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Research paper

Immune status and function in harbor seal pups during the course of rehabilitation

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ABSTRACT

Routine hematological and serum chemistry parameters are important tools for the evaluation of health and the treatment of marine mammals admitted to rehabilitation centers. The evaluation of phagocytosis, oxidative burst and immunoglobulin G (IgG), as markers of immune system function, and haptoglobin (Hp), as a stress marker, were evaluated alongside routine hematology and chemistry as potentially informative diagnostic tools for marine mammal health assessments. Blood samples from harbor seal pups (*Phoca vitulina*) admitted to ($n=46$), and released from ($n=28$), the Vancouver Aquarium's Marine Mammal Rescue Center (VAMMRC) were collected (1) to perform routine and novel functional approaches to evaluate the health of pups at admission; (2) to determine how these parameters changed during the rehabilitation process; and (3) to generate baseline values for reference purposes. Sodium was the only blood parameter which differed between seal pups that survived and those that died, with the surviving pups exhibiting higher levels on admission diagnostics. Positive correlations between total protein concentrations, IgG and Hp levels were observed with globulin concentrations of seal pups. Changes in serum chemistry values between admission and release included a decrease in red blood cells (RBCs), glucose, bicarbonate, total bilirubin, γ-glutamyltransferase (GGT) levels, and an increase in mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), lymphocytes, eosinophils, urea, potassium, anion gap, calcium, phosphorus, total protein, albumin, globulin and osmolality levels. During the rehabilitation process, phagocytosis decreased, while Hp levels increased. Age and improved health appeared to underlie changes in these parameters during the rehabilitation period.

1. Introduction

Harbor seals (*Phoca vitulina richardsi*) inhabiting the coastal waters of British Columbia (BC), Canada, are characterized by late and extended pupping seasons (July–August) (Temte et al., 1991). Mean body mass of newborns is 11.2 ± 0.31 kg and the average daily mass gained during the nursing period is 394 ± 26 g (Cottrell et al., 2002). The mean weaning mass is estimated at 23.6 ± 1.2 kg at a mean weaning age of 32 ± 1.5 d (Cottrell et al., 2002). The harbor seal population in BC is estimated to be over 100,000, 37% of which is concentrated in the Strait of Georgia (Olesiuk, 1999). Between 1990 and 1998, a net productivity rate was estimated for the Georgia Strait population at 7.2% (Olesiuk, 1999). Harbor seal survival has primarily been estimated from mortality and life tables and juvenile survival seems to be high variable with pre-weaning survival ranging from 69% to 83% (Boulva and

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McLaren, 1979; Steiger et al., 1989) and post-weaning first year survival ranging from 35% to 80% (Bigg, 1969; Reijnders, 1978).

Approximately 150 orphaned, ill and/or injured neonatal harbor seals pups are brought to the Vancouver Aquarium Marine Mammal Rescue Center (VAMMRC) each summer, which represents a very small fraction of the annual production (Olesiuk, 1999). Pups admitted to VAMMRC are often emaciated, dehydrated, and have illnesses or injuries that require treatment. Upon admission, blood samples are collected from pups for diagnostic hematology and serum chemistry evaluation. Veterinary management of each patient is based on blood results, clinical signs and age.

Although hematology and serum chemistry variables are commonly used parameters for the clinical evaluation of disease and injury in pinnipeds (Bossart et al., 2001), immunological assessments and changes in acute phase protein can also be important (Dierauf and Gulland, 2001). Previous research on the rehabilitation of harbor seal pups has shown that blood values change as they undergo a variety of clinical and physiologic changes while in captivity (Lander et al., 2003). Since no clinical hematology variables are available for harbor seals in British Columbia, this study complements the availability of data for geographically and genetically disparate populations along the west coast of North America (Greig et al., 2010).

