Epidemiol. Infect. (1998), **120**, 245–250. Printed in the United Kingdom

Prevalence of *Clostridium botulinum* type E in Finnish fish and fishery products

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(*Accepted 22 January 1998*)

SUMMARY

The prevalence of *Clostridium botulinum* type E gene in fish and fishery products of commercial importance in Finland was determined using a quantitative PCR analysis. The contamination level in 438 raw fish samples from intestines, surface and whole fish and 208 fish roe samples varied from 10–40% and from 4–14% respectively, depending on the fish species studied. The presence of *C. botulinum* in European wild freshwater fish and roe was demonstrated for the first time by isolation of the organism from PCR-positive samples. Five percent of 214 vacuum-packed and 3% of 123 air-packed fishery product samples examined at retail level were positive for the botulinum neurotoxin type E gene. A contamination level of 10% in vacuum-packed hot-smoked whitefish was detected. The results demonstrate that *C. botulinum* type E poses a serious health risk for those consuming fishery products from the Baltic Sea area.

INTRODUCTION

The increasing use of vacuum-packaging technology for fishery products, combined with chill storage to extend shelf-life, has raised concerns about the potential health risk for consumers caused by non-proteolytic *Clostridium botulinum* [1–5]. The processing of most hot- and cold-smoked and raw pickled vacuum-packed fishery products with minimal or no preservatives is insufficient to inactivate clostridial spores; however, the predominant spoilage micro-organisms are selectively eliminated or their growth inhibited [6]. The non-proteolytic toxin types of *C. botulinum* are psychrotrophic organisms which are capable of growth at low temperatures [7].

Contamination of live fish by *C. botulinum* has been recognized for many years. Worldwide prevalence studies in temperate geographical areas have shown *C. botulinum* type E as the most prevalent toxin type in aquatic environments and in fish and fishery products [8–11]. These studies have also documented the regular isolation of proteolytic types A, B and F and nonproteolytic types B and F, but the prevalences of these serotypes have been found to be considerably lower than that of type E. Type E has been implicated in the majority of botulism cases associated with the consumption of fish and fishery products [11–13].

Baltic Sea coastal waters are heavily contaminated with *C. botulinum* type E. Various surveys have shown a prevalence close to 100% in marine, freshwater and trout farm sediment samples in Denmark and Sweden [10, 14–16]. High contamination levels have also been reported in various fish species caught in the Baltic Sea near the Danish and Swedish coasts [14, 17, 18] as well as in trout originating in Danish trout farms [16]. A recent survey detected a high prevalence of *C. botulinum* type E in Finnish marine and freshwater bottom sediments [19]. No other toxin types were demonstrated.

Knowledge of the contamination level of Finnish fish is limited to a small scale survey of two trout

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farms [20], in which 4 and 10% of the intestinal samples of rainbow trout were positive. No other fish species have been studied. Very few data are available on the prevalence of type E in vacuum-packed fishery products and fish roe reaching the consumer in Europe. In the United Kingdom, Cann and colleagues [21] detected a type E contamination level of 0·77% in vacuum-packed fishery products. Due to known heavy contamination of Baltic Sea sediments we had reason to expect a considerably higher prevalence in fishery products originating from this area.

The aim of the present study was to examine which fish species and fishery products were associated with a potential *C. botulinum* hazard. A thorough understanding of the prevalence and properties of the organism is essential when considering the safety of fishery products, especially where new or modified preservation methods must be evaluated. In view of the results of the earlier bottom sediment distribution study only *C. botulinum* type E prevalence was examined [19].

METHODS

Sampling

During the period December 1994–September 1996, 438 raw fish samples of 5 different fish species and 208 roe samples of 4 fish species were examined. The raw fish samples consisted of 178 intestinal, 157 surface and 103 whole fish samples. The complete length of the intestines was included in the intestinal sample. Intestinal samples were packed individually and separately from other samples. The surface sample contained the skin, gills, fins and peritoneum of the fish. With small-sized fish species such as Baltic herring and vendace composite samples of whole fish each weighing 100–200 g were used. Sixty-two percent of the raw fish samples were non-farmed; they were purchased from local market places or retail outlets as whole or gutted fish. The burbot, whitefish and Baltic herring were mostly of marine origin. The vendace mainly originated in freshwater catching areas in Finland. The rainbow trout intestines were obtained from six freshwater and nine marine trout farms from different locations in Finland. The rainbow trout surface samples originated in marine trout farms; they were purchased from local market places or obtained directly from the farms. In all, 20% of non-farmed and 33% of farmed raw fish samples were of freshwater origin. In terms of geographical distribution, the origins of the raw fish samples were representative of the major Finnish catching and fish farming areas. Of the fish roe samples 54 were fresh with no preservatives, while 154 were frozen, containing about 3% NaCl. All the frozen and some of the fresh roe samples were purchased from local retail outlets. The fresh burbot roe samples were obtained from the intact roe pouch before evisceration.

