Epidemiol. Infect. (2000), **125**, 303–308. Printed in the United Kingdom

Comparative investigations of *Listeria monocytogenes* isolated from a turkey processing plant, turkey products, and from human cases of listeriosis in Denmark

B. OJENIYI*, J. CHRISTENSEN AND M. BISGAARD

Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University, Stigbøjlen 4, 1870 Frederiksberg C. Copenhagen, Denmark

(*Accepted 21 June 2000*)

SUMMARY

Listeria monocytogenes was isolated from critical control points in a Danish turkey processing plant, from turkey products and from cases of human listeriosis. During processing in the plant the prevalence of *L. monocytogenes* ranged from 25·9 to 41·4%. Cleaning and disinfection decreased the prevalence to 6·4%. Isolates of *L. monocytogenes* were characterized by pulsed-field gel electrophoresis (PFGE) using restriction endonuclease *Apa*I. Identical DNA types were obtained from turkey products and the processing line even after cleaning and disinfection. Two identical DNA types were demonstrated among isolates from turkey products and human cases of listeriosis. The prevalence of *L. monocytogenes* in turkey products ranged from 7·3 to 17·4% for ready-to-eat products and raw products, respectively. Since none of the 27 flocks examined before slaughter sampled positive for *L. monocytogenes* and the prevalence increased during processing, the potential risk from turkey meat was apparently due to factory hygiene rather than intrinsic contamination of the turkeys.

INTRODUCTION

Human listeriosis is believed to be caused mainly by consumption of contaminated food items [1]. Due to the ubiquitous distribution of *L. monocytogenes*, different food items including soft cheese, milk products, meat products, vegetables, and seafood have been implicated as reservoirs of infection [2]. Previous investigations in seven abattoirs in Denmark showed a prevalence rate of 9·1% of *L. monocytogenes* in ready-to-cook broilers although caecal samples from 2078 broilers representing 90 randomly selected broiler flocks were negative for the organism [3]. A case of listeriosis associated with consumption of turkey franks in USA was reported by Barnes and colleagues in 1989 [4] and an evaluation of the turkey plant involved showed the prevalence of *L. monocytogenes* in fresh turkey parts to range from 11·7–20·0% [5].

* Author for correspondence:

We report the results of a systematic investigation of turkey flocks before slaughter, on finished turkey products, and on critical control points in the abattoir during slaughtering and after a cleaning and disinfection process. Isolates from the processing line, turkey meat products and human cases of listeriosis were compared by pulsed-field gel electrophoresis (PFGE) to determine their relationships and to assess the extent of persistence of *L. monocytogenes* in the processing plant.

METHODS

The processing line of the abattoir

Samples were taken from transport crates, and machines for slaughtering of birds, scalding water, defeathering, evisceration, pluck sorting and from the working up department, where the meat was either cut into smaller pieces, removed from the bones or marinated. Samples were also taken from the area

where ready-to-eat products were processed and packed. A total of 99 and 105 samples, from the processing line during two separate visits were examined for *L. monocytogenes*. The 99 samples from the first visit were all taken during processing. On the second visit 2 months later, 78 samples were taken from the cleaned and disinfected areas of the abattoir and 27 samples during subsequent processing. Samples were taken from identical critical control points during the first and second visit to the abattoir.

Selected critical control points

Twenty-two critical control points were selected in the abattoir. Gauze tampon samples from each of these control points were taken during processing (22 samples) as well as after cleaning and disinfection (22 samples). This procedure was repeated 7 times at 3-week intervals to give a total of 308 samples. This part of the study covered a period of 18 weeks and sampling was performed by staff instructed during the first visit.

Processed turkey products

An average of 10 turkey products was examined for *L. monocytogenes* on a weekly basis over 9 weeks; 101 product samples were taken including ready-to-eat products (heat treated, smoked and dried) as well as raw products.

Gauze tampon swabs

Samples were obtained from selected critical control points along the processing line by swabbing a 100 cm^2 area with a gauze tampon moistened in sterile buffered peptone water. The samples were taken in the abattoir during processing and after the cleaning and disinfection process.

Cleaning and disinfection of the processing plant

After processing, the abattoir was cleaned and disinfected by the following routine. Surfaces were hosed down with water at 50 °C , washed each time with either 1% Topaz 33 or 1% Topax 66 alkaline detergents (Henkel-Ecolab, Valby, Denmark), or 1% Kombinon (SFK, Hvidovre, Denmark) following a fixed cleaning protocol for a minimum of 15 min, followed by hosing with 50 °C water, and then treated with either an acid disinfectant, 1% Oxonia, (Henkel-Ecolab) or a neutral disinfectant 1% Rodalon (SFK) in cold water for 15 min, followed by a final hosing with cold water.

