

Isolation and Molecular Detection of *Listeria monocytogenes* in Minced Meat, Frozen Chicken and Cheese in Duhok Province, Kurdistan Region of Iraq

Saman Said Taha Said Ahmed^{*}, Bizhar Ahmed Tayeb, Alind Mahmood Ameen, Sowaila Mekaeel Merza and Yousif Hamed Mohammed Sharif

^{*}Laboratory Department, Veterinary Directorate in Duhok 1, 1000 Jn Galy Way, 42001 Duhok Province, Iraq

^{*}Corresponding author: Saman Said Taha Said Ahmed, Laboratory Department, Veterinary Directorate in Duhok 1, 1000 Jn Galy Way, 42001 Duhok Province, Iraq, E-mail: shokri.mustafa@uqconnect.edu.au

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Abstract

Aims: *Listeria monocytogenes* (*L. monocytogenes*) is the food-borne pathogen responsible for listeriosis. It is considered a serious public health risk, and is spread through the consumption of food products. The disease can be fatal to humans and animals. The objective of the present study was to isolate and identify *L. monocytogenes* from frozen chicken, minced meat, and cheese in Duhok province, Iraq.

Methodology and Results: Between March and October 2015, equal numbers (n=50) of samples of minced meat, frozen chicken, and cheese were collected (total n=150). Biochemical and microbiological tests, including real-time PCR, were performed to determine the prevalence of *L. monocytogenes* in the samples. Out of 150 samples, 20 displayed greyish/black colonies with black halos on Oxford and Palcam agar. Of these putative *Listeria*, 12 were subsequently identified as *L. monocytogenes* by PCR. *L. monocytogenes* was detected in 1 (2%), 7 (14%), and 4 (8%) of isolates from cheese, minced meat, and frozen chicken, respectively.

Conclusion: Significance and Impact of study: Detection of pathogenic bacteria in foods, such as those analysed in this study, is crucial for safeguarding public health. The qPCR technique offers a rapid and reliable method for isolation of many foodborne pathogens in different kinds of foods.

Keywords: *Listeria monocytogenes*, Real time PCR; Identification; Detection; Food

Introduction

Listeria spp. are broadly distributed in the environment. They can be isolated from soil and water Jersik, et al. [1]. A wide range of animal species can become contaminated by *L. monocytogenes*, including mammals, domesticated animals, pets, fish, birds, and crustaceans Alsheikh et al. [2].

In mammals, *L. monocytogenes* can cause premature births and meningoencephalitis Aznar et al. [3]. *L. monocytogenes* is a concern to public health because of its capability to survive or even grow under harsh conditions [4,5]. The increasing rate of *L. monocytogenes* involvement in food-borne outbreaks has led to the requirement for a rapid method for testing food products. Almost all cases of *listeriosis* tend to be food-borne, and a number of food items can become contaminated by *L. monocytogenes*, including raw chicken meat, raw minced meat, soft cheese, and fish. Most of these items are widely consumed in Iraq's Kurdistan and Duhok provinces.

A number of molecular biological methods have been described for detection of *L. monocytogenes*, including DNA probes and PCR techniques. Direct detection of *L. monocytogenes* in food products by PCR has been reported in several cases Bansal et al. [6], Manzano et al. [7], Agersborg et al. [8], O'Connor et al. [9] and Hudson et al. [10].

To our knowledge, there is no published data regarding *L. monocytogenes* prevalence in food samples from Duhok province. In fact, there are no official data of food-borne *listeriosis* recorded in

Duhok, as *L. monocytogenes* is rarely tested for in food samples. The objective of the present research was to assess the use of PCR for the detection of *L. monocytogenes* in food products, and to determine the prevalence of *Listeria spp.* in food products in Duhok, by using molecular and biochemical methods.

Materials and Methods

Samples (n=150) of different food products, including chicken (n=50), minced meat (n=50), and cheese (n=50), obtained from different supermarkets, restaurants and veterinary quarantine centres in Duhok province, were tested. All samples had been correctly stored, and were placed in separate sterile plastic bags to prevent spilling and cross contamination. The samples were brought to the laboratory on crushed ice, and were kept at 4°C until testing (within 4 h).

Food samples were analysed for the presence of *Listeria spp.* using a selective enrichment and isolation protocol, as recommended by the United States Department of Agriculture (USDA) [11].

Microbiological investigation

Samples (25 g) were added to 225 mL of ½ strength Fraser broth (Conda, Spain), to obtain a 1:10 dilution. All samples were homogenized (30-60 sec) and incubated (30°C, 24 h). From this primary enrichment, 0.1 mL was then inoculated into 10 mL of Fraser broth, and incubated (37°C, 48 h) with shaking. A loopful of the subsequent culture was streaked on the surface of different agars (PALCAM *Listeria* agar (Conda, Spain) with supplement, and OXFORD agar (Lab UK) with X122 supplement). Agar plates were then incubated (37°C, 48 h) and observed (at 24 to 48 h incubation)

for suspected colonies (greyish colonies surrounded by black halos, with sunken centres with possible greenish sheen on Oxford, or black colonies on PALCAM). The following tests were used for confirmation; Gram's stain, motility test, catalase and oxidase reactions, and qPCR.

