INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

INFLUENCE OF MILK COMPONENTS FROM DIFFERENT ANIMAL SPECIES AND INCUBATION TEMPERATURE ON SURVIVAL OF *LISTERIA MONOCYTOGENES IN VITRO*

Moutaz A.W. Abdul Mounam
Zoonotic Diseases Unit / Coll. Vet .Med / Baghdad University-Iraq

ABSTRACT
A study was carried out to investigate the influence of milk components from different animal's species and different incubation temperatures on *in vitro* growth of *Listeria monocytogenes*. A total of 25 samples from *Cows, Buffalos, Ewes*, *Does* (Goats) and imported UHT milk (5for each, 250 ml for each) were collected from Baghdad markets andprocessed according to research design in accordance with food hygienic methodologies with some modifications during *February & March 2012*.Each sample was divided into 3 parts in which 50ml of each was inoculated experimentally with 1ml*log5*(3-5*10^5 cfu \ml)of *Listeria monocytogenes* and incubated overnight (18-24 hrs.)at 4C°, ambient room temperature and 37C°, then counted according to Miles & Misra procedure. The results showed significant inhibitoryeffect of *Goats*milk especially at 4C° followed by *Ewes, Cows* ,UHT and *Buffalos* milk with no fluctuations at 37 C° and this may support the idea that *Goats*milk may contain inhibitory molecules such as fatty acids in concentration and quality better than that of other species especially during retarding growth at 4C°(increasing *Lag* phase)of contaminant psychrophilic *Listeria monocytogenes* ,while *Buffalos* milk enriched the growth of *Listeria monocytogenes* may be due to high lipid content especially those that are resuscitate multiplication of them as well as the nature of rearing of *Buffalos* in Iraq. These findings suggest presence of combined effect (inhibition or potentiation) of ambient growth temperature and different milk components especially fatty acids on *in vitro* growth of gram positive contaminant *Listeria monocytogenes*.

KEY WORDS: *Listeria monocytogenes***,** milk Anti-microbial, food microbiology *etc*.

INTRODUCTION

Gastrointestinal infections provoked by food-borne bacteria are an enormous problem for public health. Newborns, the elderly and immunocompromised subjects are particularly at risk. Due to the increasing resistance of pathogens to antibiotics, efforts to enhance the host's resistance to pathogens by a nutritional approach deserve attention [1,2,3,4]. The host immune system consists of constitutive nonspecific defenses and inducible specific antibody-mediated defenses. Because full expression of specific defenses takes at least one week, supporting nonspecific defenses could be successful in fighting intestinal infections. Luminal factors such as gastric acidity, antimicrobial bile salts and pancreatic enzymes, together with intestinal motility, epithelial mucin secretion, exfoliation of epithelial cells and autochthonous microflora contribute to intestinal nonspecific defenses by killing pathogens and preventing their colonization [5]. We hypothesize that changing the composition of the diet and thus changing the composition of the gastrointestinal contents may affect survival and colonization of pathogens. For example, whole milk consumption in children is associated with fewer gastrointestinal infections than is consumption of low fat milk[6]. This may be attributed to the antimicrobial activity of milk lipids towards bacteria and viruses that has been observed *in vitro* [7]. Triglycerides in milk fat are not toxic in themselves but become active upon treatment with lipase [7], implying involvement of free fatty acids and partially hydrolyzed glycerides. Unlike di-glycerides, fatty acids and monoglycerides are powerful antimicrobial agents *in vitro* [8]. The bactericidal effects of free fatty acids and monoglycerides depend on properties of the bacterial cell wall. Although some exceptions have been described, gram-positive bacteria are more sensitive than gram-negative bacteria; this is because the lipopolysaccharide rich outer membrane protects gram-negative bacteria against cytotoxic surfactants [9]. Despite the well-known *in vitro* bactericidal effects, evidence of lipid-mediated protection against gastrointestinal infections *in vivo* is scarce. Because fat digestion yields fatty acids and monoglycerides, protection against microbes mediated by lipolytic products is a likely phenomenon *in vivo* [1]. *Listeria monocytogenes* has been recognized as an important food-borne pathogen that causes listeriosis in man and animals. Outbreaks of listeriosis have been associated with milk, cheese, vegetables and salads, and meat products [10]. The organism is particularly problematic for the food industry because it is widespread in the environment [10, 11] and because of its ability to grow within a wide range of temperatures (1.5 to 45°C), pH values [4.39 to 9.4], and osmotic pressures (NaCl concentrations up to 10%).So, extensive research was carried out on predictive models describing the behavior of this pathogen in foods. Today, the models describing the environment effect on growth rate of *L. monocytogenes* are sufficiently accurate to be used by manufacturers, regulators, and scientists to assess microbial risks associated with the consumption of foods or to evaluate the relevance of risk management options [12]. Because *L. monocytogenes* can grow slowly at refrigeration temperature, control of the organism is of particular

