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Authors: Jores, Joerg, Derocher, Andrew E., Staubach, Christoph, and Aschfalk, Ansgar

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Occurrence and Prevalence of *Clostridium perfringens* in Polar Bears from Svalbard, Norway

Joerg Jores,[1,5,7] **Andrew E. Derocher,**[2,6] **Christoph Staubach,**[3] **and Ansgar Aschfalk**[3,4] [1] Institut für Mikrobiologie und Tierseuchen, Freie Universität Berlin, Philippstrasse 13, 10115 Berlin, Germany; [2] Norwegian Polar Institute, Polarmiljøsenteret, 9296 Tromsø, Norway; [3] Federal Research Institute for Animal Health, Institute of Epidemiology, SeestraBe 55, 16868 Wusterhausen, Germany; [4] Institute of Food Safety and Environment, Section for Arctic Veterinary Medicine, Stakkevollveien 23b, 9292 Tromsø, Norway; [5] Current address: International Livestock Research Institute, PO Box 30709, Nairobi 00100, Kenya; [6] Current address: Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada; [7] Corresponding author (email: jores@web.de)

ABSTRACT: To obtain insight into the occurrence and prevalence of *Clostridium perfringens* and its major toxins in polar bears (*Ursus maritimus*), we took fecal samples for bacteriologic analysis from live-captured bears in the Svalbard Archipelago, Norway, in 2001. *Clostridium perfringens* was isolated from 40 of 92 samples (44%). Thirty strains were further characterized by determining toxin type and were classified to be type A, while one was also positive for the gene encoding β2-toxin. Despite the fact that *C. perfringens* type A has been associated with fatal diseases in several animal species as well as in humans, our data indicate that *C. perfringens* type A is an normal inhabitant of the gastrointestinal tract of polar bears.

Key words: β2-toxin, *Clostridium perfringens*, polar bear, prevalence, toxin type A, *Ursus maritimus*.

Clostridium perfringens is a normal inhabitant of the gastrointestinal tract of many warm-blooded animal species, as well as humans, and it has been frequently isolated from soil. However, *C. perfringens* is associated with the ability to cause gas gangrene, food poisoning in humans, and myositis, enterotoxemic, and diarrheagenic diseases (Hirsh and Biberstein, 2005). This dichotomy of disease patterns can be partly explained by different settings of virulence traits within *C. perfringens*. Additionally, host factors, such as shifts in microbial environments due to altered diets or anaerobic conditions at wounds, can play a crucial role in the development of disease. Numerous toxins have been reported in *C. perfringens*, and, according to the expression of its four major toxins (α, β, ε, and ι), *C. perfringens* can be grouped into five toxin types A–E (Hirsh and Biberstein, 2005).

There is very little information available on the occurrence and impact of this important bacterium in Arctic animals. To date, there are no reports on this bacterium in free-ranging polar bears (*Ursus maritimus*). Furthermore, there are no reports on the pathogen's involvement in causing disease of any free-ranging bear species. However, fatal clostridial myonecrosis caused by other *Clostridium* species (*Clostridium septicum*, *Clostridium chauvoie*, and *Clostridium novyi*) has been reported in free-ranging black bear (*Ursus americanus*) (Barnes and Rogers, 1980). In addition, antibiotic-associated colitis due to *Clostridium difficile* has been reported in a captive Kodiak bear (*Ursus arctos*; Orchard et al., 1983), and, very recently, two fatal cases of enterotoxemia in two captured Asiatic black bears (*Selenarctos thibetanus*) due to type A strains have been described (Greco et al., 2005). In order to obtain more insight into the bacterial flora of the gastrointestinal tract of free-ranging polar bears, we investigated the occurrence and toxin type of *C. perfringens* in fecal samples.

Fecal samples from 92 wild, free-ranging, and clinically healthy polar bears of both sexes ($n_{\text{females}}=37$; $n_{\text{males}}=55$) from Svalbard (under Norwegian administration) were collected in 2001 (Fig. 1). Three of the animals were younger than 1 yr. All bears had been immobilized for

