Science of the Total Environment 408 (2010) 5801–5807

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Trans-generational and neonatal humoral immune responses in West Greenland sledge dogs (*Canis familiaris*) exposed to organohalogenated environmental contaminants

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ARTICLE INFO

Article history:
Received 17 January 2010
Received in revised form 19 July 2010
Accepted 30 July 2010
Available online 15 September 2010

Keywords:
Balaenoptera acutorostrata
Canis familiaris
Humoral immunity
Minke whale
Organohalogen contaminants
PCB
Sledge dogs
Trans-generational study
West Greenland

ABSTRACT

Previous investigations in the Arctic have suggested OHC (organohalogen contaminant) induced immune toxic effects on *e.g.* polar bears (*Ursus maritimus*). We therefore studied the dietary impact from minke whale blubber (*Balaenoptera acutorostrata*), rich in polyunsaturated fatty acids and OHCs, on the humoral immunity of 7 captive West Greenland sledge dog (*Canis familiaris*) bitches and their 4 pups constituting a sentinel model species for polar bears. A control group was composed of 8 bitches and their 5 pups all fed pork (*Suis scrofa*) fat. The study included serum IgG measurements (bitches and pups) and specific immune responses towards tetanus toxoid (bitches) and diphtheria toxoid (pups) as well as influenza virus (pups). The analyses showed that IgG concentrations were non-significantly lowest in exposed bitches and pups (t-test: all $p>0.05$). In addition, significant lower antibody response was detected in exposed pups immunized with influenza virus at age 3 months (t-test: both $p<0.05$). No clear group differences were found for tetanus toxoid in bitches and diphtheria toxoid in pups. The results suggest that the humoral immune system of sledge dogs may be suppressed by the dietary blubber composition of OHCs and polyunsaturated fatty acids while a larger follow-up study is recommended in order to investigate this relationship further.

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1. Introduction

Exposure to environmental organohalogen contaminants has in mammals been associated with a range of adverse effects on the immune system that is recognized as being particularly sensitive especially in its pre- and neonatal developmental phases, and may lead to irreversible dysfunction also in humans (Carpenter, 1998; DeRosa et al., 1998; Dewailly et al., 2000; Holladay and Smialowicz, 2000; Lyche et al., 2004, 2006; McFarland and Clarke, 1989; Morein et al., 2002; Mos et al., 2006; Safe, 1994; Thomas and Hindsdill, 1978; Tryphonas, 1994; Vos and Luster, 1989). Despite most of these studies have been conducted on laboratory animals, comprehensive *in vivo* studies of harbour seals (*Phoca vitulina*) fed contaminated Baltic fish have shown that OHCs at environmental concentrations suppress antibody response and cell-mediated immunity (De Swart et al., 1994, 1995; Ross et al., 1995, 1996). This is supported by mitogen-induced lymphocyte response and IgG challenges in other OHC contaminated marine mammals including bottlenose dolphins (*Tursiops truncatus*) (Lahvis et al., 1995), striped dolphins (*Stenella coeruleoalba*), harbour seal (Troisi et al., 2001; Mos et al., 2006) and in the St. Lawrence beluga whale (*Delphinapterus leucas*) (Martineau et al., 1994; De Guise et al., 1995, 1998).

Due to marine and atmospheric long-range transport, numerous OHCs bioaccumulate preferentially in adipose tissue of Arctic organisms and subsequently polar bears (*Ursus maritimus*) eating blubber from mainly ringed seals (*Phoca hispida*) and bearded seals (*Erignathus barbatus*) are among the most OHC polluted species (Norheim et al., 1992; Bernhoft et al., 1997; Norstrom et al., 1998; Dietz et al., 2004, 2007; Smithwick et al., 2005; Verreault et al., 2005; Muir et al., 2006; Letcher et al., 2009, 2009). East Greenland and Svalbard subpopulations hold the highest concentrations in the Arctic and several studies report on the immune toxic properties of OHCs in Svalbard polar bears that predate on the marine environment (Bernhoft et al., 2000; Skaare et al., 2001; Lie et al., 2004, 2005). For example, serum immunoglobulin G (IgG) concentrations, antibody

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doi:10.1016/j.scitotenv.2010.07.076

formation and cellular immunity seem to be impaired with consequences for disease resistance not least because OHCs also may interfere with the endocrine systems of Svalbard polar bears (Skaare et al., 2001; Haave et al., 2003; Oskam et al., 2003; Braathen et al., 2004; Oskam et al., 2004).

In controlled studies of captive West Greenland sledge dogs (*Canis familiaris*), used as sentinels for polar bears, it has previously been shown that oral OHC exposure causes an impairment of the innate and cellular immune systems (Sonne et al., 2006, 2007). As OHC exposure of pregnant bitches may impair the development and maturation of the neonatal immune systems, as well as influencing maternal immunity, we decided in the present study to further investigate the humoral immunity of the West Greenland sledge dog cohort. The study reports the effects of dietary minke whale blubber (*Balaenoptera acutorostrata*) on adult and neonatal humoral immune responses by assessing the levels of total serum IgG and specific antibody production following immunization mirroring the capacity for specific immune responses to protect against lethal infections.