DNA Extraction

Listeria DNA was extracted from a sweep of several colonies grown on PALCAM, using the boiling method. One loop of *Listeria* from the agar plates was suspended in 100 µL of sterile de-ionized water in a 1.5 mL microcentrifuge tube and vortexed, boiled (95-100°C, 10 min), and centrifuged (10,000 × g, 10 min). The supernatant was used as a DNA template for PCR Nessa et al. [12]. Purification of DNA was achieved by using a genomic DNA purification kit (Qiagen, Germany), according to the manufacturer's instructions. The DNA was measured with the Nanodrop Spectrophotometer QIAxpert (QIAGEN), and stored at 4°C.

Real-Time PCR

Screening of suspected *Listeria* was conducted with a Food proof *Listeria* detection kit (Biotecon Diagnostics, Catalog), which uses real-time PCR for the qualitative detection of *Listeria spp.* This analysis was performed with a final volume of 25 µL of reaction mixture in each well of a 96-well plate, containing 20 µL of PCR Master Mix (provided) and 5 µL of DNA template, with positive and negative controls also being run. DNA was then amplified in the standard mode running (2 h) on a 7500 fast real-time PCR machine (Applied Biosystems, Foster City, CA, USA). Amplification conditions were optimized Table 1. The amplification results were analysed during the last 50 cycles of the amplifications, using the 7500 fast software V.1.4.0 (Applied Biosystems).

Stage	Temperature	Time	Cycles
Holding stage	37°C	4 min	1
Holding stage	95°C	5 min	1
Denaturation	95°C	5 sec	50
Annealing	60°C	60 sec	50

Table 1: RT-PCR conditions and cycling for *L. monocytogenes* detection.

Results

Of the 150 tested samples, 13.3% were presumptively positive *Listeria spp.* by the culturing method, while 8% were confirmed as *L. monocytogenes* by PCR. All samples were initially streaked on Palcam and Oxford agar. On Palcam, which is a selective media recommended for the isolation of *L. monocytogenes* from foods, *Listeria* colonies appeared grey-green with black-sunken centers and a black halo Figure 1. Twenty putative *L. monocytogenes* colonies were confirmed as *Listeria spp.* by being positive for catalase and motility, and negative for oxidase Table 2. *Listeria* was found in 3 samples of cheese (6%), 6 of chicken (12%), and 11 of meat (22%).

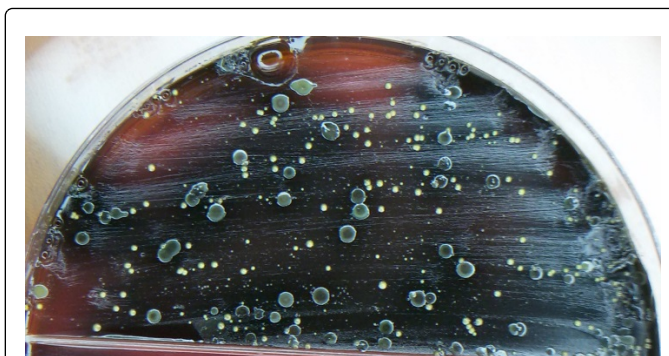


Figure 1: *L. monocytogenes* colonies on Palcam agar (arrows) appears as grey-green with black-sunken centres and a black halo colonies.

Sample	Positive samples by culture	Catalase	Oxidase	Motility
Cheese	3	Positive	Negative	Motile
Meat	11	Positive	Negative	Motile
Chicken	6	Positive	Negative	Motile
Total	20			

Table 2: Microbiological and Biochemical tests of *L. monocytogenes*.

Confirmation of *L. monocytogenes* by real-time PCR

Real-time PCR analyses of the 20 suspected *L. monocytogenes* samples are shown in Figure 2. The majority (12) of samples were confirmed as positive Table 3.

Sample	No. Of Sample	Suspected <i>L. monocytogenes</i> culture (Oxford and Palcam)	By <i>L. monocytogenes</i> Amplifying by PCR
Cheese	50	3	1
Meat	50	11	7
Chicken	50	6	4
Total	150	20	12

Table 3: Number of Positive sample according to real-time PCR.

