M., & Almeida, R. A. (2005). Foodborne pathogens in milk and the 342 dairy farm environment: food safety and public health implications. *Foodborne Pathogens343 & Disease*, 2, 115-129.
- Pirie, J. H. (1940). *Listeria*: change of name for a genus bacterium. *Nature*, 145(3668), 264-264.
- Portnoy, D. A., Chakraborty, T., Goebel, W., & Cossart, P. (1992). Molecular determinants of *Listeria monocytogenes* pathogenesis. *Infection and immunity*, 60(4), 1263-1267.
- Robinson, R.K.; Batt, C.A. and Patel, P.D. (2000). *Encyclopedia of Food Microbiology*, San Diego, CA.
- Safana A.S.Yassin, Ban N. Nadhom , Nagham M.Al-Gburi. (2021). College of Veterinary Medicine, University of Baghdad, Bagdad, Iraq Detection of *Listeria monocytogenes* in Several Types of Frozen Meat in Baghdad city.62.3.4.
- Schlech, W.F., Lavigne, P.M. and Bortolussi, R.A. (1983). Epidemic listeriosis: evidence for transmission by food. *New. Engl. J. Med.* 308: 203 - 206.
- Seeliger H P R, Jones D. (1986) *Listeria*. In: *Bergey's Manual of Systematic Bacteriology* Vol2, eds Sneath P H A, Mair N S, Sharpe M E & Holt J G. Williams & Wilkins, Baltimore.
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, 6(1), i-v. <https://doi.org/10.53730/ijhs.v6n1.5949>
- Vitas A, Garcia-Jalon V. (2004). Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). *Int J Food Microbiol.* 90: 349–356.
- Warburton, D.; Boville, A.; Pagotto, F.; Daley, E. and Chow, C. (2003): The detection of *Listeria* spp. in food and environmental samples using PALCAM broth, *health and food branch1*, 6(2): S85 – S89a.