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Preliminary Haematological and Blood Chemistry Values in Wedge-tailed Eagles

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Haematological and plasma chemistry reference values are unavailable for many Australian native birds, including the Wedge-tailed eagle (*Aquila audax*). Blood samples taken on an opportunistic basis from free-living birds undergoing rehabilitation at a local facility were used to establish a preliminary set of reference intervals. Data collection is ongoing, and these initial reference ranges are expected to be revised in the future. Increased sample numbers are expected to decrease the variability in values caused by age, sex, condition, season, nutritional, reproductive and health status.

Introduction

Haematological and plasma chemistry reference values are unavailable for many Australian native birds. For veterinarians treating wildlife, this means interpretation of laboratory results is often based on extrapolation from related or taxonomically distant species. Currently, most veterinary laboratories interpret a sample from a Wedge-tailed eagle (*Aquila audax*) based on established reference values for the Golden eagle (*Aquila chrysaetos*), whose six subspecies span the Northern Hemisphere. Reference ranges for the Golden eagle currently in use include those published by the International Species Information System (ISIS), Polo et al. (1992), Jennings (1996), and Nazifi et al. (2008). It is unclear how similar the reference values of these two species are, and therefore desirable that values for the Wedge-tailed eagle be established.

The Wedge-tailed eagle is Australia's largest raptor and one of the largest in the world. Like the Golden eagle, it is listed by CITES in Appendix II (http://checklist.cites.org/) and while the mainland subspecies (*Aquila audax audax*) is not currently threatened, the Tasmanian subspecies (*Aquila audax fleayi*), is considered endangered (Commonwealth of Australia Environment Protection and Biodiversity Protection Act 1999; Tasmanian Threatened Species Protection Act 1995; Austin et al., 2014).

The authors provide *pro bono* veterinary services to local wildlife care groups, including a local facility specialising in the care of Australian raptors (Higher Ground, run by Australian Raptor Care and Conservation Inc.), which receives birds from veterinary clinics, other wildlife care groups and members of the public throughout New South Wales and the Australian Capital Territory. On average, between 12 and 24 Wedge-tailed eagles are admitted to the facility each year, and veterinary assessment on admission to the facility includes, but is not limited to, full physical examination, radiographs, faecal analysis and blood sampling. Depending on the length of rehabilitation, further sampling may be indicated. This arrangement enables the acquisition and collation of a dataset which may be used to establish biochemical and haematological reference ranges for a multitude of native raptor species. Due to the consistency of the numbers of Wedge-tailed eagles presenting each year, this species was the first in which data has been examined.

Methods

Birds

In the period March 24th, 2015 to February 29th, 2016, a total of 13 Wedge-tailed eagles came into care and were assessed and treated at the South Highlands Veterinary Centre. Blood samples were taken from all at the time of admission, and serial blood samples were taken from four birds during their rehabilitation period. The birds ranged in age from fledgling to adult; four were successfully released and two are still in care. Six birds were euthanased either at the initial veterinary assessment

or during the rehabilitation process. The work was carried out under an Animal Research Authority issued by the Animal Care and Ethics Committee of the NSW Secretary of Trade and Investment, Department of Primary Industries.

Sample collection and analysis

All birds were anaesthetised with isofluorane and oxygen via face mask; blood was collected from the vena cutanea ulnaris superficialis with a 25G needle on a 3ml non-heparinized syringe into a 1ml green-topped lithium heparin tube; several smears were made directly from the collection syringe. Samples were immediately refrigerated and submitted to Vetnostics veterinary laboratories (North Ryde, NSW) via courier. Time from sample collection to analysis was less than 36 hours in all cases. Biochemical assays were run on the EVO Chemical Analyzer (Roche, Basel, Switzerland) except for bile acids, which used an assay from Vital Diagnostics (Lincoln, RI, USA)(Dr Doug Hayward, pers. comm., 11 June 2016). Haematocrit was performed manually, and total and differential leukocyte counts were obtained manually from the blood films. Haemoglobin and erythrocyte counts were not reported.

Calculation of reference intervals

Seventeen data sets were obtained over a 12-month period from 13 individuals. For four individuals, two consecutive data sets were obtained, at least five months apart. For the purposes of this calculation, each data set was considered as a separate entity, such that n=17. To account for variation in health and nutritional status at the time of sampling, extreme outliers were removed from the raw data prior to calculating mean, standard deviation and percentiles. Two samples were removed prior to calculating values relating to measurements of bile acids, as it was highly likely that the bird had consumed a meal in the preceding two hours. One sample was removed from the serum glucose data set due to the animal presenting with clinical signs of starvation (hypoglycaemia). Two samples were removed from the calculations for creatinine kinase, one from amino alanine transferase and one from calculations for aspartate amino transferase due to significant soft tissue injury. Five sets did not have manual haematocrit results due to insufficient sample remaining. Two samples were removed from calculations of total white cell count and heterophil differential due to leukaemoid reaction and florid heterophilia associated with severe inflammation.

