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Survival of *Listeria monocytogenes* in Raw Milk

***May A. Al-Allaf** ****Amera M. Al-Rawi** ***Abdul Muhsin S. Al-Qazzaz**
** Environmental and Pollution / Control Research Center / University of Mosul*
*** Department of Biology / College of Science / University of Mosul*
E- mail:** ***Mayallaf2@yahoo.com

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ABSTRACT

This study was conducted on different raw milk samples (cow, buffalo, sheep, goat) and pasteurized "Nada" milk was used as a control sample after confirming that it is free from any microbial contamination. Mesophilic aerobic bacteria (microbiota) was enumerated in all raw milk samples. Different concentrations of *Listeria monocytogenes* were inoculated at different dilutions of raw milk to detect the survival of this bacterium with the presence of microbiota in milk .The results showed the ability of *Listeria monocytogenes* to survive in raw milk samples with low levels of microbiota while it can not grow in the samples with high levels of microbiota and this survival differs with different origins of raw milk samples under study.

Keywords : Autochthonous microbiota, *Listeria monocytogenes*, Raw milk.

بقاء جرثومة *Listeria monocytogenes* في الحليب الخام

الملخص

أجريت هذه الدراسة على عينات حليب خام مختلفة (بقر، جاموس، غنم، ماعز) وأستخدم حليب ندى المبـستر بوصـفها عينات سيطرة بعد التأكد من خلوه من أي تلوث جرثومي. تم حساب أعداد البكتريا الهوائية (microbiota) الموجودة أصـلا في الحليب الخام بأنواعه. لقحت عينات الحليب بتراكيز مختلفة من جرثومة *Listeria monocytogenes* لدراسة مدى مقاومة هذه الجرثومة وقدرتها على البقاء مع الجراثيم الموجودة أصلا في عينات الحليب. أظهرت النتائج قـدرة جرثومـة *Listeria monocytogenes* للبقاء حية في عينات الحليب الخام التي احتوت على تركيز قليل من الجراثيم الموجودة أصلا في الحليـب في حين لم تتمكن من النمو في العينات الحاوية على تركيز عالٍ من هذه الجراثيم مع ظهور تفاوت في هذه المقاومة في أنواع الحليب الخام قيد الدراسة.

INTRODUCTION

Listeria monocytogenes is an important pathogenic bacteria in medical and veterinary medicine that has an issue of global concern (Kalorey *et al*., 2008). It has an increased presence in milk and other food products (Jay *et al*., 2005). This bacteria can enter the host body and cause many hygienic problems as a serious opportunistic pathogens for both human and animals (Aygun and Pehlivanlar, 2006), it may cause a high mortality 20-30% associated with a mild illness (called listeriosis) that most prevalent in the elderly, pregnant women, AIDS patient and other immune-compromised patients. (Kells and Gilmour, 2004).

Listeria is a gram positive rods, facultative anaerobic, non-spore forming bacteria that lives in extreme and sever environments. This bacteria can survive in low temperature and it has a wide range of temperature for growth (0-45)°c, and it can grow over a wide range of pH values (4.3-9.1) (HPA, 2009 ; Thevenot *et al*., 2005).

L. monocytogenes frequently contaminate food products of animal origin meat, milk especially those of poultry and eggs. (McCarron and Heaney, 2004).

Milk is a complete and enrichment food that contains protein, minerals and vitamins, that are necessary for the human body at different periods of growth and due to its high content of wet and natural acidity it is considered a good nutrient suitable for the growth of microorganisms, including pathogenic bacteria that may be transferred to human by using contaminated milk (Kasalica, 2000; Huth *et al*., 2006).

L. monocytogenes was isolated from raw milk of ruminants as a result of animal infection or contamination during collection and processing under poor hygienic conditions, machines, workers hands or clothes (Arques *et al*., 2005). Pasteurization can not protect milk from pathogenic bacteria, so it can survive during manufacture and storage due to inadequate pasteurization or post-process contamination (Gameiro *et al*., 2007).

In view of the significance of *L. monocytogenes* economically and hygienically, the study aimed to detect the survival of this bacterium in raw milk samples of different origins after the determination of the levels of microbiota in these samples.

MATERIALS AND METHODS

1. Bacterial strains

Cultures of *L. monocytogenes* were obtained from Bacterial Strains Bank Unit in Biology Department/ College of Science /Mosul University.

2. Raw milk samples

The raw milk samples were collected from College of Agriculture/Mosul University, it includes (Cow, Buffalo, Sheep, Goat). The samples were transported immediately in a sterile containers under cooled condition to the bacteriological laboratory to perform the tests (Benson, 2012).