During the period November 1995–January 1997, 214 vacuum-packed raw pickled, cold- and hot-smoked rainbow trout and salmon and hot-smoked whitefish samples, and 123 air-packed hot-smoked Baltic herring, vendace and river lamprey (*Petromyzon fluviatilis*) samples were examined. The samples were purchased from local retail outlets. The vacuum-packed fishery product samples were produced by 25 different Finnish manufacturers. Six of these 25 manufacturers hold a major market share of Finnish fishery products. In this study 74% of the samples came from these six producers, 70% of the whitefish were imported from Canada for processing in Finland, and 28% of the hot-smoked salmon (*Oncorhyncus spp.*) were of Alaskan origin. The NaCl concentration of the products as stated by the manufacturers was 1·1–4·1%. No other preservatives were included, with the exception of sodium benzoate in the hot-smoked products of some manufacturers. There was no means of knowing either the manufacturers' names or the NaCl concentration of the air-packed products.

PCR-detection and quantification

Samples were examined for the presence of *C. botulinum* type E neurotoxin gene (BoNT/E) using a quantitative PCR analysis, following the method described by Hielm and colleagues [22]. DynaZyme™ DNA polymerase (cloned from *Thermus brochianus*; Finnzymes, Espoo, Finland) and a 96-well PTC-100 thermal cycler (MJ Research, Watertown, MA, USA) were employed. The size of the amplified PCR products was determined in agarose gels by comparison with standard DNA fragments (DNA molecular weight marker VI, Boehringer–Mannheim, Mannheim, Germany).

All PCR-positive vacuum-packed fishery product samples were also examined for the presence of botulinum type E neurotoxin, using a mouse bioassay and following the method of the Nordic Committee

https://doi.org/10.1017/S0950268898008693 Published online by Cambridge University Press

Table 1. *Prevalence of* C. botulinum *type E gene in Finnish raw fish, fish roe and fishery product samples as determined by PCR and* C. botulinum *spore count in PCR-positive samples*

Sample type	No. of samples examined	No. of positive samples (%)	Mean MPN spores kg^{-1} in positive samples (range)
Raw fish			
Rainbow trout (*Oncorhyncus mykiss*)			
Intestines	117	18 (15)	240 (30–1900)
Surface*	51	10 (20)	110 (30–580)
Burbot (*Lota lota*)			
Intestines	61	9 (15)	280 (40–620)
Surface*	56	10 (18)	100 (40–290)
Whitefish (*Coregonus lavaretus*)			
Surface*	50	9 (18)	380 (50–2730)
Vendace (*Coregonus albula*)			
Whole fish†	50	5 (10)	30 (30–40)
Baltic herring (*Clupea harengus membras*)			
Whole fish†	53	21 (40)	120 (30–580)
Fish roe			
Rainbow trout (*Oncorhyncus mykiss*)	55	3 (5)	50 (30–90)
Burbot (*Lota lota*)	51	7 (14)	50 (30–100)
Whitefish (*Coregonus lavaretus*)	51	4 (8)	100 (100–120)
Vendace (*Coregonus albula*)	51	2 (4)	30
Fishery products			
Vacuum-packed products			
Raw pickled rainbow trout	50	1 (2)	40
Cold-smoked rainbow trout	64	2 (3)	160 (40–290)
Hot-smoked rainbow trout or salmon	50	2 (4)	30 (30–40)
Hot-smoked whitefish	50	5 (10)	40 (30–60)
Air-packed products			
Hot-smoked Baltic herring‡	50	0 (0)	0
Hot-smoked vendace‡	50	3 (6)	30 (30–40)
Hot-smoked river lamprey§	23	1 (4)	60

* Skin, gills, fins and peritoneum.
† A composite sample of 100–200 g of fish.
‡ A composite sample of 200–300 g of product.
§ A composite sample of 4–5 fish.

on Food Analysis [23]. This method detects all *C. botulinum* neurotoxin types. The bioassays were approved by the Faculty of Veterinary Medicine's Committee on Animal Experimentation. A part of the PCR-positive samples was submitted to a *C. botulinum* isolation procedure, following the method of the Nordic Committee on Food Analysis [24] modified by using PCR detection instead of mouse bioassay.