Sock samples

Over a period of 3 months, gauze sock samples (elastic cotton tubes pulled over the operator's boots) were collected from 27 turkey flocks by walking around in the turkey house [6]. Flock sizes ranged from 3000 to 11 300 birds and included both females and males aged between 14 and 17 weeks.

Isolation and identification of *L. monocytogenes*

The samples were incubated in L-Palcamy broth for 2 days at 37 °C [7] and 5 μl of each broth culture were streaked on Palcam agar and incubated aerobically for 2 days at 37 °C. Suspect colonies were identified as *L. monocytogenes* according to Barrow and Feltham [8] as: Gram-positive rods, that were catalase-positive and motile. Colonies were also confirmed as CAMP-positive on blood agar cross-streaked with *Staphylococcus aureus* and CAMP-negative with *Rhodococcus equi*. All were β-hæmolytic on blood agar and produced acid from L-rhamnose and α-methyl-D-mannoside, but not from D-mannitol and D-xylose. Sugar fermentation tests were carried out in meat extract-peptone-broth containing 0·5% of D-mannitol, D-xylose, L-rhamnose and α-methyl-D-mannoside, respectively, with bromthymol blue as indicator [9].

L. monocytogenes from cases of human listeriosis

Forty-one isolates of *L. monocytogenes* from human cases of listeriosis in Denmark were kindly provided by Dr Peter Gerner Smidt (State Serum Institute, Copenhagen, Denmark). The human strains were isolated during and after the period the abattoir and turkey products were examined.

Epidemiological investigation

Molecular characterization of *L. monocytogenes* was carried out by PFGE according to the method of Grothues and colleagues [10], modified by Ojeniyi and colleagues [11] and Brosch and colleagues [12]. The restriction enzyme used was *Apa*I (Boehringer Mannheim, Germany) in a CHEF DRIII apparatus (Biorad, USA). Band patterns were compared visually and distinct patterns differing by one or more band positions were designated type A through T.

Table 1. *Number of positive samples of* L. monocytogenes *and PFGE type obtained from critical control points of the cleaned and disinfected abattoir and during subsequent processing of turkey meat*

	Number of isolates			PFGE Type	
Visit	Disinfected abattoir	During process	Total number of strains (percentage)	Disinfected abattoir	During processing
1	1	7	8/44 (18·2)	D	D,D,D,B,B,B,B
2	3	5	8/44 (18·2)	B,B,D	B,B,B,B,G
3	0	2	2/44 (4·5)		B,B
4	2	7	9/44 (20·2)	B,B	D,D,D,D,B,B,E
5	1	5	6/44 (13·6)	E	D,D,B,B,B
6	2	1	3/44 (6·9)	B,B	B
7	0	10	10/44 (22·7)		D,D,D,D,D,D,B,B,B,E
Total (%)	9/154 (5·8)	37/154 (24·0)	46/308 (14·9)		

Table 2. *The prevalence and PFGE type of* L. monocytogenes *in raw and processed turkey products*

	Number of *L. monocytogenes* positive samples	PFGE type	Raw or heat treated products
Retail department			
Minced turkey breast	2/6	B,B	Raw
Turkey slices	1/17	B	Raw
Minced turkey meat from legs	5/23	B,D,D,D,E	Raw
Working up department			
Turkey wings	1/1	D	Core temp 75 °C
Smoked turkey fillet, ready to eat	3/14	B,B,B	Smoked 38–39 °C
Cooked smoked turkey breast	0/18		Core temp 75 °C
Turkey parts	0/1		Core temp 75 °C
Cooked smoked turkey in dices	0/2		Core temp 75 °C
Smoked turkey cuvette	0/17		Smoked 38–39 °C
Turkey breast, smoked	0/2		Smoked 38–39 °C
Totally	12/101 (11·9 %)		
Heat treated products	4/55 (7·3 %)		
Raw products	8/46 (17·4 %)		

RESULTS

Prevalence of *L. monocytogenes*

Processing line

Forty-one of 99 (41·4 %) and 7 of 27 (25·9 %) samples from the processing line during processing were positive for *L. monocytogenes*, while only 5 of 78 (6·4 %) samples after cleaning and disinfection yielded the organism.

Critical control points

Recovery rates before processing (after cleaning and disinfection) ranged between 0–13·6 % (average 5·8 %) and during processing from 4·5–45·5 % (average 24·0 %) (Table 1).

Turkey products

L. monocytogenes was isolated from 4 of 55 (7·3 %) ready-to-eat products, and 8 of 46 (17·4 %) raw products (Table 2). None of the sock samples from the 27 turkey flocks was positive for the species.