3. Control milk samples

Nada milk (Al-Othman Company/ King Saudi Arabia), was used as a control sample after examining it to insure the efficiency of its pasteurization. Then Nada milk was used to dilute the raw milk samples as follows:

A : 100 ml raw milk

B : 10 ml raw milk A + 90 ml Nada milk to obtain 10^{-1} dilution

C : 10 ml from B + 90 ml Nada milk to obtain 10^{-2} dilution

D : 100 ml Nada milk (control)

4. Preparation of *L. monocytogenes* suspension

Tryptic Soy Agar with 0.6% Yeast Extract (TSA-YE) was prepared as mentioned in the manufacture company information (Himedia). *L. monocytogenes* was inoculated on this medium and incubated for 24 hrs at 37 ˚C , isolated colonies were transferred to tryptic soy broth with 0.6 % yeast extract and incubated for 18 hrs at 37 ˚C. Viable *L. monocytogenes* count was performed using TSA-YE medium. Serial dilutions were done using normal saline, Serial dilutions were done from the stock solution to prepare 10^{-1}, 10^{-2} and 10^{-3} to obtain different pathogen concentrations to be tested (Nero *et al*., 2009).

5. Enumeration of mesophilic aerobes (microbiota) in raw milk samples

A, B and C raw milk samples were submitted to serial dilutions in normal saline. The dilutions of 10^{-5} , 10^{-6} were cultured on nutrient agar medium and incubated for 48 hrs at 37 ˚C to enumerate the mesophilic aerobic bacteria.

6. Raw milk and control samples treatment with pathogen

A, B, C and D were subdivided into four equal divisions, each one of these were inoculated with four different concentrations of *L. monocytogenes* including the original sample, 10^{-1},10^{-2} and 10^{-3}.

7. **Detection of *L. monocytogenes* in different concentrations of different milk samples**

After inoculating the different concentrations of the bacteria in A, B, C and D milk samples, one ml of each of them was transferred to 5 ml of TSB-YE and incubated for 48 hrs at 37 °C. Then the cultures were transferred to TSA-YE media to perform morphological and biochemical tests according to (Nester *et al*., 2004 ; Mims *et al*., 2006), the results were recorded as positive or negative for growth.

RESULTS AND DISCUSSION

The viable count of *L. monocytogenes* in the stock solution was 36×10^6 (7.56 log 10) and the numbers in 10^{-1},10^{-2} and 10^{-3} were 3.2×10^6 (6.51 log 10), 1.1×10^6 (6.04 log 10), and 0.6×10^6 (5.7 log 10) respectively.

The results showed that Nada milk samples were free from any microbial contamination which assure the efficiency of pasteurization.

The numbers of mesophilic aerobes in raw milk samples (autochthonous microbiota) were listed in (Table 1).

Table 1: Number of mesophilic aerobes microbiota in raw milk samples

Milk origin	A (Raw)	B (10^{-1})	C (10^{-2})
Cow	32	21	7
Buffalo	39	29	21
Sheep	21	11	4
Goat	27	16	7

The numbers $\times 10^6$

The results showed that all raw milk samples were contaminated with different types of bacteria as shown in (Table 1) as autochthonous microbiota.

Buffalos milk samples were more contaminated type than the others: cow, sheep and goat milk samples. This may due to the differences in the chemical constituents of the different milk origin as well as to the physiological and genetical differences between the animals (Adesiyun *et al*., 2007).

Also the buffalo milk contains a high fat ratio (8%) as phospholipids as well as it contains phospholipases that enhance the growth of many types of bacteria, while sheep and goat milk contains a high level of fatty acids such as capric, caproic, caprylic and butyric which inhibit the microbial growth (Robinson *et al*., 2000). Many studies referred that the differences in the microbiota in different animals were due to the nature of their environments, breeding and hygienic care especially types of food as well as the contaminated water (Quinn *et al*., 2006 ; Radostits *et al*., 2007).

In our study the results also showed that *L. monocytogenes* was recovered in all Nada milk samples regardless of the inoculum concentration of this pathogen. In addition the recovery of *L. monocytogenes* occurred in all raw milk samples with different variation depending on the concentration of the pathogen inoculums and the levels of microbiota (Figs 1-4).