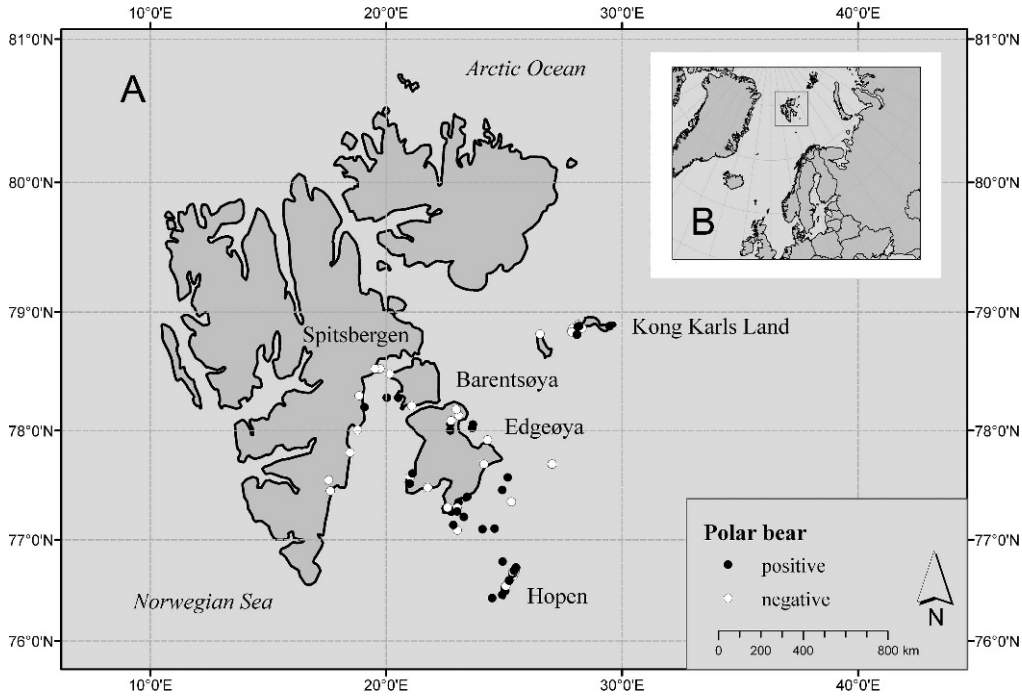

FIGURE 1. A. Map of Svalbard displaying the sites where the polar bears were immobilized for sampling. Black and white dots indicate the site of *Clostridium perfringens* positive and negative polar bears, respectively. B. Svalbard in relation to the European continent.

other scientific purposes and were handled using standard capture methodology (Stirling et al., 1989) in accordance with approved methods consistent with humane animal-handling protocols. A global positioning system (GPS) unit was used to determine the coordinates where the polar bears were immobilized.

Fecal specimens were collected from the rectum of the immobilized bears with cotton swabs and were kept cool (4 C) until processed. Specimens (about 1 g) were cultured anaerobically (AnaeroGen, Oxoid Ltd., Basingstoke, England) at 40 C for 24 hr in 10 ml cook meat medium (Oxoid Ltd.). The crude cultures were subcultured onto blood-glucose agar (Blood agar base, Merck GmbH, Darmstadt, Germany) containing 5% sheep blood and 1% glucose (Merck). After 24 hr at 40 C, colonies with typical growth characteristics were subcultured and identified further by biochemical means. Isolates were stored at −80 C in glycerol stock until further processed. For toxin type determination, isolates were grown anaerobically in brain heart infusion broth (Oxoid Ltd.) for 12 hr at 37 C, and 300 μl of culture were mixed with 700 μl aqua bidest and incubated at 100 C for 10 min. After centrifugation at 16,000 × G for 10 min, DNA was extracted from the supernatant using standard protocols (Sambrook and Russell, 2001). The same procedure was applied for the following *C. perfringens* toxin reference strains: ATCC13124 (type A), ATCC3626 (type B), NCTC3180 (type C), ATCC3628 (type D), ATCC27914 (type E), and E484/97 (type A plus toxin β2).

Genes for toxins α, β, ε, ι, and enterotoxin were detected using previously described primers (Meer and Songer, 1997). The gene encoding β2-toxin was detected using primers Beta 2/1 (GAAAG GTAATGGAGAATTATCTTAATGC) and Beta 2/2 (GCAGAATCAGGATTTTGAC-CATATACC). For polymerase chain reaction (PCR) analyses, 100 ng of *C.*

perfringens genomic DNA was used in a total volume of 50 μl employing a Perkin Elmer 2400. A relational database was created that included *C. perfringens* isolation results with the geographic position of each animal. The geographic information systems (GIS) analysis was performed using ArcGIS ArcView 9.1 software (ESRI; Redlands, California, USA).

Clostridium perfringens was isolated from 40 of 92 fecal samples, reflecting a total prevalence of 44%. Fourteen female (38%) and 26 male (47%) polar bears were carriers of *C. perfringens*. Interestingly, one of the three cubs investigated was a positive carrier as well. The relatively low overall prevalence is noteworthy, since *C. perfringens* has been reported to be ubiquitous in many warm-blooded animals.

The gene encoding for α-toxin was present in all thirty strains that were tested with the multiplex PCR; none of the strains harbored genes encoding for other major toxins. Toxin type A represents the most common toxin type for *C. perfringens*, and isolates have been reported in various diseased and healthy animal species as well as from the environment (Hirsh and Biberstein, 2005). The gene encoding the β2-toxin was detected in a single isolate of a 10-yr-old female polar bear, while the gene encoding enterotoxin was not found in any of the 30 isolates. The extent to which type A strains (with or without the β2 gene) pose a risk to polar bears is not known, but there are no reports of *C. perfringens*–associated diseases in any free-ranging bear species. This and the fact that all of these isolates were from clinically healthy animals, suggests that type A strains of *C. perfringens* belong to the normal gastrointestinal flora of polar bears.

Since polar bears migrate over very long distances (Mauritzen et al., 1002), they are exposed to a varied diet. This diet primarily consists of seals (Derocher et al., 2002), and it is possible that *C. perfringens* from this source could colonize the intestine of the bears through the food chain. This is supported by the fact that hooded seals (*Cystophora cristata*) in the Greenland Sea have been found to be carriers of *C. perfringens* (Aschfalk and Muller, 2001). Such a dietary source, however, would not explain the detection of *C. perfringens* from the single unweaned bear, and, in this case, an environmental source is suggested. The predominance of toxin type A *C. perfringens* isolates from polar bears is interesting, and it is not known if this reflects the distribution of this toxin type in the diet and environment of these bears or if it reflects a host specificity (Petit et al., 1999). Further research is needed to further define the microbial (pathogenic) flora of polar bears in order to define the interactions of this endangered species with its surrounding microorganisms.

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