concern in minimally processed refrigerated foods with an extended shelf life [13, 14]. For control of *L. monocytogenes* in these foods, it often becomes necessary to incorporate barriers, including preservatives [14]. Trace quantities of certain long-chain fatty acids have been known to inhibit microorganisms, especially gram positive bacteria [15, 16]. Susceptibility to fatty acids varies considerably among species. Raw milk contains bacterial inhibitors that can limit growth of some pathogens, such as the lactoperoxidase system, lactic acid bacteria (which are sometimes bacteriocin producers), competition for nutrients, steric limitations, *etc*. [17]. The LPS is composed of an enzyme, lactoperoxidase (LP); an oxidative agent, hydrogen peroxide (H2O2); and a substrate, the thiocyanate (SCN^-). The oxidation product ($OSCN^-$) can react with the amine and thiol groups of the enzymes essential for bacterial metabolism. LPS has a bacteriostatic effect on Gram-positive bacteria including *L. monocytogenes*, and is inactivated in pasteurized milk [18]. Bovine milk fat represents a rich source of biologically active molecules, many of which offer potential for commercial exploitation in health-promoting functional food products [19, 20]. Several milk fat-derived molecules can modulate immunity, reduce serum LDL (low density lipoprotein) cholesterol, inhibit carcinogenesis and act as an effective bactericidal agent on pathogens. The important features of milk fat from the consumer's point of view are the flavour, the traditional image, spread ability (of butter) and the health perception. These must be considered as a bundle and any changes in one characteristic should not have adverse effects on the others [20,21]. Milk fat contains a number of components having functional properties. Milk fat has fatty acids and membrane lipids that may exert antimicrobial effects either directly or upon digestion. Both sphingolipids and their active metabolites, ceramides and sphingosines, were determined as effective bactericidal agents on pathogens like *Listeria monocytogenes*. Thus, this study was designed to evaluate these differences of milk components from different animal species in combination with ambient growth temperatures on *in vitro* growth of *Listeria monocytogenes*.

MATERIALS & METHODS

This study was conducted on *Cows*, *Buffalos*, *Ewes*, *Does* (*Goats*) and imported UHT milk (5 for each, 250ml for each) were collected into sterile bottles from different locally markets in Baghdad and transported aseptically to the laboratory of Zoonotic Unit in Baghdad Veterinary College, then processed according to research design in accordance with the food hygienic methodologies [22,24,25,27] with some modifications during *February & March*2012. Each sample was divided into 3 parts in which 50ml of each was inoculated experimentally with 1ml*log* 5 [3-5 ∗105 cfu\ml) of *Listeria monocytogenes* and incubated overnight (18-24 hrs.) at 4Cº, ambient room temperature and 37Cº. A *log* 5 of *Listeria monocytogenes* was prepared according to standard protocols of antibiotics susceptibility test and McFarland's opacity tubes [23] in which each 4-5 pure colonies obtained from chromogenic ALOA (OCLA) Agar Listeria Ottaviani and Agosti (Oxoid Chromogenic *Listeria* Agar) were inoculated in 10-15 ml Trypton Soya Yeast Extract Broth (HiMedia, India) for 8-12 hours then a milk samples were warmed inside water bath at 40Cº for 30 minutes to ensure homogenization of their components especially triglycerides, before contamination with 1ml *log* 5 of *Listeria monocytogenes* inside sterile bottles, mixed well by shaker then incubated overnight with ambient growth temperatures according to research design. At the second day of incubation, each experimentally contaminated sample was mixed thoroughly and diluted decimally with sterile PBS in which a portion of analytic sample was added to 9 portions of PBS then mixed thoroughly by rot mixture for 2-3 minutes then a 20 microliter of each dilution was droplet on ALOA agar until absorbed from it before incubation overnight at 37Cº then counted according to method of Miles & Misra [26].

