THE ISOLATION OF LISTERIA SPP. IN MEAT AND MEAT PRODUCTS

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SUMMARY

By means of the FDA-method, modified by a sub-enrichment (1:100) after 24 h and the introduction of a new selective medium, we could demonstrate that in Switzerland 44.2% of beef, pork, and poultry as well as 20.3% of raw meat products are contaminated with Listeria spp. 20.5% of the meat samples and 5.9% of the meat products respectively revealed the presence of *L.monocytogenes*.

INTRODUCTION

There is evidence of an increasing prevalence of listeriosis in humans, not only among the risk groups - such as e.g. pregnant women or immunocompromised patients - but also among persons who have previously been healthy.

Several infections with *L.monocytogenes* have been associated with the consumption of contaminated food such as coleslaw (Schlech et al. 1983), pasteurised milk (Fleming et al. 1985), Mexican style soft cheese (CDC 1985), or soft cheese (Bannister 1987). In New Zealand an outbreak of listeriosis among newborns was connected with the consumption of raw fish by their mothers (Lennon et al. 1984).

Up to now no Listeria - infections could be traced to meat or meat products. Therefore we conducted a survey on the contamination rate of meat and raw meat products in Switzerland.

EXPERIMENTAL METHODS

So far we have tested 103 samples of beef, 31 samples of pork, 56 samples of poultry, and 187 samples of different raw meat products.

Listeria spp. were isolated after an 48-hour-enrichment in Acriflavin-broth (EB) at +37C. In parallel a sub-enrichment (1:100) after 24 h of incubation in EB was run, which was incubated for another 24-hour-period at +37C. For cold enrichment each sample was stored in EB at +4C during 4 weeks.

Sub-cultures from all enrichment-broths were plated on a modified McBride-agar (MCBA) according to the FDA-method and on Acriflavine-Ceftazidime-agar (ACA) proposed by Bannerman and Bille (1988). MCBA and ACA were both incubated at +37C. After 24 and 48 h of incubation the plates were checked for the growth of Listeria spp. by means of obliquely transmitted light, as described by Henry (1933). Using this technique, Listeria spp. appear on MCBA in blue coloured colonies and on ACA as small, translucent, yellow colonies with a blue border.

Suspicious colonies were identified biochemically according to the FDA-method and all isolates of *L.monocytogenes* were serotyped (Dr. Bille, Lausanne).

RESULTS AND DISCUSSION

122 (32.4%) out of the 377 samples of meat and raw meat products contained Listeria spp. (Tables 1 and 2). 36 (29.5%) isolates were identified as *L.monocytogenes* and in another 14 (11.5%) samples *L.monocytogenes* was isolated together with *L.innocua* (13 samples) or together with °L.seeligeri and *L.innocua* (1 sample). In 3 (2.5%) samples °L.seeligeri was found and one (0.8%) sample contained °L.seeligeri and *L.innocua*. 68 (55.7%) isolates were identified as *L.innocua*.

Table 1: Occurrence of Listeria spp. in raw meat

(L.m = L. monocytogenes, L.s = L.seeligeri, L.i = L. innocua)

	n examined	L.m	L.m + L.i	L.m + L.s + L.i	L.s + L.i	L.i
Mixed minced meat	85	13 15,3%	5 5,9%	1 1,2%	0	15 17,6%
Beef	18	1 5,6%	2 11,1%	0	0	3 16,7%
Porc	31	1 3,2%	3 9,7%	0	0	10 32,3%
Poultry	56	10 17,9%	4 7,1%	0	1 1,8%	16 28,6%
Total	190	25 13,2%	14 7,4%	1 0,5%	1 0,5%	44 23,2%

Table 2: Occurrence of Listeria spp. in raw meat products

	n examined	L.monocytogenes	L.seeligeri	L. innocua
Air dried meat	44	4 = 9,1%	0	3 = 6,8%
Uncooked ham	19	0	0	2 = 10,5%
Salami	46	0	2 = 4,8%	7 = 15,2%
Smoked sausage	55	3 = 5,5%	1 = 1,8%	5 = 9,1%
"Mettwurst"	19	4 = 21,1%	0	7 = 36,8%
Other products	4	0	0	0
Total	187	11 = 5,9%	3 = 1,6%	24 = 12,8%

Table 3: Isolation of 109 Listeria spp. on MCBA and ACA after enrichment, subenrichment, and cold enrichment

MCBA			ACA		
enrichment	subenrichment only	cold enrichment only	enrichment	subenrichment only	cold enrichment only
59	25	17	77	14	16
(58,4%)	(24,7%)	(16,8%)	(72,0%)	(13,1%)	(15,0%)

The serotyping of the *L.monocytogenes*-strains revealed that 5.1% of the isolates belong to the serovar 1/2 a, 38.5% to serovar 1/2 b, 25.6% to serovar 1/2 c, and 30.8% to serovar 4 b.

On ACA 98.2% of the Listeria spp. were recovered versus 92.7% on MCBA (Table 3). Since most Listeria spp. have grown as a pure culture, the ACA proved also to be more specific. This may explain why after 48 h of enrichment the isolation rate on ACA was significantly higher (72.0% versus 58.4%) than on MCBA.

CONCLUSIONS

The ACA proves to be a sensitive medium for recovering Listeria spp. from heavily contaminated food such as raw meat and fermented meat products. Due to the fact that after 48 h of incubation the pH in EB approaches 5.5 to 5.0, a sub-enrichment after 24 h of incubation in EB is highly recommendable. In our material 13.7% of Listeria spp. were detected after sub-enrichment only. The cold enrichment reveals another 15.0% of isolates, this however necessitates at least 4 weeks of incubation. This technique therefore is suitable for scientific purposes only and cannot be recommended for the bacteriological control of meat or meat products.

REFERENCES

Bannerman, E.S. and Bille, J. (1988). *Applied and Environmental Microbiology* **54**:165.

Bannister, B.A. (1987). *Journal of Infection* **15**:165.

Centers for Disease Control (1985). *Morb. Mort. Weekly Rep.* **34**:357.

Fleming, D.W., Cochi, S.L., MacDonald, K.L., Brondum, J., Hayes, P.S., Plikaytis, B.D., Holmes, M.B., Audurier, A., Broome, C.V. and Reingold, A.L. (1985). *New England Journal of Medicine* **312**:404.

Henry, B.S. (1933). *Journal of Infectious Diseases* **52**:374.

Lennon, D., Lewis, B., Mantell, C., Becroft, D., Dove, B., Farmer, K., Tonkin, S., Yeates, N., Stamp, R. and Mickleson, K. (1984). *Ped. Inf. Dis.* **3**:30.

Schlech, W.F. III, Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S. and Broome, C.V. (1983). *New England Journal of Medicine* **308**:203.