There are several methods of reporting reference intervals from data collected from this type of population. Rümke and Bezemer (1972) recommended that as most biological variables do not follow Gaussian distribution, reference ranges should be reported as the inner limits of the percentiles P2.5 and P97.5, with a probability of 95%. Reference values in several avian species have been reported using this methodology (Lierz, 2003; Lierz and Fenske, 2004; Lierz & Hafez, 2005; Lierz & Hafez, 2006). Reference intervals derived from very small samples ($n < 25$) have been reported as an arithmetic mean and standard error (SEM) (Polo et al., 1992; Nazifi et al., 2008). Recommendations by the American Society for Veterinary Clinical Pathology are that for data sets where $20 \leq n < 40$, and the data follows a non-Gaussian distribution, robust methods should be used to calculate reference intervals with 90% confidence interval. Samples where $10 \leq n < 20$ are considered too small to report reference intervals. Instead, data should be reported in the form of a histogram, with mean or median, and minimum and maximum; alternatively, a table of all reference values can be provided along with the histogram (ASVCP, 2011; Friedrichs et al., 2012).

For these preliminary data, the following parameters are reported: mean and SEM, median, P2.5 and P97.5, and range.

Results

Table 1: Preliminary reference ranges for selected biochemical parameters in the wedge-tailed eagle (*Aquila audax*)

Parameter	Unit	N	Mean	SEM	Median	P2.5	P97.5	Range
Bile acids	µmol/L	15	6.27	±1.392	3	1.35	15	1-15
Urate	mmol/L	17	0.34	±0.052	0.29	0.1	0.84	0.07-0.97
Glucose	mmol/L	16	18.2	±0.798	18.55	11.5	22.7	10.2-23.3
AST	U/L	16	193.19	±17.206	182.5	101.63	338.63	87-372
ALT	U/L	16	22.63	±1.809	21	13	35.88	13-37
ALP	U/L	17	107.41	±22.066	62	26.4	306.2	24-331
Total protein	g/L	17	35.59	±1.361	36	25.4	43.6	25-44
Albumin	g/L	17	16.53	±0.713	17	12	20.6	12-21
Globulin	g/L	17	19.06	±1.481	19	11.4	30.2	11-31
Amylase	U/L	17	831.82	±92.067	774	441.2	1691.6	240-1926
Creatinine kinase	U/L	15	1093.73	±154.95	901	482.65	2134.7	462-2155
Cholesterol	mmol/l	17	3.68	±0.260	3.7	1.66	5.52	1.5-5.8
GDH	u/L	17	3.76	±0.650	3	0.4	8.6	0-9

Table 2: Preliminary reference ranges for selected haematological parameters in the wedge-tailed eagle (*Aquila audax*)

Parameter	Unit	N	Mean	SEM	Median	P2.5	P97.5	Range
HCT	L/L	12	0.37	±0.014	0.39	0.28	0.43	0.27-0.43
TWBC	$x10^9$/L	15	19.65	±2.535	18.4	5.81	36.5	4.90-40.00
Heterophils	%	15	73.27	±3.242	75	46.6	88.9	41-91
Heterophils	$x10^9$/L	15	14.13	±1.992	13.8	4.79	30.4	4.46-34.00
Lymphocytes	%	17	16.94	±2.931	14	3.4	43.8	3-53
Lymphocytes	$x10^9$/L	17	3.83	±0.765	2.7	0.55	11.16	0.20-13.00
Monocytes	%	17	5.59	±1.198	5	1	16.8	1-22
Monocytes	$x10^9$/L	17	1.84	±0.625	1.2	0.07	8.56	0.05-10.0
Eosinophils	%	17	1.63	±0.523	1	0	5.88	0-7
Eosinophils	$x10^9$/L	17	0.29	±0.112	0.11	0	1.38	0.00-1.54
Basophils	%	17	0.88	±0.296	0	0	3.6	0-4
Basophils	$x10^9$/L	17	0.24	±0.092	0	0	1.07	0.00-1.12

Discussion

The American Society for Veterinary Clinical Pathology recommends that in the determination of *de novo* reference intervals, a minimum of 20 individuals should be used, and as many variables (gender, age, source of animals, time of sampling, fasting status and health status) as possible controlled (ASVCP, 2011; Friedrichs et al., 2012). Unfortunately, in a study such as this where animal recruitment and sampling is essentially opportunistic, the best way of eliminating variation due to these variables is by increasing sample size. Therefore, as this project is ongoing and more data continues to be added to the larger set, the more defined the published reference ranges will become.

There are considerable differences in the published reference ranges for the Golden Eagle (ISIS, 2002; Polo et al., 1992; Jennings, 1996; Nazifi et al., 2008), though it is not known whether these differences are statistically significant. In addition, there are differences in methodology - not only in sample sizes of animals - between studies. For example, Polo et al. (1992) sampled only five individuals; Nazifi et al. (2008) reached the recommended sampling threshold with n=21 but reported reference ranges as (mean, ± standard deviation) instead of the percentile range (P2.5, P97.5). Therefore, direct comparison of the described preliminary reference ranges for Wedge-tailed Eagles and published ranges for Golden Eagles is not likely to be helpful at this stage.

While the authors are aware of the high number of variables associated with data collection, the ongoing nature of this project is likely to produce sound and consistent reference ranges for this, and other native raptor species, which can be reliably used in the clinical setting.

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