Type E botulism in the Canadian Arctic is frequently associated with the consumption of traditional native foods, especially aged marine mammal products (1–3), yet the distribution of *Clostridium botulinum* type E in the Canadian Arctic is largely unknown. The geographic distribution of *C. botulinum* type E has been studied in the coastal waters of Alaska, Greenland, and Scandinavian countries (4–6), but not along the Canadian Arctic coastline. It has been shown that the pattern of human botulism generally follows the distribution of *C. botulinum* type E in marine sediments (6, 7). Bacterial contamination of marine mammal meat from the coastal environment could occur during butchering, particularly when tissues are cut and placed in direct contact with shoreline soil or rocks by Inuit hunters. Shoreline water collected along beaches also contained *C. botulinum* (6), which could contaminate meat if water is used to rinse the meat.

Another possible source of *C. botulinum* type E is the marine mammal itself. Fish and shellfish are known to be contaminated with *C. botulinum* type E spores (6, 8, 9). Consumption of fish and shellfish by marine mammals could lead to contamination of the intestinal tract with *C. botulinum*. Miller (6) reported the presence of *C. botulinum* type E in one beluga whale harvested in Alaska. If handling precautions are not taken during evisceration, contamination of marine mammal meat with intestinal contents containing *C. botulinum* type E may occur. No studies have examined the skin of marine mammals as a potential source of *C. botulinum* type E, which could contaminate the traditional aged marine mammal meat preparations called igunaq. The skins of walruses and seals harvested in Nunavik, in northern Quebec, are commonly used as chambers for aging meat and fat into igunaq (10) and could possibly introduce the microorganism.

Identification of environmental and animal sources of *C. botulinum* type E is necessary to assess the contamination risks and determine the transmission routes occurring at butchering sites in northern regions where food-borne botulism remains a public health problem. The objective of this study was to determine the geographic distribution and levels of *C. botulinum* type E in the environment of Nunavik. The prevalence in harvested seals was also investigated as a potential animal source of bacterial contamination. Initial results from the field survey showed a distinct cluster of shoreline butchering sites located in southern Ungava Bay that tested positive for *C. botulinum* type E. This area receives discharges from rivers draining large land masses. To determine the influence of the freshwater and terrestrial environments on the occurrence of *C. botulinum* type E in the coastal environment, the Koksoak River, which is the largest freshwater river flowing into Ungava Bay, was also included in the survey.

**MATERIALS AND METHODS**

**Study area and sampling sites.** The local Hunting, Fishing, and Trapping Associations (HFTAs) from each village of Nunavik provided the locations of the two most frequently used butchering sites for bearded seals (*Erignathus barbatus*) and ringed seals (*Phoca hispida*) on 1:250,000 maps. The sites were visited in October 2000 using the Department of Fisheries and Oceans’s helicopter and precisely positioned for latitude and longitude by using the helicopter’s global positioning system (GPS). The selection of the butchering area was based on the type of shoreline, its proximity to water, and ease of access by canoes. In addition to these sites, samples collected from a number of butchering sites visited during a preliminary study conducted in 1999 along the Hudson Bay and Hudson Strait coasts,
and subsequently in July 2001 along the southern Ungava Bay coast, were included in this study. Field surveys were also conducted in the environment of the Koksos River, near the village of Kuujjuaq, during the summers of 2001 and 2002. The marine portion of the river extends to Kuujjuaq, which is approximately 50 km inland from southern Ungava Bay. The freshwater portion of the river extends from the 50-kilometer mark to the 136.5-kilometer mark, where the Caniapiscau and Larch Rivers merge. Each sampling site from the Koksos River was geographically positioned with a portable Ensign GPS (Trimble Navigation Ltd., Sunnyvale, CA).

