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Original investigation

Hematology and serum biochemistry reference ranges of healthy captive Tasmanian devils (*Sarcophilus harrisii*) and their association with age, gender and seasonal variation

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ABSTRACT

The Tasmanian devil (*Sarcophilus harrisii*) is the largest extant carnivorous marsupial. The Tasmanian devil is currently listed as endangered and is under threat from a contagious cancer. The aims of the study were to determine hematology and blood chemistry reference intervals for captive Tasmanian devils and determine the influence of three biological factors on blood variables. Hematology and blood chemistry data were analyzed retrospectively from medical reports obtained from Taronga Zoo. Samples were analyzed using current technology at the time of collection. Thirty seven variables were analyzed for 104 blood samples from 1992 until 2015. Data were statistically analyzed for differences between age, gender and season. Generally Tasmanian devils have higher serum concentrations of albumin (ALB) and creatinine and lower alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and amylase (AMY) compared with other dasyurids. Younger animals tended to have significantly higher serum concentrations of ALP, AST and phosphorus, while total protein and globulin activity in younger animals was less than in older animals. Hemoglobin, total protein and AST concentrations were influenced by season, with higher concentrations observed in either spring or summer. Lymphocyte, and erythrocyte counts, and serum concentrations of lipase and AMY were significantly higher in females compared with males. The reference ranges determined here can be used in the health assessment of captive Tasmanian devils and for those used in translocation programs in the future.

Introduction

The Tasmanian devil (*Sarcophilus harrisii*) is the largest extant carnivorous marsupial, ranging up to 10 kg body weight (Jones, 2008). Devils eat medium sized mammals, fish and birds (Jones, 2008; Pemberton et al., 2008). In the wild, the geographical range of the devil is currently restricted to the island state of Tasmania; however they were once more widespread across mainland Australia. In the past Tasmanian devils were persecuted for killing livestock and now they are under threat from a contagious cancer (Hawkins et al., 2006; McCallum et al., 2007). The Tasmanian devil population has declined by >60% and is listed as 'Endangered' on the IUCN Red List (Hawkins et al., 2008).

Current data available on hematology and blood chemistry of Tasmanian devils is limited, with gaps in the variables reported and small samples sizes (Bartels et al., 1966; Parsons et al., 1970; Nicol, 1982; Clark, 2004; Holz, 2008). Recently data have become available for wild devils (Peck et al., 2015). However, thus far these studies have shown that Tasmanian devils have notably high serum concentrations of acid phosphatase, phosphorous and urea (Parsons et al., 1970). Devils also have a significant proportion of alkali resistant hemoglobin compared with eutherian species (Parsons et al., 1970). Preliminary investigation of a lactating female shows they have lower calcium and alkaline phosphatase (ALP) compared to males, but in that study only one lactating female and two males were sampled (Parsons et al., 1970). Devils generally conform to the typical marsupial serum chemistry having high

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blood glucose and urea when compared to humans, but specific ranges are species dependent (Parsons et al., 1971).

Abnormal hematology and blood chemistry values can indicate poor health and disease affecting an individual. Baseline reference data is required for determining health and illness in individuals. At this stage suitable reference data is not available for captive Tasmanian devils, although there are reference intervals available for wild Tasmanian devils and other dasyurids (for example, Schmitt et al., 1989; Bradley, 1990; Haynes and Skidmore, 1991; Clark, 2004; Stannard et al., 2013; Peck et al., 2015). Blood variables can be influenced by age, season, gender, nutrition, and illness/disease, with the effects of these factors on blood variables varying from species to species (for example, Schmitt et al., 1989; Haynes and Skidmore, 1991; Bradley, 1990; Wells et al., 2000; Hall et al., 2007). Large populations of devils are being kept in captivity as part of the insurance population and this provides an opportunity to collect and develop baseline data from these animals as a preliminary step in monitoring translocated or reintroduced animals and can be used as a comparison for wild individuals.

The present study involved compiling hematology and blood chemistry data available for healthy captive Tasmanian devils from Taronga Zoo, with the aim of developing baseline reference ranges. Comparisons were made between gender, season and age to determine how these factors influenced the blood variables studied.

Material and methods

Animals and blood sample analysis

Records were collected from Taronga Zoo Medical Reports on their Tasmanian devils from 1992 to 2015 from captive-bred animals. Blood samples were taken as part of routine health checks, during quarantine or as part of an enquiry into clinical signs of illness. Results from clinically ill animals (based on veterinarian notes) were not included in the analysis. During blood collection animals were sedated with Isoflurane (IsoFlo, Abbott Australasia Pty Ltd., Botany NSW) and blood was collected from the saphenous, jugular, cephalic or tail vein. The blood collected was split between a serum separator tube for clinical chemistry (Becton Dickinson, North Ryde, NSW) and an ethylenediamineteraacetic (EDTA) tube for hematology analysis (Becton Dickinson, North Ryde, NSW). Tasmanian devils were maintained in outdoor enclosures and exposed to natural light cycles and temperatures at either Taronga Zoo (Mosman, NSW) or Taronga Western Plains Zoo (Dubbo, NSW). All devils were maintained on a carnivorous diet that included whole quail, chicken wings, kangaroo pieces, and gutted rabbit carcass.