RESULTS

Of 438 raw fish samples, 82 (19%) and of 208 fish roe samples 16 (8%) were positive for the BoNT/E gene. Of the fish species studied, the highest prevalence of *C. botulinum* type E gene was in Baltic herring raw fish samples and burbot roe samples (Table 1). The estimated number of *C. botulinum* type E spores per kg was 30–2730 (mean ± standard deviation: 180 ± 390) in the PCR-positive raw fish samples, and 30–120 (60 ± 40) in the fish roe samples. The presence of the bacterium was confirmed by isolation of *C. botulinum* type E strains from the PCR-positive samples. The PCR-positive raw fish samples were evenly distributed throughout the main Finnish catching areas. The prevalence of *C. botulinum* type E gene in the non-farmed marine and freshwater raw fish samples was 23 and 9%, respectively. The contamination level of the farmed raw fish samples at both marine and freshwater farms was 13%. Fish samples from 5 out of 9 marine farms and 4 out of 6

https://doi.org/10.1017/S0950268898008693 Published online by Cambridge University Press

freshwater farms were contaminated by *C. botulinum* containing the type E gene. The total prevalence in non-farmed fish was 20% and in farmed fish 17%.

Of the 214 vacuum-packed and 123 air-packed fishery product samples studied, 10 (5%) and 4 (3%) were positive for the BoNT/E gene, respectively. The highest prevalence was in vacuum-packed hot-smoked whitefish (Table 1). The estimated count of *C. botulinum* type E spores per kg was 30–290 (60 ± 80) in the PCR-positive vacuum-packed samples and 30–60 (40 ± 10) in the PCR-positive air-packed samples. Again, the presence of the bacterium was confirmed by isolation of *C. botulinum* type E strains from the PCR-positive samples. The mouse bioassays did not detect any botulinum neurotoxin in the PCR-positive vacuum-packed fishery product samples. The PCR-positive vacuum-packed samples were produced by four manufacturers who are among the largest producers in Finland. Six out of seven positive hot-smoked samples were produced by the same manufacturer, and the raw fish of these samples was of Canadian or Alaskan origin. The *C. botulinum* type E gene contamination level of the vacuum-packed hot-smoked products of the plant was 19%.

DISCUSSION

A moderately high prevalence of *C. botulinum* type E in Finnish non-farmed fish was detected in this survey. Earlier studies conducted in the Baltic Sea area [14, 17, 18] had shown variable prevalences depending on the fish species examined, but in general the contamination level has been found to be high. We are the first to demonstrate *C. botulinum* type E in wild freshwater fish in Europe, detecting type E gene prevalence of 10% in freshwater vendace. We were also able to isolate *C. botulinum* type E strains from these PCR-positive samples. There seems to be a considerable difference in the contamination levels of marine and freshwater fish. Definite conclusions, however, should be avoided, since a comparison of marine and freshwater contamination levels was not made within a single fish species. The feeding habits of the fish appeared to have an influence on the level of contamination. The lowest prevalence was recorded for vendace, which feed only on plankton. Of the other non-farmed fish species studied, burbot is a predatory fish and whitefish a bottom feeder. During the early stage of life Baltic herring is a plankton feeder, but later it also feeds at the bottom on crustaceans and fish fry [25]. *C. botulinum* type E appeared to be slightly more prevalent in fish surface samples than in the intestines, but the difference was not statistically significant. Huss and Pedersen [18] reported a considerably higher prevalence of type E in bottom-feeding fish species as compared to plankton-feeders; they concluded that the sea bed was the primary source of contamination. On the basis of these results we agree with this conclusion.

