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First health and pollution study on harbor seals (*Phoca vitulina*) living in the German Elbe estuary

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ABSTRACT

The Elbe is one of the major rivers releasing pollutants into the coastal areas of the German North Sea. Its estuary represents the habitat of a small population of harbor seals (*Phoca vitulina*). Only little is known about the health status and contamination levels of these seals. Therefore, a first-ever seal catch was organized next to the islands of Neuwerk and Scharhörn in the region of the Hamburg Wadden Sea National Park. The investigations included a broad set of health parameters and the analysis of metals and organic pollutants in blood samples. Compared to animals of other Wadden Sea areas, the seals showed higher γ-globulin levels, suggesting higher concentrations of pathogens in this near-urban area, elevated concentrations for several metals in particular for V, Sn, Pb, and Sr, and comparable ranges for chlorinated organic contaminants, except for elevated levels of hexachlorobenzene, which indicates characteristic inputs from the Elbe.

1. Introduction

Due to their role as top predators within the marine food web, marine mammals such as harbor seals (*Phoca vitulina*) can be used as indicators for ecosystem change (The Trilateral Wadden Sea Cooperation, 2010). Increasing commercial use, e.g., fisheries and offshore wind parks, as well as ongoing inputs of pollutants strongly influence the North and Baltic Sea ecosystems. The German states Schleswig–Holstein, Hamburg and Lower Saxony have declared their Wadden Sea areas as National Parks. The Hamburg Wadden Sea area includes also parts of the Elbe estuary, where harbor seals play an important role for the regional tourism.

The harbor seal population in the Elbe estuary is relatively small in comparison to other populations that can be found along the Wadden Sea coast line. In one of the latest aerial surveys conducted in 2008, on an average 427 animals were counted in the area of the Hamburg Wadden Sea (Hellwig and Krüger-Hellwig, 2008). Most animals (371) were present on the western haul out sites "Robbenplate" and "Wittsandloch". Fifty-six animals were counted on the eastern haul out site "Hundebalje". Beside regular aerial surveys since 2002, no further investigations, e.g., of the health status of these animals, have been carried out.

In 2002, the phocine distemper virus (PDV) epizootic reduced the harbor seal population to 50% in this and other areas of the Wadden Sea (Reijnders et al., 2005). Since the epidemic impact, the seal population of the Hamburg Wadden Sea area has grown continuously. However, the size of the population has not yet reached its original size before the virus outbreak (Hellwig and Krüger-Hellwig, 2008).

Whether environmental pollution-related immunosuppression might have contributed to the severity and extent of morbillivirus-caused mass mortalities among marine mammals is still under discussion (Härkönen et al., 2006; Ross, 2002). However, several studies have shown a relationship between contaminant body burdens and immunological dysfunctions (Beckmen, 1999; De Guise et al., 2006; De Swart et al., 1994; Kakuschke and Prange, 2007). Despite partly decreasing inputs of contaminants into the North Sea, the Elbe River is still the primary contributor to the contamination of its estuary and of the German Bight (Loewe et al., 2006).

Several studies concerning the health status (Hasselmeier et al., 2008; Kakuschke et al., 2010; Siebert et al., 2007) and/or

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contaminant body burdens (Ahrens et al., 2009; Griesel et al., 2008; Weijs et al., 2009) of harbor seals were conducted in the Wadden Sea. To our knowledge, we report for the first time results for seals of the Elbe estuary. Our investigation included a common set of health parameters and pollutants, applied in the studies mentioned above. In addition, a new method for the determination of transferrin (Tf) isoforms (established markers for specific disorders in humans) as a potential new biomarker for seals was applied.

2. Material and methods

2.1. Animals

The seal catch was carried out in the estuary of the river Elbe next to the islands of Neuwerk and Scharhörn in the area of the Hamburg Wadden Sea National Park (Germany) in October 2008 (Fig. 1).

The seal catch was coordinated from on board the GKSS research vessel "Ludwig Prandtl" and carried out with two Zodiac boats. Harbor seals were captured using a 120 m × 8 m net with a mesh size of 10 cm × 10 cm, adapted from a method described by Jeffries et al. (1993). Briefly, the net was spread out slowly between both Zodiac boats in a distance of around 100 m to the animals. Due to the low water depth, the net reached to the ground and the animals were not able to dive below the net. Both boats moved simultaneously towards the beach, trapping the seals within the net. After the landing of the two boats, the net was moved manually onto the shore line. The caught animals were removed from the net, transferred into tube nets, and restrained manually to assess length, weight, sex and age and to collect anal smears and blood samples. The handling for measurements and blood collection took 10–15 min for each seal. During the procedure the animals were continuously under observation of two veterinarians. After completing the investigations, the animals were released back into the wildlife. The time span between transferring all animals in tube nets and releasing back into the wildlife took 1 h.

