

Prevalence of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 in Raw Milk

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ABSTRACT

The present study was undertaken to investigate the incidence of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 in 350 composite milk samples (four quarters in one collection vial) obtained from five different farms in Istanbul. Each sample was aseptically taken straight from udder of individual cattle. The results showed that 0.57% (2/350) of milk samples were contaminated with *L. monocytogenes*. *Salmonella* spp. and *E. coli* O157:H7 were not detected in any samples. In conclusion, considering that the mortality of listeriosis is very high, low prevalence rate of *L. monocytogenes* should not be disregarded. Therefore, it is essential to ensure improving the quality of production technology and developing the sanitation strategies for enhancing the safety of food. **Keywords:** Raw Milk, *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7

Introduction

Milk is the fluid secreted by mammals for the nourishment of their young. The principal components of milk are water, fat, protein and lactose. High water activity, moderate pH (6.4-6.6) and ample supply of nutrients make milk an excellent culture medium for microorganisms, in particular certain pathogens (Adams and Moss, 2008). Pathogenic bacteria in raw milk have been a major factor for public health concern. The main sources of contamination are the dairy cattle; food handler, dairy equipments and consumption of raw milk are considered to be the main cause of several

outbreaks of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7. Each year millions of cases occur that most of these infections cause mild illness, severe infections and serious complications including death (Robinson et al., 2000).

L. monocytogenes is a psychrotrophic microorganism that is capable of growing at refrigeration temperature and at relatively low pH. These characteristics make *L. monocytogenes* particularly difficult to control in food therefore contamination could lead to high risk factor (Hobbs and Roberts, 1993). In Turkey, several studies have indicated that *L.*

monocytogenes is present in raw milk and the reported rates of contamination vary from 0% to 18.2 % (Sharif and Tunail, 1991; Unlu et al., 1998; Aygun and Pehlivanlar, 2005; Soyutemiz et al., 2001; Gun, 1994).

Salmonella spp. is one of the most important pathogenic genera implicated in foodborne bacterial outbreaks. It rarely produces clinical disease that include nausea, vomiting, septicemia, headache, diarrhea, abdominal cramps and fever; additional complications associated with septicemia and arthritis (Downes and Ito, 2001). In the last few years, several outbreaks of salmonellosis have been reported in Scotland, England and Wales due to the consumption of contaminated raw milk (De Buyser et al., 2001).

E. coli O157:H7 is neurotoxin producing enterohemorrhagic *E. coli*. It rarely produces clinical disease in animals but associated with hemolytic uremic syndrome, thrombocytic thrombocytopenic purpura and hemorrhagic colitis in human (Roberts et al., 1998). There have been some reports on the incidence of *E. coli* O157:H7 in raw milk in Greece (Dontorou et al., 2003), in France (Decludt, 1995) and in the USA (Keene et al., 1997).

The present study was undertaken to determine the prevalence of *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 in raw milk samples obtained from different farms in Istanbul, the biggest city of Turkey.

Materials and Methods

Sample Collection

Raw milk samples were collected at intervals between September 2007 and December 2008. A total of 350 composite milk samples (four quarters in one collection vial) aseptically taken from udder of individual cattle were investigated for the presence of *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 which were obtained from five different dairies at different regions of Istanbul. All samples (approximately 200 ml) were collected in sterile jars and immediately

transferred to the laboratory in cold boxes at 4 °C.

Microbiological Analysis

For the isolation of *L. monocytogenes*; 25 ml of milk samples were pummeled with 225 ml of Listeria Enrichment Broth (LEB) (Oxoid CM0862) containing Listeria selective supplement (Oxoid SR 141). Samples were homogenized in a stomacher bag for 60 sec. at normal speed and incubated at 32 °C for 24 h. A 0,1 ml portion of the enrichment broth was streaked on to Chromogenic Listeria Agar (OCLA) (Oxoid CM1080) supplemented with Listeria Selective Supplement (Oxoid SR0227) and Listeria Differential Supplement (Oxoid SR0228). After incubation typical colonies were transferred to Tryptic Soy Agar with Yeast Extract (TSYE) (Oxoid CM0131) and incubated for 24-48 h at 30°C. The typical colonies were identified by Gram's staining, catalase reaction, tumbling motility at 20-25 °C, Methyl Red-Vogues Proskauer (MR-VP) reactions, CAMP test, nitrate reduction and fermentation of sugars (Hitchins, 1995).

For detection of *Salmonella* spp., pre-enrichment was done by suspending 25 ml of sample in 225 ml buffered peptone water (BPW) (Oxoid CM0509) followed by incubation at 37°C for 16-20 h. 0,1 ml mixture was transferred to Rappaport-Vassiliadis (RV) (Oxoid CM0866) and Muller KaufmannTetrathionate Broth (MKTTn) (Oxoid CM0343). MKTTn and RV broth was incubated for 24h at 42°C. After incubation samples were streaked on Hectoen Enteric Agar (HE) (Oxoid CM0419) and XLD Agar (Oxoid CM0469), incubated for 24h at 35°C. The typical colonies were identified by Triple Sugar Iron Agar (TSI) (Oxoid CM0277), Lysin Iron Agar (LIA) (Oxoid CM0381), urease test (Oxoid CM0071) and confirmed with *Salmonella* antiserum (O and H-Vi polyvalent antiserum) (Harrigan, 1998).

For detection of *E. coli* O157:H7, each sample were carried out by combining 25 ml of each sample with 225 ml of modified Tryptone Soya Broth with novobiocin (m-TSB) (Oxoid CM0989) into a stomacher bag, homogenized

for at least 2 min into a stomacher and incubated at 37°C for 24 h. Enriched culture was streaked on to Sorbitol MacConkey Agar (Oxoid CM0813) supplemented with Cefixime Tellurite Selective Supplement (CT) (Oxoid SR172) which were incubated at 37 °C for 18–24 h. Following the incubation period, the colorless colonies were tested by *E. coli* O157 latex kit (Oxoid DR0620) (Hitchins et al., 2000).