RESULTS & DISCUSSION

Recalls, illnesses, and deaths associated with *Listeria* in food products have been reported over the last years. These incidences have increased the awareness that additional techniques may be needed for controlling *Listeria* in food [8] processing plants and, especially, those producing Ready to Eat (RTE) products [28]. Experimental conditions can affect the outcome of bacterial stress-tolerance assays. Growth conditions that optimize microbial recovery should be established to help evaluate the effectiveness of treatment conditions for food safety [29]. The results showed significant inhibitory effect of *Does* milk especially at 4Cº followed by *Ewes*, *Cows*, UHT and *Buffalos* milk with no fluctuations at 37Cº and this may support the idea that *Does* milk may contains inhibitory molecules such as fatty acids in concentration and quality better than that of other species especially during retarding growth at 4Cº (increasing *lag* phase) of contaminant psychrophilic *Listeria monocytogenes*, while *Buffalos* milk enriched the growth of *Listeria monocytogenes* may be due to high lipid content especially those that are resuscitate multiplication of them as well as the nature of rearing of *Buffalos* in Iraq [30].

Incubation Temperature	Means Log Count of *Listeria monocytogenes* after overnight inoculation of Milk				
	Cows	*Buffalos*	*Ewes*	*Does* (*Goats*)	*Imported UHT*
4 C°	7.096	8.176	6.477	5.903	7.243
Ambient Room C°	8.301	8.079	7.591	7.439	9.041
37 C°	9.217	9.00	9.690	9.060	9.875

These results showed similarities with some studies [14] in which the pathogen was more susceptible to monolaurin in skim milk at low temperature, and monolaurin was bactericidal at 4°C but was not inhibitory in skim milk at 30°C or in whole milk at 4 or 30°C [9]. The effectiveness of monolaurin was influenced by the fat content of milk and by potentiating agents, including antioxidants. Medium and long-chain fatty acids have long been recognized as

inhibitors of spores and vegetative cells of gram positive bacteria. Decreasing effectiveness with longer chain length may be related to increased hydrophobicity and decreased solubility. Inhibitory fatty acids must be sufficiently water soluble to reach an effective concentration in the aqueous solution and yet sufficiently hydrophobic to interact with hydrophobic proteins or lipids on the bacterial cell surface. The composition of a food could affect the inhibitory activity of fatty acids and monoglycerides. Certain nutrients in relatively high concentrations, such as lipophilic proteins (e.g., albumin), fat globules, starch, or others, could interact with fatty acids and decrease their availability. In some foods, the use of fatty acids as inhibitors could be undesirable because they can affect the organoleptic and functional properties. Many factors are known to influence microbial thermo tolerance in foods such as the composition of the food and the physiological condition of vegetative cells and spores [31]. Bacterial thermo tolerance can also increase after exposure to a variety of environmental stress conditions including heating at sub lethal temperatures, presence of deleterious chemicals in the growth medium (e.g., hydrogen peroxide, dyes, and antibiotics, etc., viral infections, and osmotic and acidic shocks [31].In Conclusion, this may indicate variability in the thermo growth of *Listeria monocytogenes* according to type of media and environmental temperature present in it, and my findings suggest presence of combined effect (inhibition or potentiation) of ambient growth temperatures and different milk components especially fatty acids on *in vitro* growth of gram positive contaminant *listeria monocytogenes.*

REFERENCES

[1]. Sprong, R.C., Hulstein, M.F. and Van der Meer, R. (1999) High Intake of Milk Fat Inhibits Intestinal Colonization of *Listeria* but not of *Salmonella* in Rats. J. Nutr. 129: 1382–1389.

[2]. Gershwin, M. E. (2004) Handbook of Nutrition and Immunity. 1st ed., Humana. Press Inc., USA.

[3]. Park, Y.W. (2009) Bioactive Components in Milk and Dairy Products. 1st ed., Wiley-Blackwell, USA.

[4]. Incidence of Listeria in table-eggsin Baghdad Markets (2011) International Journal for Sciences and Technology (IJST).6 (2): 93-98.

[5]. Kindt, T.J., Osborne, B.A. and Goldsby, R.A. (2006) Kuby Immunology. 6th ed., W. H. Freeman Comp., USA.

[6]. Koopman, J. S., Turkish, V. J., Monto, A. S., Thompson, F. E. & Isaacson, R. E. (1984) Milk fat and gastrointestinal illness. Am. J. Public Health 74: 1371– 1373.

[7]. Isaacs, C. E., Litov, R. E. & Thormar, H. (1995) Antimicrobial activity of lipids added to human milk, infant formula, and bovine milk. J. Nutr. Biochem. 6:362–366.

[8]. Kabara, J. J., Swieczkowski, D. M., Conley, A. J. & Truant, J. P. (1972) Fatty acids and derivatives as antimicrobial agents. Antimicrob. Agents Chemother, 2: 23–28.

[9]. Sheu, C. W. & Freese, E. (1973) Lipo polysaccharide layer protection of Gram negative bacteria against inhibition by long-chain fatty acids. J. Bacteriol., 115: 869–875.