Blood was collected into monovettes after puncture of the epidural vertebral vein using a 20 mL syringe and a 12 mm × 100 mm needle (TSK-Supra, TSK Laboratory, Japan). The tubes were carefully agitated and kept at room temperature until further sample processing. Most blood samples were processed within 1–12 h. Swabs taken from the anus were used for microbiological investigations.

During this catch five animals were caught and coded sequentially (Table 1). The age was estimated based on length and weight and the animals were grouped into seals <1 year, between 1 and 2 years, and >2 years.

2.2. Hematology

For hematology, EDTA monovettes (Sarstedt AG & Co., Nümbrecht, Germany) were used. A basic hematology profile (white blood cells [WBC], red blood cells [RBC], hemoglobin [HGB], hematocrit [HCT], mean cellular volume [MCV], mean cellular hemoglobin [MCH], mean cellular hemoglobin concentration [MCHC], thrombocytes, and reticulocytes), was analyzed at Synlab.vet Hamburg in Geesthacht, Germany, using a Sysmex XT – 2000 analyser (Sysmex Deutschland GmbH, Norderstedt, Deutschland). The leukocyte subgroups (neutrophiles, eosinophiles, lymphocytes, and monocytes) were counted manually.

2.3. Lymphocyte proliferation assay

The MELISA® (Memory Lymphocyte Immunostimulation Assay), a modification of the lymphocyte transformation test (LTT), was performed as previously described in the Laboratory Center

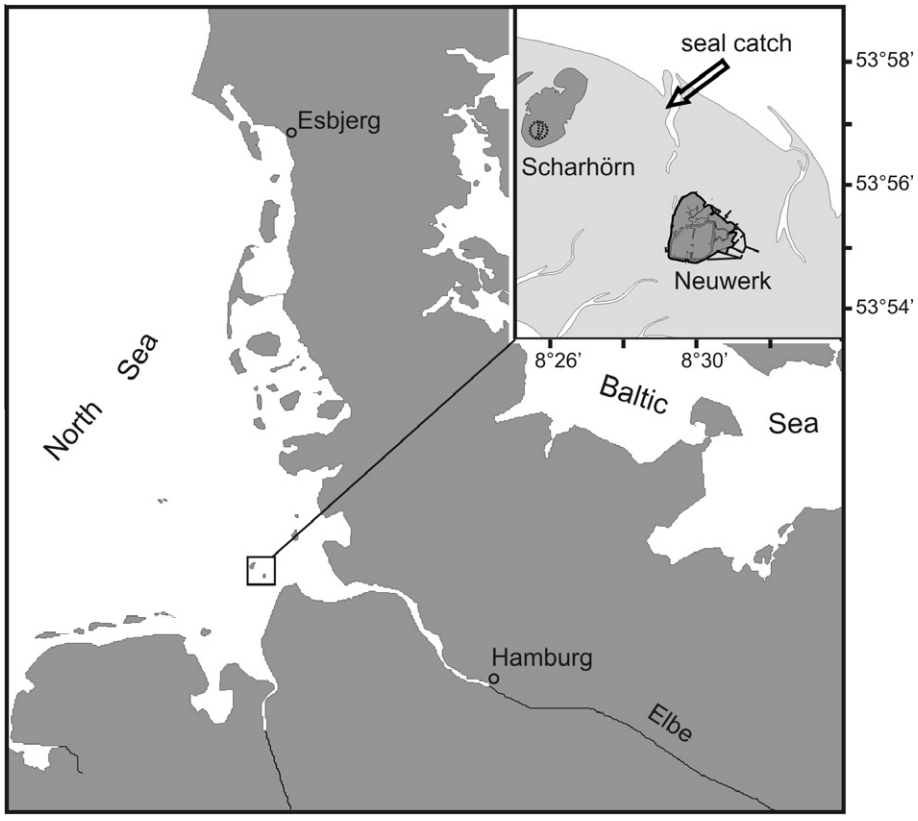

Fig. 1. Sampling location in the estuary of the river Elbe.