**Environmental sample collection.** From each butchering site, a 1-m² area was delineated on a large flat rock or beach surface and gently swabbed in a cross-hatched pattern by using two sterile cellulose sponges (bio-spo; Solar Biologicals Inc., Ottawa, Ontario, Canada) premoistened with 10 ml of buffered peptone water. Near the waterfront, a shoreline soil sample of about 300 to 500 g and from approximately 10 cm deep was collected by using a metal scoop or simply by pushing the 500-ml jar into the ground. Within 2 feet from the shore, seawater was collected 10 cm below the surface into a 2-liter polyethylene wide-mouth bottle by using a forward sweeping motion to avoid potential contamination from gloves. Sediment samples were collected every 5 to 10 km from the mouth to the fork of the Koksos River by using a Petite Ponor grab sediment sampler (Wildlife Supplier Company, Buffalo, NY). The jaws of the sampler were gently opened, and a sample of 300 to 500 g was transferred from the grab into a 500-ml polyethylene wide-mouth container by using a large spatula. For sediments located in small tributaries, samples were collected by simply pushing a 500-ml polyethylene jar into the bed until it filled up. In addition to sediment samples, terrestrial soil samples of dry earth of 300 to 500 g were collected at locations approximately 25 to 100 m inland from the main river or its tributaries by using a large spatula. Small numbers of freshwater and seawater samples were also collected according to the described procedure for coastline waters. All environmental samples were stored in coolers with frozen cold packs pending shipment to the laboratories of Health Canada in Ottawa, where they were kept at 4°C until tested.

**Intestinal contents from harvested seals.** Hunters originating from 8 villages of Nunavik participated in the collection of the intestinal tracts of harvested ringed seals and bearded seals between 1999 and 2002 while hunting for subsistence food. With the use of sampling kits provided by the Nunavik Research Centre (NRC) in Kuujjuaq, hunters were asked to wear laboratory gloves and milk the entire intestinal content down toward the rectum by using their fingers, until the contents reached a pretied end of the intestine near the anus. The other end of the intestine was then tied with a string to make a small intestinal bag and then removed. The intestinal segments were put into small plastic bags, and their contents were later transferred to a suitable plastic container at the NRC. A 5-cm-wide strip of abdominal skin from the navel to the tail was also excised by the hunters, put into a plastic bag, and sent along with the intestinal samples in an insulated cooler with ice packs to the NRC, where they were kept at 4°C until they were sent to Health Canada’s laboratories. Upon reception at the Health Canada laboratories, samples were stored at 4°C until cultured (for testing within 2 weeks) or frozen at −20°C if testing was delayed.

**Preparation of enrichment broths from samples.** Samples were initially cultured with and without pretreatment with 50% ethanol for 1 h (11). To reduce the number of cultures tested by mouse bioassay, the protocol was changed such that samples were first cultured without pretreatment. Any negative samples were then treated with 50% ethanol. Finally, samples that tested negative for *C. botulinum* by both initial enrichment and 50% ethanol treatment were then subjected to a heat pretreatment. Enrichment broths consisted of special peptone-peptone-gluco-yeast extract (SPGY) broth containing 5% special peptone (Oxoid, Basingstoke, England), 0.5% peptone (Bacto, Becton, Dickinson and Company, Sparks, MD), 2% yeast extract (Bacto, Becton, Dickinson and Company), 0.4% glucose, and 0.1% sodium thioglycolate, with the pH adjusted to 7.2 (12).

Water samples were filtered through sterile 0.22-μm nitrocellulose filters (Millipore Canada Ltd., Nepean, Ontario, Canada). Membrane filters were then asepically transferred to the original sample bottles, and 60 ml of SPGY broth was added to each bottle. Cellulose sponges were asepically cut in half, transferred into sterile 250-ml bottles, and cultured in SPGY broth.

Shoreline, sediment, and terrestrial soil samples collected during the field surveys were washed by suspension in gelatin phosphate buffer (GPB) to remove potential soluble inhibitors. Suspensions were centrifuged at 18,500 × g for 20 min (4°C). Supernatants were filtered through 0.22-μm filters, and filters were added back to the sediment pellets. SPGYT was added to the tubes containing the pellets and filters.

**Detection and typing of BoNT in cultures.** All enrichment broths were incubated at 20°C for 7 days in an anaerobic chamber with an atmosphere of 10% H₂, 10% CO₂, and 80% N₂ and then assayed for botulinum neurotoxin (BoNT) in a mouse bioassay (12). To minimize the number of mice required for the assay, supernatants from three cultures were combined and diluted 1:2 (or 1:6 for an individual sample) with GPB. Confirmation of serotype was performed by seroneutralization using monovalent botulinum antitoxins. Toxic broth cultures were streaked on *Clostridium botulinum* isolation agar (13), and colonies producing botulinum toxins were detected in a colony blot immunobassay using affinity-purified antibodies (14).