2. Experimental section

2.1. Design

The present study is a part of a larger study about effects of contaminants in minke whale blubber in West Greenland sledge dogs previously described by Sonne et al. (2006). The experimental design was conducted as a randomized blind intervention study on West Greenland sledge dogs in Aasiaat, Disco Bay in West Greenland (Fig. 1) using real life exposure as described by Sonne et al. (2006). Briefly, the parent generation (P) was composed of an exposed (EXP, n = 7) and a control (CON, n = 8) group and their 9 pups (4 EXP and 5 CON; all sisters and brothers) (Table S1). The exposed group was fed 50–200 g/day of West Greenland minke whale blubber with significant OHC concentrations and rich in vitamin A/D and polyunsaturated lipids, while the control group was fed pork (*Suis scrofa*) fat (significantly lower in OHCs, vitamin D/A and polyunsaturated fatty acids) (Table S2). Bitches were fed exposed and control lipid source, respectively, immediately after entering the project at an age of 2 months. Pups were exposed to OHCs *in uteri* and via lactation transfer earlier in life and they were likewise fed exposed and control lipid source, respectively, immediately after weaning. The exposed group was fed minke whale blubber while the controls were fed pork fat, and both groups had a mean intake of 112 g/day. Both groups were likewise supplemented with standardized Royal Canin Energy 4300/4800 diets (http://www.royalcanin.com/) to compensate for differences in fatty acid, vitamin and microelement composition (e.g. iron, zinc and selenium) between the minke whale blubber- and pork fat-based diets (Sonne et al., 2006), and to cover the essential nutritional needs of these regularly exercised dogs inhabiting the cold Arctic climate (Hill, 1998). Due to field logistics all humoral immunological analyses were based on serum samples taken during the nearly 2 year period and subsequently shipped frozen from West Greenland as one batch for further analyses at the Food Safety and Infection Biology laboratory, The Norwegian School of Veterinary Science, Oslo, Norway. The animal experiments were performed on a licence granted by the Self Government of Greenland and The Kingdom of Denmark.

2.2. Chemical analyses

Results from the chemical analyses are given in Table 2 and in Supporting information. Analysis of organohalogen contaminants (organochlorines and several brominated flame retardants) in blood plasma was conducted at the Letcher Research Laboratory at the National Wildlife Research Centre, Carleton University, Ottawa, Canada, according to methods described in detail elsewhere (Gebbink et al., 2008; Verreault et al., 2008; Gauthier et al., 2009). The sum of 59 PCB (Σ_{59}PCB) congeners in blood plasma was based on the sum of the following 59 congeners (CB16/32, 17, 18, 22, 31/28, 33/20, 42, 44,

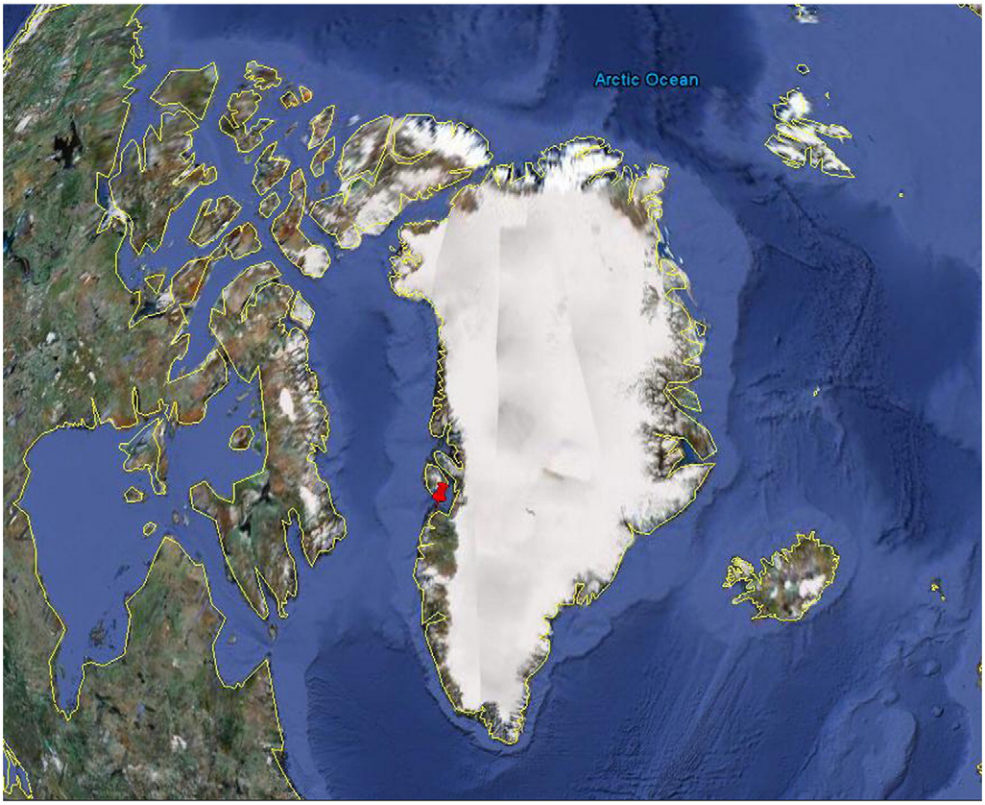

Fig. 1. Location of Aasiaat (Egedesminde; 68°42′N. 52°50′W) in West Greenland where the sledge dog study was conducted during year 2004–2005. Modified from www.google.com.

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