Haematology and biochemistry values for Temminck's pangolins (*Smutsia temminckii*) from Zimbabwe

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Abstract

Blood biochemistry values are routinely employed during clinical examinations to assess the health of the patient and to identify potential underlying conditions. No blood biochemistry data are currently available for Temminck's pangolin (*Smutsia temminckii*), a species that is being confiscated from both the domestic and international trade with increasing frequency, and this lack of data is hampering rehabilitation efforts. We determined haematological and serum biochemical values for ten Temminck's pangolins rescued from the illegal wildlife trade in Zimbabwe and placed in the care of the Tikki Hywood Foundation as part of their rehabilitation. Our results suggest a large overlap in serum biochemistry and haematology values with previously reported values for other pangolin species, but also suggest some

apparent differences. Haemoglobin, mean corpuscular haemoglobin and albumin:globulin ratio were positively correlated with mass, while alkaline phosphatase and amylase were negatively correlated with mass. Lymphocytes and monocytes were positively correlated with body condition, while mean corpuscular volume, alanine aminotransferase and total bilirubin were negatively correlated with condition. These results suggest that at least some parameters are independent of mass and are directly correlated with body condition and may therefore be informative in rapid health assessments of confiscated individuals.

Keywords: Blood analysis; Electrolytes; *Manis temminckii*; Pholidota

Introduction

The accurate interpretation of clinical data is reliant on normal values for each test being available, as well as the underlying physiological mechanisms and the effects of diseases on these parameters being known (Thrall 2004). Haematology values are regularly used during clinical examinations to draw inferences on the apparent health of an individual, to support clinical diagnoses and to facilitate the early detection of infections or other environmental disturbances. To facilitate the appropriate interpretation of these results, however, specific reference intervals need to be available for that species (Meyer and Harvey 2004; McKeown 2008; Deem et al. 2009; Flint et al. 2010; Shrivastav et al. 2012).

Pangolins (Pholidota: Manidae) are unique mammals that are covered in keratinous scales rather than hair (Gaudin et al. 2009). They are extensively traded internationally for their scales, which are used in various traditional medicines, while their meat is considered a culinary delicacy in Asia (Kang and Phipps 2003; Wu et al. 2004; Liou 2006; Yongping 2009; Yue 2009; Challender and Hywood 2012; Challender et al. 2019; Shairp et al. 2016; Xu et al. 2016). Pangolins are also extensively used as a protein source and for traditional African medicine, especially in Central and West Africa (Fa et al. 2006; Boakye et al. 2014, 2015, 2016; Ingram et al. 2018). The collapse of the Asian pangolin populations has resulted in a dramatic shift in trade to Africa to supply the insatiable Asian demand for scales and derivatives, placing local pangolin populations under increased pressure (Duckworth et al. 1999; Yue 2009;

Challender 2011; Challender and Hywood 2012; TRAFFIC 2014; Challender et al. 2014; Xu et al. 2016; Mwale et al. 2016; Heinrich et al. 2016, 2017; Ingram et al. 2018).

Due to their rarity and the inability to maintain pangolins in captivity for any length of time (Hoyt 1987; Yang et al. 2007; Hua et al. 2015), there is a paucity of data regarding their haematology and biochemistry. Previous studies have established baseline values for some of the Asian and tropical African pangolin species (Heath 1986; Oyewale et al. 1997, 1998; Chin et al. 2015; Khatri-Chhetri et al. 2015), although to the best of our knowledge no studies have yet published blood chemistry values for Temminck's pangolin (*Smutsia temminckii* Smuts, 1832). This lack of data hinders rehabilitation efforts as there are no reference values to use when assessing the general health of confiscated Temminck's pangolins. As a start to remedying this situation, we determined blood chemistry values from healthy and compromised individuals as part of routine veterinary assessments.

Methods

Ten Temminck's pangolins (three males and seven females) that were rescued from the illegal wildlife trade in Zimbabwe by the authorities and placed in the care of the Tikki Hywood Foundation for rehabilitation were examined during this study. Each individual's body condition at the time of sampling was visually scored by two experienced rehabilitators (EC and LH) and the condition expressed on a scale of 1–5 with five representing a wild, healthy individual. Although this scoring is somewhat subjective, it does provide an indication of body condition in a species that is otherwise difficult to assess.

Four individuals had been in care for more than a year, were walked daily and appeared in good health (body condition score ≥ 4.5; Supplementary Table S1) at the time of sampling. The remaining six individuals had been in trade for an unknown period of time and had body condition scores of 3–4 (Supplementary Table S1). One individual was pregnant while a second had a dorsal cranial abscess but did not show any clinical signs of illness (Supplementary Table S1).

Temminck's pangolins were anaesthetised using Isoflurane (Piramal Healthcare, India) delivered via a facemask at a concentration of 5% in concert with clinical oxygen delivered at a flow rate of 250–1 000 mL/minute via an open circuit (Khatri-Chhetri et al. 2015; Connelly et al. 2019). The more fractious individuals were premedicated with medetomidine hydrochloride 1 mg/mL (Domitor®, Pfizer Animal Health, Australia) at a concentration of

0.125 mg/kg body mass, not including scales, which were estimated to account for 30% of the total body mass (Pietersen 2013; Tikki Hywood Foundation, unpubl. data). Once anaesthesia had been induced, a light plane was maintained at a rate of 2–3% isoflurane vaporized in 750 mL/min medical oxygen (Khatri-Chhetri et al. 2015; Connelly et al. 2019). After medical examinations, procedures and sampling had been completed, delivery of isoflurane seized and animals received pure medical oxygen at a flow rate of 750 mL/min.