Molecular characterization of isolates

PFGE pattern types B and D were detected in all parts of the processing line including crates, the departments for slaughtering, evisceration, working-up,

Table 3. *Prevalence and PFGE type of* L. monocytogenes *demonstrated during two visits to the turkey abattoir*

PFGE type	First visit: during processing	Second visit: during processing	Second visit: after cleaning and disinfection of the abattoir
A			2 (40·0 %)
B	27 (65·9 %)	2 (28·6 %)	3 (60·0 %)
D	11 (26·8 %)	4 (57·1 %)	
C		1 (14·3 %)	
E	1 (2·4 %)		
F	1 (2·4 %)		
G	1 (2·4 %)		
Totally	41/99 (41·4 %)	7/27 (25·9 %)	5/78 (6·4 %)

processing products and packing, on both visits to the abattoir, while types A and B were recovered from the cleaned and disinfected abattoir (Table 3). On the first visit, 27 of 41 (65·9 %) isolates were of type B, 11 (26·8 %) of type D, and 3 of types E–G. Of the 7 isolates from the second visit, 2 and 4 were of types B and D, respectively, and the remaining strain was type C. After disinfection 3 of 5 isolates were of type B, and 2 of type A (Table 3). Two additional PFGE types (type E and G) were identified in the evisceration department and type E persisted after cleaning of this department.

PFGE types B and D were the only strains identified from ready-to-eat products and these types were also predominant in raw products. A type E strain was also recovered from raw products.

A total of 15 PFGE types were found among the 41 isolates of *L. monocytogenes* from human cases of listeriosis. Type B was found in 9 cases (22·0 %) and type D was represented by a single case. Type H was identified in 11 cases, type I in 6 and types J–L in 2 cases each. The remaining strains were unique.

DISCUSSION

Since it was shown that food-borne transmission of listeriosis occurs [13] isolates of *L. monocytogenes* from various food items have been characterized by a number of methods including serotyping [14], phage typing [15], multilocus enzyme electrophoresis [16], and various DNA fingerprinting techniques [17–19]. In this study we used PFGE which is highly discriminatory for *L. monocytogenes* [3, 19, 20]. The technique has also been shown in a WHO multicentre study to be reproducible between laboratories in different countries [12].

The prevalence of *L. monocytogenes* in turkey products in this study (11·9 %), is within the range observed by others [21, 22] and similar to that previously detected in broilers [3]. As was the case with broilers and fish processing plants [18], relatively few strains, defined by PFGE, were found in the turkey abattoir suggesting that *L. monocytogenes* might be endemic in certain situations. An attempt to link fish sources with human listeriosis failed to demonstrate similarity of strain types [18] but Nakama and colleagues [19] found that 4 of 20 isolates from different foods (2 from raw sausages, 1 from salmon flakes and another from smoked salmon) were of 3 PFGE types also found in human disease. Indeed, similar DNA types are known to predominate among human and animal strains [15].

The absence of *L. monocytogenes* in the turkey flocks before slaughter agrees with the results of a similar study of broiler abattoirs in Denmark [3] where caecum samples and bedding in the broiler pens were negative for the organism. Since identical DNA types were obtained from both the cleaned and disinfected abattoir as well as during processing, it appears that *L. monocytogenes* is able to persist in the processing plant environment although it can not be ruled out that it may have been reintroduced at a low level either with the flocks or from the natural environment. The fact that the prevalence of *L. monocytogenes* was higher in the abattoir after processing had begun than in the disinfected abattoir seems to indicate multiplication and subsequent spread during processing. The machinery is difficult to clean and fat and protein deposits on numerous surfaces probably provides good growth conditions for bacteria in a biofilm. The presence of relatively few frequently occurring strains in the abattoir even

during processing, and that identical types were constant in the critical control points over 18 weeks, and finished products for 9 weeks, underlines the persistence of *L. monocytogenes* in an abattoir environment. The persistence of a few clones may also be explained by the selection of clones having the capacity to form biofilms, or the presence of common clones in the environment of the processing plant, from where it spreads easily to turkey products. That 1/55 (1·8%) of fully cooked products (core temperature 75 °C) sampled positive for *L. monocytogenes* was most likely due to post-processing contamination rather than to inadequate cooking, as the temperature during the process was monitored. Smoked turkey fillet (Table 2) appeared to be a more risky product due to insufficient heat treatment (smoked and dried at 38–39 °C) but post-processing contamination cannot be ruled out.

The finding of identical DNA types in turkey products as well as in human cases of listeriosis suggests that turkey meat may constitute a reservoir and a risk for compromised patients. As the potential risk from turkey meat is apparently due to factory hygiene, rather than intrinsic contamination of the turkeys, thorough cleaning and disinfection of an abattoir is strongly recommended. Enhanced mechanical cleaning with brushes or high pressure hosing with either cold or hot water in combination with different detergents and disinfectants should be carried out to assess which procedure is most efficient to eliminate the organism. Work is in progress to investigate whether strains found in human listeriosis and in turkey products represent common types of the species that are also found in other food products and in the environment, and whether these strains are more virulent than their counterparts not associated with disease.

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