[10]. Farber, J. M., and P. I. Pertenkins. (1991) *Listeria monocytogenes*, a foodborne pathogen. Microbiol. Rev. 55:476–511.

[11]. Guillier, L., Pardon, P. and Augustin, J.C. (2005) Distributions of *Listeria monocytogenes* Influence of Stresson Individual Lag Time. Appl. Environ. Microbiol. 71(6): 2940-2948.

[12]. Anonymous (2003)Quantitative assessment of relative risk to public healthfrom foodborne *Listeria monocytogenes* among selected categories of ready to- eat foods. http://www. foodsafety.gov/ _dms/ lmr2-toc.html.

[13]. Ryser, E. T., and Marth, E. H. (1991) *Listeria*, listeriosis, and food safety. Marcel Dekker, Inc., New York.

[14]. Wang, L.L and Johnson, E.A. (1992) Inhibition of *Listeria monocytogenes* by Fatty Acids and Monoglycerides. Appl. Environ. Microbiol 58(2): 624-629.

[15]. Nieman, C. (1954) Influence of fatty acids on the growth of microorganisms. Bacteriol. Rev. 18:147-163.

[16]. Kabara, J. J. (1978) Fatty acids and derivatives as antimicrobial agents. A review, p. 1-14. In J. J. Kabara (Ed.). The pharmacological effect of lipids, vol. I. American Oil Chemists Society, Champaign, Ill.

[17]. Pitt W.M., Harden T.J., Hull R.R. (2000) Investigation of the antimicrobial activity of raw milk against several foodborne pathogens, Milchwissenschaft, 55: 249–252.

[18]. Gay, M. and Amgar, A. (2005) Factors moderating *Listeria monocytogenes* growth in raw milk and in soft cheese made from raw milk. INRA, EDP Sciences. 85, 153–170.

[19]. Akaln, S., Gonc, S. and Unal, G. (2006) Functional Properties of Bioactive Components of Milk Fat in Metabolism. Pakistan Journal of Nutrition 5 (3): 194-197.

[20]. Boland, M., Gibbon, A. M. and Hill, J. (2001) Designer milks for the millennium. Livest. Prod. Sci., 72: 99- 109.

[21]. Gill, H. S. (2002) Bovine Milk as Source Immunomodulatory Ingredients. Bulletin of IDF No: 370.

[22]. Jay, J.M., Loessner, M. J. and Golden, D.A. (2005) Modern Food Microbiology, 7th ed., Aspen Pub. Gaithersburg MD, USA.

[23]. Quinn, P. J., Carter, M. E., Markey, B. and Carter, G.R. (2004) Clinical Veterinary Microbiology. 2nd ed., Mosby Int., USA.

[24]. Wehr, H.M. and Frank, J.F. (2004) Standards methods for the examination of dairy products. 17th ed., APHA, Washington, D.C., USA.

[25]. Hitchins, A.D. (2003) Detection and Enumeration of *Listeria monocytogenes* in Foods. Chapter 101. In: Jackson, G.J. (coordinator), Bacteriologial Analytical manual. 10th ed., Revision A., AOAC Int., Gaithersburg, M.D., USA.

[26]. Miles, A. A., Misra, S. S. and Irwin, J.O. (1938) The estimation of the bactericidal power of blood. J. hyg. Camp. 38: 739-749. (Cited by Quinn et al., 2006).

[27]. Pagotto, F., Daley, E., Farber, J. and Warburton, D. (2001). Isolation of *Listeria monocytogenes* from all food and environmental samples. MFHPB-30, Health products and food branch, HPB Method, Ottawa, Canada.

[28]. Doyle, M. E. (1999) Literature survey of the various techniques used in *Listeria* intervention .*FRI BRIEFINGS:* Control of *Listeria* in Meat. http://fri.wisc.edu/docs/pdf/listeria.pdf.

[29]. Shearer, A. E. H., Neetoo, H. S. and Chen, H. (2010) Effect of growth and recovery temperatures on pressure resistance of *Listeria monocytogenes*. International Journal of Food Microbiology. 136(3): 359-363.

[30]. Al-Shammary, A.H.A. (2009) Detection of *Listeria monocytogenes* in milk and some dairy products in Baghdad. PhDThesis, college of Vet. Med., Baghdad University.

[31]. Rowan, N. J. and Anderson, J. G. (1998) Effects of Above-Optimum Growth Temperature and Cell Morphology on Thermotolerance of *Listeria monocytogenes* Cells Suspended in Bovine Milk. Appl. Environ. Microbiol. 64(6): 2065-2071.