Discussion

Listeria monocytogenes is a highly pathogenic bacterium, contaminating a wide range of food products, and with a high mortality rate. From a total of 150 samples of various types of food products from Duhok province, Iraq, soft cheese 1 (2%), raw chicken 4 (8%), and red minced meat (Lamb) 7 (14%), were positive for *L. monocytogenes* by PCR. These results indicate that meat products are considerably more likely to be contaminated with *L. monocytogenes* than other food products. This could be due to preparing and processing minced meat (used, for example, in kebabs) in poor hygienic conditions Akya et al. [13]. Although other factors such as

equipment and food additives can also have a role in contamination, kebabs tend to be well cooked prior to consumption, reducing the chance of acquiring *listeriosis* in this kind of food Akya et al. [13].

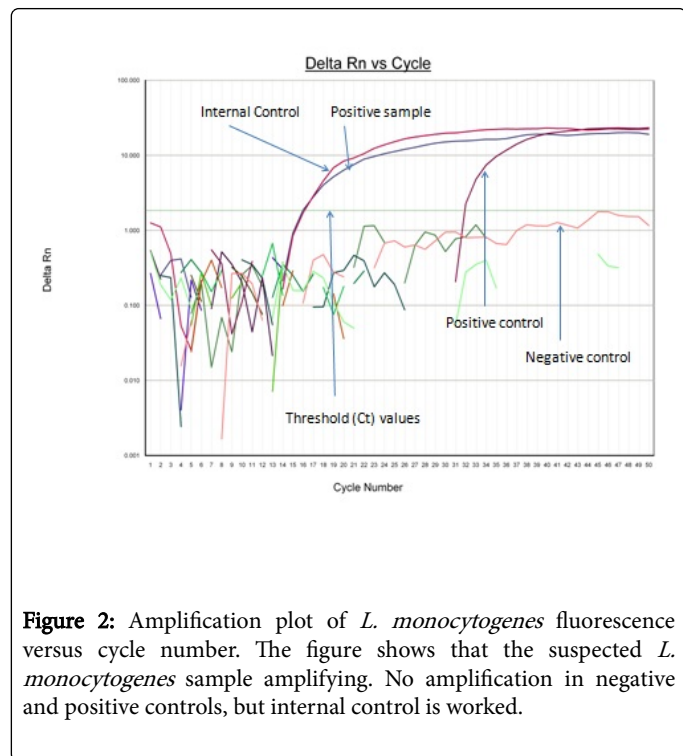


Figure 2: Amplification plot of *L. monocytogenes* fluorescence versus cycle number. The figure shows that the suspected *L. monocytogenes* sample amplifying. No amplification in negative and positive controls, but internal control is worked.

Our results showed differences when compared to other studies. For example, a research was conducted by [14] in Isfahan, Iran on various food products including dairy products, meat, and ready-to-eat food found a 4.7% contamination rate with *Listeria spp*, which is lower than our result (16%) [14]. Interestingly [15] also found that the highest contamination rate was in meat products.

Regarding chicken meat, our results showed the lowest incidence (4%) of samples containing *Listeria spp*. These values are in agreement with a study performed by Mahmood and his colleagues Mahmood et al. [16]. However, the occurrence of *L. monocytogenes* in the current research was lower than that found in work carried out by Goh, et al. [17]. Another research conducted by Ayaz et al. [18], showed a higher incidence of *L. monocytogenes* in chicken meat (20.4%), although the numbers of samples were higher compared to those for the current study Ayaz et al. [18].

In soft cheese samples, we show that the prevalence of *Listeria* is not significant (2%). Our results were contradicted in another study conducted in Jordan by Osaili et al. [19] who reported approximately 30% *L. monocytogenes* sample contamination rate. It should be noted that the contamination rate of *Listeria* in dairy products in our study was consistent with the results of [14]. A low level (1.1%) of *L. monocytogenes* contamination was found in soft cheese by [13]. Those results are different from the ones obtained in our study. This might be due to contamination during the process of cheese-making, as well as the effectiveness of pasteurization. The acceptable contamination rate of *L. monocytogenes* ranges between 10% and 15.3% for cheese and raw milk [20,21]. A research was done by Turgay et al. [22], mainly focused on detection of *E. coli* O157 strains in cheese samples in Duhok area, found that the prevalence of the *E. coli* O157 was 4%. This

shows that the *E. coli* O157 rate in cheese is double than that compared to *L. monocytogenes* in the same food source.

Conclusions

Although our results indicate that *L. monocytogenes* is present in food products in Duhok governorate, the contamination level of this pathogen was comparatively low compared to other foodborne pathogen. While it was clear that meat products showed the highest level of contamination (14%), cheese was least contaminated (2%), with chicken (8%) being intermediate between these. PCR is often considered more dependable than conventional identification methods, since it is based on stable genotypic characteristics rather than relying on biochemical or physiological traits. According to this study, hygienic conditions are highly recommended in order to minimise the presence of *L. monocytogenes* in foods. We recommend that further research on *L. monocytogenes* in Duhok is carried out, in order to provide a better background of contamination rate and the routes of transmission for this bacterium.

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