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Occurrence of *Listeria monocytogenes* in Raw Milk and Dairy Products in Noorabad, Iran

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Abstract: The presence of *Listeria monocytogenes* was investigated in a total of 360 raw milk and dairy product samples including white cheese, yoghurt and Iranian yoghurt drink (Doogh) that were collected during five months from two traditional dairy manufacturer in Southern Iran. The prevalence of *Listeria monocytogenes* in raw milk and white cheese samples of manufacturer A was found to be 1.7 and 3.3%, respectively and of manufacturer B was found to be 3.3 and 6.7%, respectively. No *Listeria monocytogenes* was isolated in any of the yoghurt and Doogh samples of both manufacturer probably because of their low pH values.

Key words: *Listeria monocytogenes*, doogh, occurence, low pH, yoghurt, Iran

INTRODUCTION

Listeria monocytogenes is an important pathogen in medical and veterinary medicine, causing abortion and encephalitis in sheep and cattle and a variety of diseases in other mammals, birds and fish (Kalorey *et al*., 2008). Human acquisition of listeriosis from animal sources has been shown to occur as an occupational hazard specially in farmers, butchers, poultry workers and veterinary surgeons but most human infections are probably food-borne primarily associated with the consumption of contaminated milk and dairy products (Kells and Gilmour, 2004).

Mild symptoms in man including diarrhea, fever, headache and myalgia but in the cases of invasive listeriosis, meningitis and septicemia are commonest form of disease which in pregnant women can lead to abortion or stillbirth (Aygun and Pehlivanlar, 2006).

The genus Listeria is ubiquitous in the farm and food industrial environment and therefore, control of this bacterium during food processing is extremely difficult (Kells and Gilmour, 2004). Numerous studies have shown that the survival or growth of *Listeria monocytogenes* depends on the conditions during manufacture (Gameiro *et al*., 2007), differenet physical and chemical stresses and storage conditions of dairy products (Arques *et al*., 2005; Faleiro *et al*., 2003). Pathogen bacteria have a variable resistance to the processes involving acid stress, for instance *Listeria monocytogenes* was found to survive at pH close to 4.5 (Thevenot *et al*., 2005). The ability to grow or survive at low temperatures, low pH and low water activities makes *L. monocytogenes* an important hazard in foods (Hwang and Tamplin, 2005). In 2004, 1267 cases of listeriosis were reported in Europe. Fifty one percent of reported cases were in the people aged 65 years and older and there were 55 cases associated with pregnancy, mothers and babies. A total of 107 (8.3%) deaths were reported (Vermeulen *et al*., 2007).

Although, occurrence of *Listeria monocytogenes* in various foods have been investigated in several countries, but the incidence and frequency of this bacterium in Iran is still unknown. Therefore, we aimed this study to determine the prevalence of *Listeria monocytogenes* in milk and some dairy products in Noorabad, South of Iran and evaluate the amount of hazard due to this pathogen.

MATERIALS AND METHODS

Sample collection: In this study, a total of 360 raw milk and dairy product samples including 120 raw milk, 60 native white cheese, 80 yoghurt and 100 Iranian yoghurt drink (Doogh) were collected during 5 months from two traditional dairy manufacturer in Noorabad, located in Southern Iran and the presence of *Listeria monocytogenes* was investigated. All samples were made from raw cow milk and were sold unpackedly. The samples were transported to the laboratory in sterile plastic bags under refrigerated conditions using ice bags and were processed within 4 h of collection.

Isolation of Listeria: First, samples of milk, yoghurt and Doogh were homogenized for 3 min and pH of all samples were measured using pH meter (Orion 701 A). About 25 mL of each sample (25 g concerning blended white cheese) were inoculated in 225 mL of sterile Listeria enrichment broth, homogenized completely and incubated

at 30°C for 48 h. A loopful of enriched medium was surface streaked in duplicate on modified oxford agar and incubated at 30°C for 48 h. All colonies surrounded by a bluish-brown to black halo were taken as possible *Listeria* sp. Three typical Colonies from each plate were surface streaked on tryptic soy agar containing 0.6% yeast extract and incubated at 35°C for 24-48 h.

Confirmation of isolates: Suspected colonies of Listeria were subcultured on 5% sheep blood agar. After 24 h incubation typical colonies were verified by Gram's staining, catalase reaction, tumbling motility in SIM medium at 25°C, methyl red-Voges Proskauer reactions, nitrate reduction, fermentation of sugars (glucose, mannitol, rhamnose, xylose and α-methyl-D-mannopyranoside), hemolysis and CAMP test.

RESULTS AND DISCUSSION

In the present study, a total of 360 raw milk and dairy product samples from two traditional dairy manufacturer were examined. The analysis showed that 1.7% of raw milk and 3.3% of white cheese samples from dairy manufacturer A and 3.3% of raw milk and 6.7% of white cheese samples from dairy manufacturer B were contaminated with *Listeria monocytogenes*, whereas no *Listeria monocytogenes* was isolated from yoghurt and Doogh samples of both dairy manufacturer (Table 1).