The prevalence of *C. botulinum* type E did not differ greatly between wild and farmed fish. Nor was there any difference in contamination levels between freshwater and marine trout farms. A study of four Danish freshwater trout farms [16] showed a very high type E prevalence of 65% in whole rainbow trout. A considerably lower contamination level of 11% was detected in Norwegian freshwater trout farms [27]. There is no information available on the situation in marine farms. The variance in contamination levels between different countries may be due to differences in fish farm construction and feeding systems. One potential source of contamination is Baltic herring, which is widely used as additional feed in rainbow trout farms in Finland.

The presence of *C. botulinum* type E was also demonstrated in the Finnish fish roe and fishery products, although the numbers of spores found were generally lower than in the raw fish samples. The reason for the high contamination level of burbot roe remains obscure. The finding is especially peculiar in that the sampling was mostly performed directly from the intact roe pouch of fresh burbot. The roe samples of the other fish species were mainly frozen and salted. One possible contamination route of the roe pouch is through the urogenital opening, which lies just next to the anus of the fish.

The temperature applied in current commercial hot-smoking treatments is generally insufficient to eliminate natural contamination of fish with *C. botulinum* type E spores. The temperature of the deep fish flesh usually increases to 60–80 °C and remains at this level for approximately 30 min. The heat resistance of the spores is increased by a high fat and protein content, brining and a dry environment [27, 28]. All these factors are usually present in a fishery product. With the exception of the Baltic Sea area, the European levels of contamination of processed fish by type E appear to be much lower than levels in the USA [2, 21, 29, 30]. Surveys from the Baltic countries report prevalences of 13 and 20% in Swedish hot-smoked Baltic herring [31] and eel [32], respectively. Huss and colleagues [16] found type E in

5% of hot-smoked farmed trout. The contamination levels of raw fish detected in the present study seemed to have little effect on the contamination levels of processed fishery products; we could not demonstrate *C. botulinum* type E gene in hot-smoked Baltic herring samples, although fresh herrings were heavily contaminated. This could be due to the hot-smoking process for herrings, during which temperatures high enough to inactivate botulinum spores are found throughout the small-sized product.

In this study, vacuum-packed hot-smoked whitefish products were associated with a fairly high botulism risk. The processing method for this product probably contributed to the high contamination level. Eviscerated whitefish are typically smoked whole. Spores are likely to survive better in the gills or on the peritoneum of a large whole fish during the hot-smoking process than for example on the surface of a fillet. In addition, the spore load of a fillet would be lower after proper cleaning. The hot-smoking process also eliminates competing microflora and may give a heat shock to the botulinum spores, which is not the case for cold-smoked or raw pickled products. All the positive hot-smoked whitefish samples originated from the same plant. This could have been due to the sampling, as 36% of the whitefish products studied came from this plant. Alternatively it could have been an indication of a problem in the manufacturing process of this plant. Spores brought into the plant along with raw fish could have contaminated the production environment. The sodium benzoate used as a preservative in the hot-smoked products of the plant may have further inhibited the growth of competing microflora. The hot-smoking process used at the plant may have favoured the survival of spores, for example, if the relative humidity applied during the heat treatment was low.

The results of this survey show that *C. botulinum* type E is prevalent in many fish species caught from the Baltic Sea, posing a health risk for consumers and an economic risk for the fishing industry, as shown by two Swedish outbreaks during the 1990s [3, 4]. In most foods, botulinum spores are of no consequence unless they are able to grow and produce toxin. At present, the inhibition of botulinum toxigenesis in fishery products relies almost solely on NaCl and refrigeration below 3 °C. It is questionable whether this strict temperature requirement is achievable in retail and domestic refrigeration. There would be good reason to limit the shelf-lives of vacuum-packed fishery products, and to re-introduce the use of nitrite as an antibotulinogenic substance in fishery products [33–35]. The development of reliable time-temperature indicators for refrigerated long-storage fishery products would do much to restore customer confidence in these products.

ACKNOWLEDGEMENTS

The authors wish to thank Kirsi Ristkari and Maria Stark for their excellent technical assistance and DVM Maija Hatakka and DVM Nina Mäki-Petäys in Vantaa City Food Inspection for supply of the vacuum-packed fishery product samples. This research was supported by the Academy of Finland, the Walter Ehrström Foundation and the Finnish Veterinary Foundation.

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