COMUNICACIONES

Occurrence of the bacteria Listeria spp. in raw milk in Costa Rica

Ma. Laura Arias¹, Rafael Monge², Florencia Antillón¹, Eduardo Glenn¹. ¹ Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica.

² Departamento de Control de Alimentos, Ministerio de Salud, San José, Costa Rica.

(Rec, 14-IV-1994. Acep. 8-VI-1994)

Resumen: De julio 1993 a febrero 1994 se estudió la presencia de *Listeria* spp. en 220 muestras de leche cruda recolectadas directamente en tanques de almacenamiento de lecherías seleccionadas aleatoriamente en el área metropolitana, San José, Costa Rica. Las muestras fueron analizadas con la metodología recomendada por la International Dairy Federation. Se aisló *Listeria* spp. de 14 (6.4%) de las muestras, el 4.5% de las cepas fue *L. innocua*, el 1.4% *L. welshimeri* y el 0.5% *L. gravi*. No se aisló *L. monocytogenes*. Tampoco se determinaron variaciones estacionales en la presencia de esta bacteria.

Key words: Listeria spp., L. monocytogenes, raw milk.

Seven species of *Listeria* spp. have been identified, but *L. monocytogenes* is the main pathogen for humans and animals, although *L. innovii* has also been reported to produce disease in animals and man (Busch 1971, Rocourt and Seeliger 1985). *L. seeligeri* and *L. welshimeri* are generally considered non-virulent species, but each one has been identified in human infections (Andre and Genicot 1987, Rocourt *et al.* 1986).

Occurrence of *Listeria* spp. in pasteurized and raw milk, seafood, fresh products, meats and manufacturing plants has been reported in industrialized countries, even though little is known about the distribution of this bacteria in food in other nations.

The aim of this study was to determine the prevalence of *Listeria* spp. in raw milk obtained from bulk tanks on dairy farms in the metropolitan area of San José, Costa Rica.

A total of 220 raw milk samples from bulk storage tanks were analyzed from July 1993

through February 1994 for the presence of Listeria spp., according to the methodology recommended by IDF (Anonymous 1986). Briefly, 25 ml of each sample were transfered to 225 ml of enrichment broth and incubated for 7 days at 30°C. This medium consists of tripticase soy broth (TSB) to which acriflavin (10 mg/L), nalidixic acid (40 mg/L) and cycloheximide (50 mg/L) have been added. After the enrichment, a loop was streaked on the surface of Oxford agar, incubated at 37°C for 48 h and evaluated for the presence of typical Listeria colonies. These were confirmed by Henry illumination, morphology and Gram staining, motility, hemolysis properties, CAMP (Chriestie, Atkins and Munch-Peterson) behavior with Staphylococcus aureus, xylose and rhamnose utilization.

Listeria was found in 6.4% (14/220) of the samples analyzed. From these, 4.5% (10/220) were L. innocua, 1.4% (3/220) L. welshimeri and 0.5% (1/220) L. gravi. We did not isolate L. monocytogenes.

Listeria species have been shown to be common in raw milk from bulk tanks in different countries. Reports of *L. monocytogenes* vary from high prevalence percentages found in countries such as Spain (45.3%) (Domínguez et al. 1985), Canada (5.4%) (Slade et al. 1988), United Kingdon (3.6%) (Schaak and Marth 1988), to very low or null ones in Australia (0.5%) (Venables 1989), Italy (0%) (Massa et al. 1990) and New Zealand (0%) (Stone 1987) for example. Excluding Spain's data, the overall prevalence worldwide of this bacteria appears to be 2.2% (Farber and Peterkin 1991).

These results, and others from Costa Rica where a 20% prevalence of *L. monocytogenes* was detected in raw milk (Oreamuno 1994) contrast with our results. Both Costa Rican studies used the same laboratory methodology, which has a sensibility of 10 CFU/ml, but an important difference was observed in the milking mechanism; in our work it was mechanical, not by hand.

Our negative results for *L. monocytogenes* may be caused by the hygiene and good manufacturing practices of dairy-men using mechanical equipment. Hand milking has a greater chance for contamination with bacteria, either from the cow skin, the surroundings or the hands, because *Listeria* is widely distributed in the environment as reported in the literature (Farber and Peterkin 1991, Seeliger and Finger 1983). The negative results can also be caused by the antagonic relationship between *L. innocua* and *L. monocytogenes* (Petran and Swanson 1993).