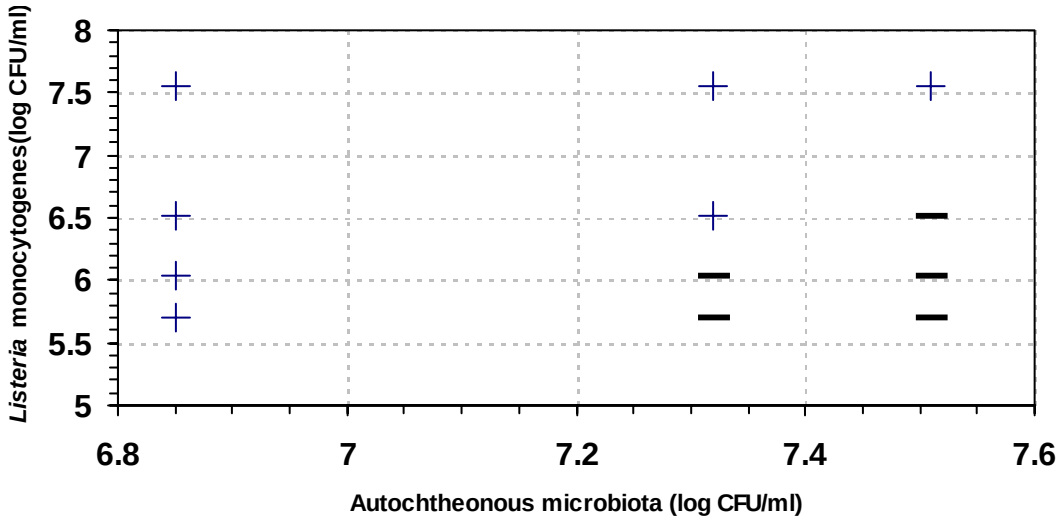

Fig. 1: Recovery of *L. monocytogenes* in cow milk (+ ability to survive and – inability to survive)

The recovery of *L. monocytogenes* from cow milk occurred with all treatments that presented concentrations of 7.5 log CFU/ml of *L. monocytogenes*. For the treatment inoculated with 6 log CFU/ml and less of *L. monocytogenes*, the recovery occurred when the autochthonous microbiota contamination was less than 7.2 log CFU/ml ,while the recovery was possible at concentration of 7.32 log CFU/ml microbiota with 6.5 log CFU/ml of *L. monocytogenes* Fig. (1).

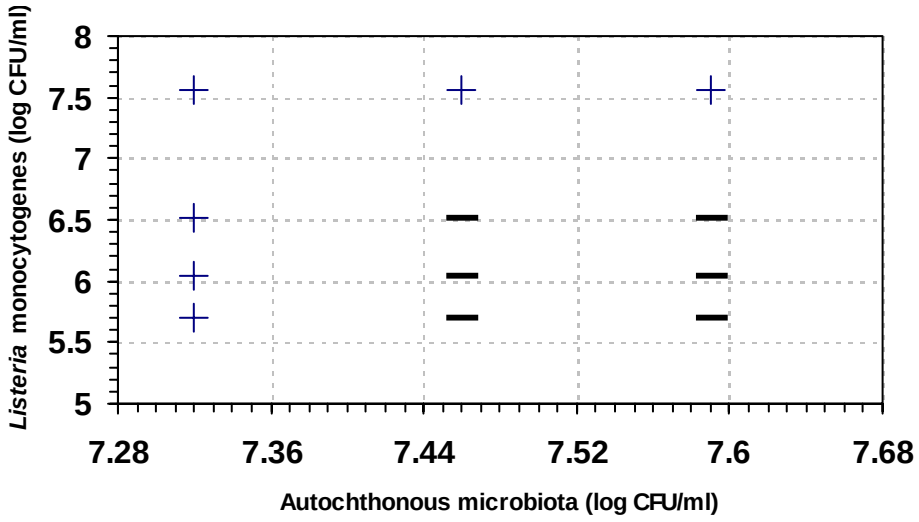

Fig. 2: Recovery of *L. monocytogenes* in buffalo milk (+ ability to survive and – inability to survive)

For buffalo milk, the recovery of *L. monocytogenes* obtained at all treatments that presented concentrations higher than 7.5 log CFU/ml of the pathogen, but when the autochthonous microbiota contamination was higher than 7.32 log CFU/ml the recovery did not occur in the treatment inoculated with 6.5 log CFU/ml of *L. monocytogenes* or less Fig. (2).