Routine hematology evaluates the cellular components of blood including cells involved in immune function. As suggested by Ross and De Guise (2007), the developmental immunology of marine mammals reflects a number of species-specific adaptations with respect to their habitat needs. Harbor seals, with their short period of maternal care, are born with a relatively competent immune system as demonstrated by strong lymphocyte proliferation and antibody responses (Ross et al., 1993, 1994) and competent phagocytic activity (Frouin et al., 2010). Maternal antibodies are mainly delivered to the newborn via colostrum, providing passive and temporary protection against pathogens during their early days (Ross et al., 1993, 1994). In mammals, failure to obtain colostrum by newborns is linked to increased mortality (Dyck and Swierstra, 1987; Coureaud et al., 2000).

Recently, it has been suggested that the determination of acute-phase proteins (APPs) be incorporated into routine clinical pathology evaluation (Petersen et al., 2004). Haptoglobin (Hp), one member of this group, increases markedly in response to infection, inflammation, trauma or tumors, in what is known as the acute phase reaction (Zenteno-Savin et al., 1997). Hp levels vary with several factors in marine mammals, including species-specific differences, and the influence of age, sex, and/or habitat quality (Zenteno-Savin et al., 1997; Beckmen et al., 2003; Mazzaro et al., 2004; Krafft et al., 2006; Thomton and Mellish, 2007).

The objectives of the present study were: (1) to combine routine and novel functional approaches to evaluate health of pups at admission; (2) to document changes to these parameters during the rehabilitation process; and (3) to provide baseline values for harbor seals in British Columbia.

Table 1
Visual assessment of the relative fat/muscle proportions at the admission.

Category	Description
Emaciated	Spinous processes, ribs, ischii visibly prominent. No fat palpable over lumbar vertebrae. Lack of muscle
Thin	Can feel ribs easily (may be slightly visible), can feel spinous processes easily
Moderate	Can feel spinous processes and ribs with pressure
Moderately fat	Can slightly feel ischii with pressure, spinous processes difficult to feel
Fat	Ischii difficult to feel, noticeable thickening of neck area

2. Materials and methods

2.1. Admission

Harbor seal pups for this study were stranded between July 27th, 2010 and September 25th, 2010 between Port McNeil (50°35′N, 127°06′W) and Victoria (48°24′N, 123°20′W), British Columbia (BC), Canada (Fig. 1). In 2010, a total of 160 pups were admitted to VAMMRC. Upon admission, an initial assessment included body mass to the nearest 0.1 kg, standard length and axillary girth to the nearest 1.0 cm, and in the absence of blubber-thickness measurements, a body condition score (BCS) expressed as axillary girth × 100/length (McLaren, 1958) was performed. Moreover, a visual assess confirmed the body condition score (Table 1).

Age was estimated from the state of pelage, tooth development, and umbilical regression, as well as body mass (Gulland et al., 1997; Cottrell et al., 2002). A condition index (CI) expressed as mass × 100/length (Lander et al., 2003) was calculated in addition to the body condition score (above) used during triage for most pups. Pups included in this study were categorized at admission as: moderately malnourished (as defined by CI ≥ 11.3), visibly injured or emaciated (as defined by CI < 11.3).

Blood samples were collected within 24 h of admission to MMRC for complete blood cell counts (CBC) and serum chemistry determined by IDEXX Laboratories (Delta, BC, Canada) using standard protocols. Blood samples were collected from the epidural vertebral vein of pups into Vacutainers containing ethylene-diamine-tetra-acetic acid (EDTA) or sodium heparin, as well as serum separation tubes with no additive (Becton Dickenson, New Jersey, USA) using a 20 gauge × 38 mm needle. Serum separation tubes were allowed to clot for 15 min before being centrifuged at 3500 rpm for 15 min. Plasma was separated using identical centrifugation protocols within 12 h and aliquoted samples were stored at −80 °C until assayed.

Complete blood cell counts consisted of differential counts of leukocytes (white blood cells [WBCs]), erythrocytes (red blood cells [RBCs]), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV; from which mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were derived), and platelets. Chemistry parameters measured included glucose, urea, creatinine, blood urea nitrogen/creatinine (BUN/Cr) ratio, sodium (Na), potassium (K), chloride (Cl), bicarbonate,