In a few studies that accomplished in the other parts of Iran, the incidence of *L. monocytogenes* in milk and dairy products was reported 0% in center of Iran (Jalali and Abedi, 2008) and 1.6% in west of Iran (Moshtaghi and Mohamadpour, 2007). In contrast Pintado *et al*. (2005) reported higher rate of incidence (up to 46%) in Portugal. In another study, the incidence of *L. monocytogenes* was reported 5% in 80 raw milk samples from Ankara (Aygun and Pehlivanlar, 2006). In that study, *L. monocytogenes* was isolated in 1 and 5% of raw milk and pasteurized milk samples, respectively.

In the present study, the pH of all samples were measured and average pH and Standard deviations calculated for each dairy manufacturer separately (Table 2). *Listeria monocytogenes* was not isolated in any yoghurt and Doogh samples, which is consistent with some other researchers by Brito *et al*. (2008) and Neves *et al*. (2008). Among dairy products, yoghurt received the least attention due to the fact that, its high acidity and milk pasteurization process before addition of starter, are effective barriers to the growth of pathogens including *L. monocytogenes* (Liu and Puri, 2008). The survival of *L. monocytogenes* in yoghurt depends on the sample acidity and this bacterium disappear when the pH falls to 3.5 (Vermeulen *et al*., 2007). In several studies was reported that the exposure to pH 4.0 can totally inactivate *L. monocytogenes* (Aygun and Pehlivanlar, 2006; Samelis *et al*., 2003; Tiganitas *et al*., 2009). In a study, microorganisms were exposed to lethal acidic conditions (pH 4.0-4.5) and osmotic stresses (15-20% NaCl) and demonstrated that acidic condition is more deleterious than osmotic stress on microbial activity and this is likely associated with the high energetic demands posed by low pH. Consequently, even small differences in pH, such as 0.5 unit, may have a major impact on the survival of pathogens and hence, on food safety (Samelis *et al*., 2003).

Table 1: Number of *Listeria monocytogenes* positive samples of two dairy manufacturer

Dairy	Samples	No. of all samples	No. of positive samples (%)
A	Raw milk	60	1 (1.7)
	White cheese	30	1 (3.3)
	Yoghurt	40	0 (0)
	Doogh	50	0 (0)
B	Raw milk	60	2 (3.3)
	White cheese	30	2 (6.7)
	Yoghurt	40	0 (0)
	Doogh	50	0 (0)

Table 2: Mean and standard deviation of pH values of samples

Dairy	Samples	Mean of pH	SD
A	Raw milk	6.50	0.85
	White cheese	5.13	2.17
	Yoghurt	4.32	1.33
	Doogh	3.65	0.78
B	Raw milk	6.62	1.03
	White cheese	4.83	2.61
	Yoghurt	4.07	1.70
	Doogh	3.23	1.70

The resistance of pathogen bacteria to the low pH is the outcome of their ability to maintaining intracellular pH homeostasis in acidic conditions (Millet *et al*., 2006; Wood *et al*., 2001). Moreover, the use of lactic acid as an acidulant or natural production of it (for instance in dairy fermented products) probably results in additional expenditure of energy by microorganism (Tiganitas *et al*., 2009). Indeed organic acids penetrate bacterial cell membranes through permeases or porin canals as undissociated molecules and their intracellular dissociation reduces cytoplasmic pH. Consequently, their antimicrobial activity is attributed both to the pH gradient across membrane and the reduction of intracellular pH (Hwang and Tamplin, 2005).

Several studies demonstrated that lactic acid bacteria can cause inhibition of the growth of some other bacteria notably via the production of antagonistic compounds (Arquez *et al*., 2005; Vermeulen *et al*., 2007). Production of bacteriocins by lactic acid bacteria can reduce counts of *L. monocytogenes* in cheese and yoghurt (Arques *et al*., 2005). A nisin-producing culture of *Lactococcus lactis*

decreased the counts of *L. monocytogenes* in raw milk Camembert cheese by 2.4 log cfu g^{-1} compared with the counts in the control cheese (Millet *et al*., 2006).

In the study, Doogh that is a native and traditional Iranian fermented milk drink with pH value usually <4 was investigated and demonstrated that all samples (100) were devoid of *L. monocytogenes* that could be due to the combined inhibitory effect of low pH and the antimicrobial activity of some compounds that probably secreted by lactic acid bacteria present in this fermented dairy product. The mean pH value in Doogh samples (3.23-3.65) were less than minimum tolerance limit for growth of *Listeria monocytogenes* (Faleiro *et al*., 2003).

CONCLUSION

The real situation of listeriosis in Iran is unknown and little information exist on the prevalence of *Listeria monocytogenes* in foods consumed in the country. It is also important to note that listeriosis is not a reportable disease in Iranian health program. The investigation of milk and some dairy products in this study revealed that the frequency of encountering *Listeria monocytogenes* in Noorabad was more than whatever reported in the other parts of Iran. Because of wide spread of this pathogen in nature, contamination of raw milk and dairy products easily can occure but the results of current study revealed that acidic fermented products such as yoghurt and Doogh with pH <4 because of inhibitory effect of low pH, probably had minimum risk of pathogenesis.

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