Results

In the present study, the results showed that 6.57% of raw milk samples were contaminated with *Listeria* spp. and 2 (0.57%) were *L. monocytogenes* (Table 1). All samples were negative for *Salmonella* spp. and *E. coli* O157:H7. According to Turkish Food Codex (TFC, 2006), the presence of *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 in 25 ml of raw milk is not acceptable.

Table 1. Prevalence of *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 in raw milk samples

Number of Samples	<i>Listeria</i> spp.	<i>L. monocytogenes</i>	<i>Salmonella</i> spp.	<i>E. coli</i> O157:H7
350	23 (6.57%)	2 (0.57%)	–	–

Discussion

Pathogenic bacteria in milk have been a major factor for public health concern. The role and importance of *L. monocytogenes* is becoming increasingly apparent. Raw milk samples have been examined in several countries for the presence of *L. monocytogenes* (Table 2). Gasparovic et al. (1989) collected 635 raw milk samples from Germany and 0.3% was found to be the positive for *L. monocytogenes*. Oni et al. (1989) reported 0.7% occurrence of *L. monocytogenes* in raw milk in Nigeria. Our findings showed approximately similarity with these results. Contrary to this, the studies which had higher results (44.7%, 7.0%, 15.3% and 5.0%) than ours were reported by Garayzabal et al. (1987), Lovett et al. (1987), Harvey and

Gilmour (1992) and Erol and Sireli (2002), respectively. These differences may be originated from detection methods, sampling procedures and the geographical conditions. The reason for high contamination rate should be due to the insufficient hygienic conditions of equipments, including bulk tanks, churns, separators, heating and cooling units, using at milking, storage and transport.

L. monocytogenes is an intercellular parasite and can be present within leukocytes in contaminated milk. An intracellular state may confer heat resistance to the organism and allow some listeriae within leukocytes to survive pasteurization (Doyle et al., 1987). Hence, the low concentration of *L. monocytogenes* detected in this study, can also present a potential hazard.

Table 2. Incidence of *L. monocytogenes* in raw milk in Turkey and other countries

Country	Number of Samples	Incidence	References
France	337	14 (4.2%)	Gledel (1988)
Holland	137	6 (4.4%)	Beckers et al. (1987)
Germany	635	2 (0.3%)	Gasparovic et al. (1989)
USA	200	14 (7.0%)	Lovett et al. (1987)
Turkey	100	4 (4.0%)	Gun (1994)
Turkey	47	0	Aygun and Pehlivanlar (2006)
Turkey	250	3 (1.2%)	Sagun et al. (2001)
Turkey	100	4 (4.0%)	Unlu et al. (1998)
Spain	67	30 (44.7%)	Garayzabal et al. (1987)
Nigeria	150	1 (0.7%)	Oni et al. (1989)
Canada	315	17 (5.4%)	Slade and Colins (1987)
USA	300	9 (3.0%)	Lund et al. (1991)
Turkey	100	3 (3.0 %)	Soyutemiz et al. (2001)
Mexico	100	0	Angelica et al. (1995)
Turkey	77	14 (18.2%)	Sharif and Tunail (2001)
Mexico	1300	62 (13.0%)	Carlos et al. (2001)
India	2060	105 (5.0%)	Kalorey et al. (2008)
Spain	1445	37 (2.5%)	Gaya et al. (1996)
Turkey	80	4 (5.0%)	Erol and Sireli (2002)
N. Ireland	176	27 (15.3%)	Harvey and Gilmour (1992)
Malaysia	930	18 (1.9%)	Chye et al. (2004)
India	86	7 (8.1%)	Bhilegaonkar et al. (1997)

The prevalence of *Salmonella* spp. in milk samples tested in these studies were detected by **O'Donnel (2007)** in UK (0.36%), **Chye et al. (2004)** in Malaysia (1.4%) and **Soyutemiz et al. (2000)** in Turkey (1.0%). They explained the high *Salmonella* spp. prevalence with mishandling or improper hygiene. In general, *Salmonella* spp. is rarely indigenously present in milk. In this study, no *Salmonella* spp. was isolated. These results were in acceptance to the findings reported by **Padungtod and Kaneene (2006)**, **Ekici et al. (2004)**, **Abd El-Atty and Meshref (2007)** and **Eleftheriadou et al. (2002)**.

In the present study, *E. coli* O157:H7 was not detected. Similar results were reported by **Soyutemiz et al. (2000)** in Turkey, **Coia et al. (2001)** in England, **Heuvelink et al. (1998)** in Holland and **Ansary and Kaspar (1997)** in

USA. *E. coli* O157:H7 presents sporadically at very low levels together with very high levels of competitor organisms that's why it is difficult to detect. In contrary, **Chye et al. (2004)**, **Dontorou et al. (2003)** and **Abdul-Raouf et al. (1996)** demonstrated that *E. coli* O157:H7 was isolated from 33.53%, 0.3% and 6.0% of milk, respectively. In Turkey, few studies have detected *E. coli* O157:H7 in raw milk. **Aslantas and Yildiz (2002)** and **Oksuz et al. (2004)** found *E. coli* O157:H7 in 1 out of 100 samples.

Conclusion

In conclusion, the result of this study indicated that the incidence of *L. monocytogenes* in raw milk was low. However, considering that the mortality of listeriosis is

very high, low prevalence rate of *L. monocytogenes* should not be disregarded. Therefore, it is essential to ensure improving the quality of production technology and developing the sanitation strategies for enhancing the safety of food.

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