**Quantification of *C. botulinum* type E.** *C. botulinum* was enumerated in all positive sediment, shoreline, and terrestrial soil samples from the Koksos River area and the coastal environment of Nunavik by using a 5-tube most probable number (MPN) procedure. Three 10-fold-decreasing amounts (30 g, 3 g, and 0.3 g) and five culture tubes for each amount, for a total of 15 tubes per environmental sample, were cultured in SPGYT broth. The toxicity of culture supernatants was determined by slot blot immunobassay (15) and confirmed with the mouse bioassay. To reduce the number of mice required, only one mouse and two mice were used, respectively, to confirm the positive and negative results obtained with the slot blot immunobassay.

**Field and laboratory quality controls.** A stock spore suspension composed of four laboratory type E strains (Gordon, E Russ, 8550, and Bennett) was prepared in sterile GPB broth and diluted to make a working suspension of 5 spores/μl, which was carried into the field. The stock suspension was plated in duplicate onto McClung Toabe agar (Bacto, Becton, Dickinson and Company), a nonselective medium (16), prior to the field survey, to verify its concentration. In the field, an inoculum of 500 spores was dispensed onto each control bacterial sponge (after a typical coastal rock was swabbed) and into the control shoreline soil samples, whereas each of the 2-liter seawater control samples was spiked with 2 × 10³ spores for a final concentration of 1 spore per ml. In addition, a separate stock suspension was prepared and used to spike samples of 100 ml of GPB with 10 spores when performing enrichment of seawater samples. Two inoculated GPB controls per batch of 18 seawater samples were filtered through a 0.22-μm nitrocellulose membrane and cultured with and without alcohol pretreatment according to the described procedure. Similarly, 15 intestinal and skin control samples were spiked with 10 spores and used as internal quality control samples.

**PFGE and fingerprint analysis.** DNA isolation, restriction digestion, and separation of DNA fragments by pulsed-field gel electrophoresis (PFGE) were performed as previously described (17). Comparison of DNA fragment patterns and cluster analysis were performed using BioNumerics version 3.5 software (Applied Maths, Kortrijk, Belgium). The level of similarity between DNA fragment patterns was estimated using the Dice coefficient, and cluster analysis of fingerprints was performed with the unweighted pair group method using arithmetic averages (UPGMA) method. Low fragment sizes (<23.1 kb) generated by Smal digestions were ignored in the analysis because they were generally much fainter and were present in most PFGE patterns of *C. botulinum* type E isolates. DNA fragment patterns showing similarity levels greater than 97% with less than one band difference were considered identical. Pair-
wise comparison of PFGE patterns was performed to determine the number of distinguishable genotypes of *C. botulinum* type E for each specimen, and only those genotypes were included in the cluster analysis.

**Statistical analysis.** Comparisons of counts between sediment samples collected from different regions of Nunavik or between different sampling areas of the Kokosak River were performed using the Mann-Whitney test (StatTools; Palisade Corporation, Ithaca, NY). The significance level was set at a P level of <0.05.

**RESULTS**

**Butchering sites.** Nunavik is a region of Quebec situated north of the 55th parallel. It is a vast territory of 507,000 km² bordered by Hudson Bay to the west, Hudson Strait to the north, and Ungava Bay and Labrador to the east (Fig. 1). Traditional fishing and hunting activities are practiced by native populations (primarily Inuit) of 14 isolated northern villages scattered along the Hudson Bay and Ungava Bay coasts. Overall, 49 butchering sites along the Nunavik coastline were visited for environmental sampling, when all surveys were combined (Fig. 1). A complete characterization of sites was performed during the survey in the fall of 2000, for which a minimum of two sites per village, for a total of 29 butchering sites, were investigated. Large and flat coastal rock characterized 72% of the investigated butchering sites. Gravel beaches composed of pebbles and cobbles were also used as butchering sites by the villages of Inukjuak, Puvirnituq, Salluit, and Aupaluk. Most of the shoreline soil collected in close proximity to the butchering sites was composed of sand and/or pebbles, except for three sites located in the southern part of Ungava Bay, where the samples were muddy in texture, indicating a higher water level and organic material. No butchering activities were observed during the survey, but the presence of marine mammal remains, such as oil and bones, was noted at a few sites. The tide level was high for 65% (11/17) of the butchering sites sampled along the coastline of Hudson Bay and Hudson Strait, while up to 80% (8/10) of the sites from Ungava Bay were sampled at low tide.