A seasonal variation of the occurrence of *Listeria* in milk has been described in the literature, finding a greater frequency during winter (Gitter *et al.* 1980) due to feeding practices, herd management or unknown factors affecting animal-bacteria, bacteria-environment relationship, or both (Domínguez *et al.* 1985). No seasonal variation was found in our study and this can be explained by the little feeding and climatic changes that the herd suffers throughout the year.

In conclusion, we recommend the implementation of mechanical milking and the maintenance of good hygienical conditions all throughout the process. This should decrease the incidence of this bacteria in raw milk and derivates such as soft cheese and ice-cream, in which 45% and 2% of the samples, respectively, presented *L. monocytogenes* (Monge *et al.* 1993).

ACKNOWLEDGEMENTS

We thank Sterling Company, Costa Rica and Laura Villalobos for their support.

REFERENCES

- Andre, P. & A. Genicot. 1987. First isolation of *Listeria* welshimeri from human beings. Zentralbl. Bakteriol. Hyg. A. 263: 605-606.
- Anonymous. 1986. Standard methods for the examination of dairy products. International Dairy Federation. Annual Session in the Hague, Netherlands: Report of Group E 47, 3-166.
- Busch, L.A. 1971. Human listeriosis in the United States, 1967-1969. J. Infect. Dis. 123: 328-332.
- Domínguez, L., J.F. Fernández, J.A. Vázquez, E. Rodríguez & G. Suárez. 1985. Isolation de microorganismes du genre *Listeria* a partir de lait cru destiné a la consommation humaine. Can. J. Microbiol. 31: 938-941.
- Farber, J.M. & P.I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microb. Rev. 55: 476-511.
- Gitter, M., R. Bradley & R.H. Blamsied. 1980. *Listeria* monocytogenes infection in bovine mastitis. Vet. Rec. 107: 390-393.
- Massa, S., D. Cesaroni, G. Poda & L.D. Trovatelli. 1990. The incidence of *Listeria* spp. in soft cheeses, butter and raw milk in the province of Bologna. J. Appl. Bacteriol. 68: 153-156.
- Monge, R., D. Utzinger & M.L. Arias. 1992. Incidence of Listeria monocytogenes in pasteurized ice cream and soft cheese in Costa Rica. Rev. Biol. Trop. 42: In press.
- Oreamuno, S. 1994. *Listeria monocytogenes* y su relación con el nivel de coliformes fecales durante la manufactura de queso blanco en plantas de la zona de Santa Cruz- Turrialba. Thesis, Escuela de Tecnología de Alimentos, Universidad de Costa Rica, San José, Costa Rica.
- Petran, R. & K. Swanson. 1993. Simultaneous growth of Listeria monocytogenes and L. innocua. J. Food Prot. 56: 616-618.
- Rocourt, J., H. Hof & A. Schrettenbrunner. 1986. Meningite purulente Aigue *Listeria seeligeri* chen un Adulte immunocompetent. Schweis. Med. Wochnschr. 116: 248-251.
- Rocourt, J. & H.P.R. Seeliger. 1985. Distribution des especies du genre *Listeria*. Zentralbl. Bakteriol. Mikrobiol. Hyg. A. 259: 317-330.

- Schaak, M.M. & E.H. Marth. 1988. Survival of *Listeria* monocytogenes in refrigerated cultured milk and yogurt. J. Food Prot. 51: 848-852.
- Seeliger, H.P.R. & H. Finger. 1983. Listeriosis, p. 264-289. In J. Remington (ed.). Infectious diseases of the fetus and newborn infant. W.B. Saunders, Philadelphia.
- Slade, P.J., D.L. Collins-Thompson & F. Fletcher. 1988. Incidence of *Listeria* species in Ontario raw milk. Can. Inst. Food Sci. Technol. J. 21: 425-429.
- Stone, D.L. 1987. A survey of raw whole milk for Campylobacter jejuni, Listeria monocytogenes and Yersinia enterocolytica. N.Z.J. Dairy Sci. Technol. 22: 257-264.
- Venables, L.J. 1989. *Listeria monocytogenes* in dairy products—the Victorian experience. Food Aust. 41: 942-943.