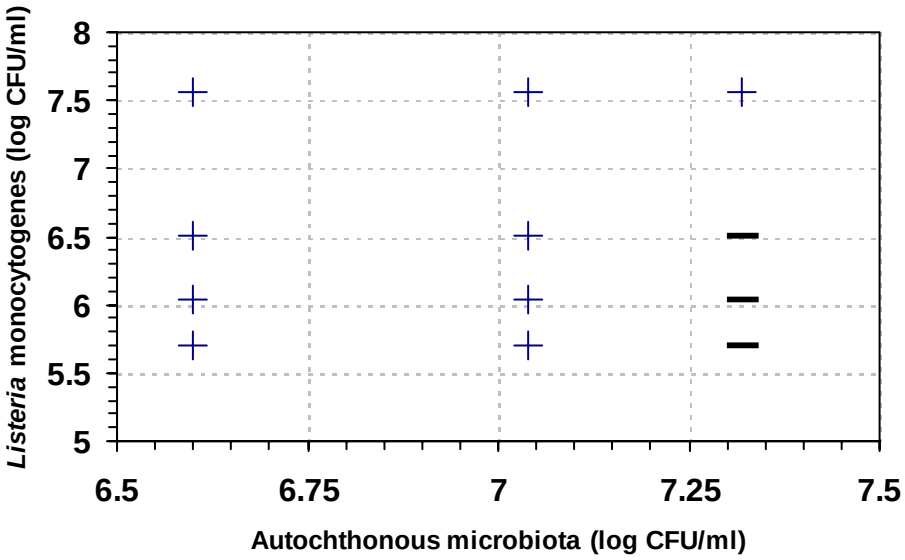

Fig. 3: Recovery of *L. monocytogenes* in sheep milk (+ ability to survive and – inability to survive)

For sheep milk, the recovery of *L. monocytogenes* obtained in all treatment that presented a concentration higher than 7.5 log CFU/ml, additionally the recovery occurred in all treatment of *L. monocytogenes* with microbiota contamination level lower than 7.25 log CFU/ml, while the recovery did not occur when the autochthonous microbiota contamination was higher than 7.25 log CFU/ml Fig. (3).

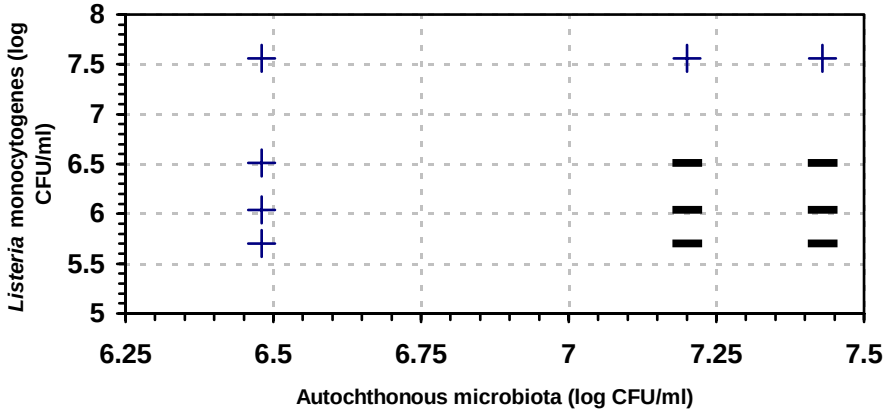

Fig. 4: Recovery of *L. monocytogenes* in goat milk (+ ability to survive and – inability to survive)

For goat milk, the recovery of *L. monocytogenes* obtained at all treatments that presented a concentration higher than 7.5 log CFU/ml of the pathogen and at a concentration lower than 6.5 log CFU/ml autochthonous microbiota for all inoculums levels of *L. monocytogenes*, but the pathogen was not recovered in other treatments.

Generally, a higher concentration of *L. monocytogenes* with a high contamination level of autochthonous microbiota lead to a great recovery of *L. monocytogenes*, while the recovery of this bacterium did not occur when the pathogen concentrations were low, but it may be possible only when the microbiota of raw milk was high . When *L. monocytogenes* present at level of 36×10^6 the recovery occurred in all treatments with low or high contamination levels of microbiota in all raw

milk samples and their dilutions, while the recovery did not occur if *L. monocytogenes* present at level less than that, and the autochthonous microbiota $18\text{-}29\times10^6$ and the recovery occurred when the contamination was less than 18×10^6 .

The recovery of *L. monocytogenes* in raw milk samples may due to the composition of the milk (Robinson *et al* ., 2000).

Autochthonous microbiota play a very important role which represents the main interfering factor such as different types of metabolites produced by microbiota in the milk as an unfavorable conditions that can inhibit the bacterial growth or survival by competition (Besse, 2002). The multiplication of autochthonous microbiota in the initial phase of *L. monocytogenes* growth may cause, decrease in pH with a consequent inhibition of the multiplication and detection of this pathogen (Carr *et al*., 2002). Moreover, many antimicrobial substances in the raw milk that contribute to this interference such as lactoferrin, whey proteins, casein fragments bacteriophages and lacto-peroxides system, all these substances associated with specific storage and processing conditions that can induce stress on *L. monocytogenes* impairing their development and recovery (Hadson *et al*., 2005).

In this study the negative results of detection of *L. monocytogenes* in raw milk samples may due to our proceeding, culture media and methodologies which were used in detection of this bacteria and this agreed with (Suh and Knabel, 2001). Alternative methods must be used such as molecular techniques and different selective media such as Half-Fraser broth to enhance the growth of this pathogen during the isolation and recovery procedures from raw milk samples (Wher and Frank, 2004).

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