**Performance of enrichment broth cultures.** All cultures from positive-control seawater, shoreline soil, and coastal rock swab samples (*n* = 38) tested positive for *botulinum* neurotoxin, indicating that the spores remained viable following transport and storage from the field to the laboratory and that the enrichment broth techniques were sensitive and reliably detected 1 spore per ml in water samples. All cultures of the seal intestinal content samples that were spiked with *C. botulinum* type E spores tested positive for *botulinum* neurotoxin in the mouse bioassay. Two of 12 skin control samples spiked with 10 spores/g did not produce toxicity in mice. The lack of toxin production from these spiked positive-control samples could have been due to the presence of competing background flora.

**Coastal environment of Nunavik.** Estimations of the prevalence and concentrations of *C. botulinum* type E in the coastal environment of Nunavik were based on 35 coastal rock, 37 seawater, and 49 shoreline soil samples from 21 butchering sites. The shoreline of Ungava Bay had a high incidence of *C. botulinum* type E, with prevalences of 87.5% in shoreline soil samples, 28.8% in seawater samples, and 18.8% in coastal rock samples (Table 1). On Hudson Bay, 50.0% of shoreline soil samples and 9.1% of coastal rock samples were positive, and no seawater samples tested positive. *C. botulinum* type E was not detected from any samples collected along the coast of Hudson Strait. Figure 1 shows the distribution of positive shoreline soil samples along the Nunavik coastline. Of the 29 isolates of *C. botulinum* obtained along the coastal environment of Nunavik, 27 were positive for *C. botulinum* type E. One strain each of *C. botulinum* type A and type B were isolated from shoreline soil samples collected near the villages of Kuujjuaraapik (Hudson Bay) and Kangiqsualujjuaq (Ungava Bay), and these were considered the first two group I strains isolated in Nunavik. The concentration of *C. botulinum* type E was highly variable in shoreline soil samples. Figure 2 shows the results of a slot blot immunoassay used for the detection of BoNT E (BoNT/E) in MPN tubes. The median concentration of *C. botulinum* in shoreline soil samples from Ungava Bay, with a count of 23 *C. botulinum*/kg, was significantly higher than in samples from Hudson Bay, with a median count of 5/kg. The highest concentrations, ranging from 270 to 1,800 *C. botulinum*/kg, were found in four mud samples collected at the mouths of the Whale River and the Koksoak River. All five positive seawater samples were also collected from the mouths of these two rivers at low or rising tide.

Isolates obtained from the coastal environment were compared using PFGE. Cluster analysis of PFGE patterns was performed using only isolates yielding different Smal or XhoI pulsortypes within each sample. Figure 3 shows a dendrogram, based on XhoI PFGE patterns, of 53 isolates of *C. botulinum* from two peat bog, seven coastal rock, five seawater, six sediment, and 33 shoreline samples. A total of 44 different PFGE genotypes were generated from 53 isolates using Smal and XhoI pulsortypes, indicating the existence of a genetically diverse population of *C. botulinum* type E within the coastal environment of Nunavik. Although very distant geographically, some isolates from the Ungava Bay and Hudson Bay coasts, two regions separated by the Hudson Strait, yielded the same PFGE profile. The PFGE patterns of isolates SO304E2 and SO305E2 from Inukjuak (Hudson Bay coast) were indistinguishable from isolate SO321E1 from Kangirsuk (Ungava Bay coast). In addition, isolates SO303E1 (Umiujaq, Hudson Bay coast) and SO325E2 (Tasiujaq, Ungava Bay coast) yielded the same PFGE profiles. Comparison of PFGE patterns of isolates recovered from the same butchering site showed that a clonal genotype was isolated from different substrates in only one of three butchering sites (Fig. 3). Isolate SO329E1 was recovered from the shoreline soil collected near the butchering site at the mouth of the Koksoak River and was indistinguishable from the coastal rock isolate SP455456E2 from the same site, suggesting that *C. botulinum* type E present on the surface of coastal rocks may originate from the shoreline soil or sediment surrounding the butchering site.