Blood variables were measured with a Reflotron Instrument (Roche Diagnostics Ltd., Switzerland) and an IDEXX VetTest Chemistry Analyzer (Idexx Laboratories, Rydalmere) from 1992 till 2010. After November 2010 blood was analyzed in a VetScan (VS2) Chemical Analyzer (Abaxis, CA, USA). Taronga Western Plains Zoo samples were sent to Pathology West (Dubbo, NSW) for analysis. The hematology variables measured were erythrocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCV), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (HGB), hematocrit (HCT), total leucocyte count (WBC) and WBC differential using a IDEXX VetAutoread (Idexx Laboratories, Rydalmere). Biochemistry variables analyzed included: anion gap, concentrations of total lipase, gamma-glutamyl transferase (GGT), cholesterol, chloride, total carbon dioxide (Total CO_2), creatine kinase (CK), glucose, urea nitrogen (BUN), creatinine, calcium, phosphorus, sodium, potassium, magnesium total protein, albumin (ALB), globulin, alanine aminotransferase (ALT), total bilirubin, amylase (AMY), alkaline phosphatase (ALP), aspartate aminotransferase (AST).

Data analysis

Reference Value Advisor v.2.1 was used to determine reference intervals and 90% confidence intervals for hematology and biochemistry (Geffre et al., 2011). For all variables where >40 samples were available non-parametric test data were used to determine reference and confidence intervals from the Reference Value Advisor program, and box-cox transformed data was used for variables with ≤40 samples. The software tested normality using Anderson–Darling and Q–Q plots, and outliers using Dixon Reed and Tukeys' tests (Geffre et al., 2011). Outliers were removed prior to further statistical analysis. ANOVAs and Tukeys' posthoc tests were used to determine significant differences between genders, ages, and seasons. Employing SPSS, a mixed-model multivariate analysis was performed on data to account for age, gender and season where enough data were available and controlled for repeated measures. Variables were grouped into season based on the southern hemisphere, for example summer: December–February.

Results

In total 104 individual Tasmanian devil blood samples were available for analysis from March 1992 to June 2015. The samples came from 50 Tasmanian devils (28 male and 22 female). Results for reference intervals, medians and confidence intervals are presented for hematology in Table 1 and those for serum biochemistry are in Table 2.

Age

WBC were significantly ($F_{7,103} = 3.9$ $P < 0.01$) higher in 1 year olds compared with 3 and 4 year olds (Table 3). HCT was significantly ($F_{7,101} = 8.2$ $P < 0.01$) lower in 7 year olds compared to 1, 2, 3 and 4 year olds, in both males ($F_{6,57} = 8.3$ $P < 0.01$) and females ($F_{6,41} = 4.5$ $P < 0.01$). The youngest two age groups had lower neutrophils ($F_{7,103} = 7.5$ $P < 0.01$), and higher lymphocytes ($F_{7,103} = 6.9$ $P < 0.01$; Table 3) than the oldest two age groups.

Age influenced globulin, total protein, ALP, AST, and P activity. Total globulins were significantly lower in animals under one year of age compared to the other ages ($F_{7,91} = 3.7$ $P < 0.01$; Table 3). Globulins were significantly different in males, with animals under one year of age having significantly lower levels than 1, 3, 4 and 7 year olds ($F_{4,49} = 3.5$ $P < 0.05$). Some age groups had a small sample size and statistical significance could not be determined for all male age groups. Globulins were not significantly different with relation to age in females ($F_{7,40} = 1.4$ $P = 0.24$). Total protein was significantly lower in 7 year olds compared to 2, 3 and 4 year olds; and in under one year olds compared to 1, 2, 3 and 4 year olds ($F_{7,91} = 7.3$; $P < 0.01$). Animals under two years of age had significantly ($F_{7,93} = 25.4$ $P < 0.01$) higher ALP activity than animals aged two years and older (Table 3). Animals under 1 year of age had significantly ($F_{7,66} = 4.2$ $P < 0.01$) higher AST concentrations than 1 and 3 year olds, and 1 year olds had significantly lower concentrations than 7 year old animals (Table 3). Phosphorus concentrations were significantly higher in animals under one year of age ($F_{7,93} = 10.6$; $P < 0.01$) in both males ($F_{6,51} = 17.3$; $P < 0.01$) and females ($F_{5,38} = 5.5$; $P < 0.01$) compared to all other ages.

Season

Hemoglobin concentrations were significantly ($F_{3,96} = 3.5$ $P < 0.05$) higher in spring compared with summer and autumn (Table 4). When controlled for gender the males had significantly ($F_{3,55} = 4.2$ $P < 0.05$) higher mean HGB levels in spring (149.0 ± 19.1 g/L, $n = 29$) compared with autumn (130.4 ± 17.9 g/L, $n = 16$). Age of the males also affected HGB ($F_{6,54} = 4.5$ $P < 0.01$) with