**Koksoak River and terrestrial environment.** A total of 94% of the freshwater (15/16) and 100% of marine (9/9) sediments from Koksoak River (Fig. 4) contained *C. botulinum* type E (Table 2). Most terrestrial soil samples (10/11) collected within 25 to 100 m from small tributaries upstream of Kuujjuaq were also culture positive. The concentration of *C. botulinum* type E was highly variable, ranging from 5 to >5,400 organisms per kg of soil or sediment in samples from the Koksoak River environment. The median concentration in marine sediment of the river (270 spores/kg) was significantly higher than in freshwater sediment (35 spores/kg) but was not significantly higher than the levels found in terrestrial soil (90 spores/kg). The data indicate that *C. botulinum* type E is abundant and widely distributed in the aquatic environment of the river and can also be encountered in inland soils surrounding the tributaries (Fig. 4).

Of 100 isolates recovered from the 41 environmental samples, 73 were compared by cluster analysis following the removal of
Fig 1. Distribution of C. botulinum type E spores in shoreline soil collected in the proximity of butchering sites commonly used by hunters of Nunavik. The distribution of positive samples was predominantly located along the shorelines of Ungava Bay and southern Hudson Bay.

Negative shoreline soil
Positive shoreline soil

(Δ) Number of sampling sites > 1
TABLE 1 Prevalence and concentrations of *C. botulinum* type E spores in the coastal environment of Nunavik

<table>
<thead>
<tr>
<th>Region</th>
<th>% prevalence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Shoreline soil spore concn (MPN spores/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seawater</td>
<td>Coastal rocks</td>
</tr>
<tr>
<td>Hudson Bay</td>
<td>0 (0/11)</td>
<td>9.1 (1/11)</td>
</tr>
<tr>
<td>Hudson Strait</td>
<td>0 (0/8)</td>
<td>0 (0/8)</td>
</tr>
<tr>
<td>Ungava Bay</td>
<td>27.8 (5/18)</td>
<td>18.8 (3/16)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Prevalences are reported as percentages, with the number of positive samples per total number of samples shown in parentheses.<br>
<sup>b</sup> Includes two shoreline soil samples containing strains of *C. botulinum* group 1, a type A strain isolated from a shoreline soil from Kuujjuaraapik (Hudson Bay), and a type B strain from Kangiqsualujjuaq (Ungava Bay).<br>
<sup>c</sup> ND, not determined.

identical genotypes originating from a common sample. Twenty-nine SmaI and 42 XhoI different PFGE fragment patterns were produced, generating 55 different genotypes of *C. botulinum* type E, indicating the presence of a heterogeneous population of *C. botulinum* type E in the Koksoak River area (Fig. 5). The largest cluster contained isolates bearing the same PFGE profile that were common to terrestrial soil, freshwater sediment, and marine sediment. Comparison of all isolates from the coastal environment and the Koksoak River environment revealed two clusters containing isolates common to both environments (data not shown). One cluster contained isolates from terrestrial soil from a tributary to the Koksoak River, freshwater sediment of the Koksoak River, shoreline soil from one of the Tasiujaq islands (Ungava Bay), and coastal rock near Puvirnituq (Hudson Bay).

Data for harvested seals from Nunavik. A total of 90 intestinal contents and 72 abdominal skin samples were collected from individual ringed seals and bearded seals harvested in the Ungava Bay (*n* = 28), Hudson Strait (*n* = 38), and Hudson Bay (*n* = 27) and cultured for *C. botulinum*. Most of the samples were from harvested ringed seals. Only 8.3% (6/72) and 10% (9/90) of skin and intestinal samples, respectively, originated from bearded seals. Overall, the prevalence of *C. botulinum* in intestinal tracts and skin samples was very low, with only 4.4% (4/90) and 1.4% (1/72) of samples, respectively, testing positive (Table 3). Two cultures of seal intestinal contents contained isolates producing BoNT/A and BoNT/E within the same sample, while the others were positive only for BoNT/E. The type A isolates were recovered from a bearded seal and a ringed seal harvested near the villages of Kuujjuaq (Ungava Bay) and Kangiqsualujjuaq (Hudson Strait), respectively. The single positive skin sample of a ringed seal harvested in Inukjuak (Hudson Bay) contained *C. botulinum* type B. These data indicate that *C. botulinum* serotypes A and B may be encountered in the environment and carried by seals along the Hudson and Ungava coasts of Nunavik.

**DISCUSSION**

Beach shores used as butchering sites for marine mammals have been identified as potential sources for food contamination with *C. botulinum* (7). Inuit hunters of Nunavik usually butcher seals near the hunting area on flat coastal rocks, but in other cases, hunters prefer to use specific sites for postharvesting activities. Hunters from Umiujaq prefer to butcher seals on the shoreline of small bays of the Richmond Gulf along the eastern shore of Hudson Bay, while hunters from Puvirnituq and Aupaluk butcher seals on a beach close to their villages. The use of gravel beaches was also observed in Inukjuak and Salluit. These sites provide ample opportunities for contact with sediment particles present on the shore when harvested meat is placed on the ground during butchering.

Of the 30 positive environmental samples collected along the Nunavik coastline, 22 (73.3%) were from the Ungava Bay region. Approximately 90% of the outbreaks of botulism in Nunavik between 1985 and 2005 (D. Leclair and J. W. Austin, unpublished data) occurred in communities of southern Ungava Bay, i.e., Tasiujaq, Kuujjuaq, and Kangiqsualujjuaq, where the concentrations of *C. botulinum* type E in shoreline soil reached levels up to 1,800 spores/kg. The geographic distribution in the coastline environment followed the spatial distribution of botulism outbreaks in Nunavik, suggesting that the environment plays a significant role in the epidemiology of the disease. In Alaska, the presence of *C. botulinum* type E was also demonstrated along the coastline of the villages where botulism outbreaks were reported (6). Interestingly, two shoreline soil samples, two intestinal tract samples, and one skin sample from seals harvested in different geographical regions of Nunavik harbored *C. botulinum* types A and B. These serotypes have been previously isolated in sediments of the Canadian Atlantic seaboard (18) and in soil from several provinces in Canada (19). In the Arctic, most of the outbreaks have been associated with type E botulism, but in Alaska, on several occasions, outbreaks have been attributed to types A and type B (20, 21).

The deposition of organic waste, such as the butchering of marine mammals on the beach, and effluents discharged from crab, fish, or shrimp canneries have been suggested to be possible sources of *C. botulinum* type E in the coastline environment of Alaska (6). The intestinal contents of marine mammals were also identified as a potential source of contamination of the butchering sites following the finding of *C. botulinum* type E in the colonic contents of 1 beluga whale out of 44 marine mammals tested (6). The prevalence of *C. botulinum* in the intestinal contents of 90

![FIG 2 Slot blot detection of BoNT/E in culture supernatants of environmental samples. Two sets of 5-tube MPN samples using three-decimal dilutions of freshwater sediments from tributaries (KR-18 and KR-22) of the Koksoak River were analyzed. Culture supernatants were diluted 1:10 in GPB and blotted onto a polyvinylidene difluoride membrane. (A) Columns 1 to 11 contained 2-fold serial dilutions of BoNT/E internal standard, from 512 to 0.5 mouse lethal doses (MLD), and column 12 contained GPB. (B) Columns 1 to 5, five 0.3-g KR-18 samples; columns 7 to 11, five 0.3-g KR-22 samples. (C) Columns 1 to 5, five 3-g KR-18 samples; columns 7 to 11, five 3-g KR-22 samples. (D) Columns 1 to 5, five 30-g KR-18 samples; columns 7 to 11, five 30-g KR-22 samples.](http://aem.asm.org/ on June 24, 2017 by guest)
seals harvested in Nunavik was also low, with only 1 bearded seal and 3 ringed seals carrying the organism. The prevalence on the surface of abdominal skin samples was even lower, at 1.4%, but this prevalence was likely underestimated, as the total surface area cultured was relatively small, being on average 136.5 ± 58.7 cm². In addition, some of the spiked skin samples used as quality control samples were culture negative, indicating that the growth of *C. botulinum* type E may have been inhibited by the background flora of the skin. Thus, the risk of contamination of seal meat with *C. botulinum* from these animal sources appears lower than that from the shoreline environment.

The presence of *C. botulinum* along the coast could result not only from the butchering of fish and marine mammals on the shore, but also from the passive accumulation of spores from the drainage of the watershed. The origin of *C. botulinum* type E in the coastal environment of Nunavik was investigated by sampling the Koksoak River, a large river flowing into the southern part of Ungava Bay, where the highest *C. botulinum* concentrations were detected along the Nunavik coastline. Up to 96% of sediment samples collected over the entire length of the river, which included several tributaries upstream and downstream of Kuujjuaq, contained *C. botulinum* type E. The highest concentration was measured in the marine sediment of the river, with more than 5,400 spores per kg found in one sampling site. These high levels may result from a higher deposition rate in the marine portion of the river, rather than from an active multiplication of a resident population of *C. botulinum* type E. The low water temperature of the Koksoak River during the summer (July, 5°C) and the short warm seasons in the Arctic regions, likely would not support active multiplication of the organism in sediments. Huss (5) suggested that the presence of spores in the Arctic zones was the result of the decomposition of stranded marine mammals on the shoreline that allowed for the growth of *C. botulinum* and its release in high numbers into the environment. However, the finding of stranded or dead marine mammals is not common in Nunavik, and such incidents would not explain the geographic distribution pattern of *C. botulinum* type E observed in this region.

*C. botulinum* type E may originate from the large southern watershed, where the organism may grow in decaying organic material. The spores may flow from the headwaters through the tributaries and rivers, accumulate downstream in cold waters, and disperse along the coast by current and tide effects. While an extensive amount of genetic diversity was indicated by PFGE analysis, isolates from the Koksoak River with the same PFGE profile

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**FIG 3** Dendrogram showing the relationship between 53 *C. botulinum* type E isolates from the coastal environment of Nunavik, based on their XhoI macrorestriction fragment patterns.
were common to terrestrial soil, freshwater sediment, and marine sediment. The concentrations of *C. botulinum* type E in terrestrial soil samples collected near the tributaries were not significantly different from those measured in marine sediments, suggesting that *C. botulinum* type E may be of terrestrial origin. The high prevalence of *C. botulinum* observed in terrestrial soils supports the terrigenous sedimentation hypothesis of Johannsen (22), who proposed that spores of *C. botulinum* type E are carried from large land masses by heavy rains into watercourses leading the organisms to the sea, rather than being a truly aquatic organism, as proposed by Bott and colleagues (23) and Huss (5). Whether the coastal type E strains were of terrestrial or aquatic origin is beyond the scope of this study, but our results indicate that the organism is widely distributed in the Koksoak River and its surrounding terrestrial soil and may reach the coastal environment either through runoff waters or the river flow, and thus contaminate the shoreline at high tide.

The influence of the freshwater environment was previously demonstrated by Craig and colleagues (8), who showed that salmon caught in the Columbia River in Oregon harbored *C. botulinum* more frequently in their viscera than did fish captured in ocean waters. The risk of contamination of seal meat would therefore be higher on butchering sites located along the river than those lining the mainland and the offshore islands away from freshwater sources. This was supported by the findings that the highest concentrations were found in shoreline soil samples collected from sites located at the mouths of large rivers, such as the Koksoak and Whale rivers.

**TABLE 2** Prevalence and concentrations of *C. botulinum* type E spores in the Koksoak River environment

<table>
<thead>
<tr>
<th>Area</th>
<th>% prevalence*</th>
<th>MPN spores per kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine sediment</td>
<td>100 (9/9)</td>
<td>270</td>
</tr>
<tr>
<td>Freshwater sediment</td>
<td>94 (15/16)</td>
<td>35</td>
</tr>
<tr>
<td>Terrestrial soil</td>
<td>92 (11/12)</td>
<td>90</td>
</tr>
</tbody>
</table>

*The percent prevalence is shown, with the number of positive samples per total number of samples in parentheses.*
FIG 5 Dendrogram showing the relationship between 73 C. botulinum type E isolates from the Koksoak River based on XhoI macrorestriction fragment patterns. Isolates were obtained from water, sediment, and shoreline soils.
In summary, the risk of contamination of marine mammal meat from environmental sources of *C. botulinum* type E during butchering is higher for animals that are butchered along the southern Ungava Bay coast, particularly near the mouths of large rivers, due to the high levels in these areas. Although much less prevalent, the endogenous spores from the seal itself, either from the skin or the intestinal contents, may pose a risk for contamination of seal meat if handling precautions are not followed. Thus, multiple sources of *C. botulinum* may be involved in the contamination of marine mammals during butchering.

**ACKNOWLEDGMENTS**

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We thank Bigitte Cadieux and Greg Sanders for their technical support in performing slot blot immunoassays and seroneutralization tests, respectively. We are also grateful to Peter May, who collected environmental samples from the freshwater portion of the Koksoak River, and to all hunters from Nunavik who participated in the seal sampling program. Finally, we thank Adam Lewis of the Makivik Corporation and Bruce Craig from the CFIA for producing maps on the distribution of *C. botulinum* type E along the Nunavik coastline and the Koksoak River.

**REFERENCES**