Blood samples (4.0 mL) were drawn from the medial caudal artery or sub-vertebral vascular plexus using a 5 mL sterile syringe and 21-gauge, 3.125 cm needle (Chin et al. 2015; Khatri-Chhetri et al. 2015; Connelly et al. 2019). Serum glucose analyses were immediately performed in duplicate, one sample each being analysed on a Solus V2 glucometer (BioSense Medical Devices, Duluth, Georgia, U.S.A.) and a OneTouch® Ultra 2 glucometer (LifeScan Europe GmbH, Zug, Switzerland). The remaining portion of each blood sample was analysed at Diagnopath Veterinary Laboratory, Harare, Zimbabwe (DVL) or Victoria Falls Wildlife Trust Laboratory, Victoria Falls, Zimbabwe (VFWT). Biochemical and haematology values were determined using an Abaxis Vetscan HM2 haematology analyser (Abaxis, Union City, California, U.S.A.) at VFWT, while a Mindray BS-200E (Shenzhen Mindray Bio-Medical Electronics, Guangdong Sheng, China) and Beckman Coulter DxH 520 haematology analyser (Beckman Coulter Life Sciences, Indianapolis, U.S.A.), respectively, were used at DVL.

The haematological blood parameters that were analysed were red blood cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLAT), mean platelet volume (MPV), white blood cell count (WBC) and differential counts of segmented neutrophils (Segs), eosinophils (Eos), lymphocytes (Lymphs) and monocytes (Monos). Measured biochemistry values were total protein, albumin, globulin, albumin:globulin ratio, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, total bilirubin (TBil), direct bilirubin (DBil), calcium (Ca^{++}), phosphorous (PO_4) and amylase. We also measured the electrolytes sodium (Na^{+}) and potassium (K^{+}).

We related mass and body condition to the blood biochemistry and electrolyte values using cor.test implemented in RStudio (RStudio Core Team 2018) and using R version 3.6.1 (R Core Team 2019). As our data were not normally distributed, we used a Spearman rank correlation with pairwise deletion of missing data.

Prior ethical clearance was not obtained for this research as the investigations were undertaken as part of the rehabilitation process and all procedures were performed by a registered veterinarian (Dr Mark Donaldson, registration number in Zimbabwe: D7711; UK: 0409930; South Africa: D05/7317), and were performed under permit from the Zimbabwe Parks and Wildlife Management Authority (permit numbers 46(1) (b) 01/2017, 46(1) (b) 02/2017, 46(1) (b) 10/2017, 46(1) (b) 02/2018, 46(1) (b) 03/2018, 46(1) (b) 04/2018 and 46(1) (b) 05/2018).

Table 1: Haematology and blood biochemistry values for ten Temminck's pangolins (*Smutsia temminckii*) from Zimbabwe.

Parameter	*n*	Mean ± SD	Range
Mass (kg)	10	8.93 ± 2.62	5.24–11.95
Body condition (5^{-1})	10	4.00 ± 0.85	3.00–5.00
RBC ($\times 10^{6}$ µl^{-1})	8	5.30 ± 0.55	4.40–5.80
HB (g dl^{-1})	8	11.23 ± 1.44	8.90–12.70
PCV (%)	8	33.93 ± 3.70	27.50–38.40
MCV (f l^{-1})	8	64.36 ± 1.48	62.50–66.60
MCH (pg)	8	21.30 ± 0.70	20.00–22.10
MCHC (%)	8	33.09 ± 1.07	31.00–34.40
RDW (%)	8	15.34 ± 1.77	13.40–19.00
PLAT ($\times 10^{3}$ µl^{-1})	8	184.5 ± 75.06	116.00–342.00
MPV (f l^{-1})	7	9.14 ± 1.60	7.30–11.30
WBC ($\times 10^{3}$ µl^{-1})	8	6.31 ± 2.20	3.60–9.20
Segmented neutrophils ($\times 10^{3}$ µl^{-1})	8	2.86 ± 2.44	0.30–6.80
Eosinophils ($\times 10^{3}$ µl^{-1})	1	0.10	–
Lymphocytes ($\times 10^{3}$ µl^{-1})	8	1.93 ± 0.70	0.80–3.00
Monocytes ($\times 10^{3}$ µl^{-1})	8	1.53 ± 1.38	0.20–3.50
Total protein (g l^{-1})	10	66.73 ± 6.64	60.00–83.40
Albumin (g l^{-1})	10	34.83 ± 4.16	25.00–39.00
Globulin (g l^{-1})	6	29.33 ± 4.27	25.00–35.00
Albumin:Globulin	6	1.18 ± 0.30	0.71–1.52
Alanine aminotransferase (U l^{-1})	10	110.35 ± 147.35	22.00–540.20
Aspartate aminotransferase (U l^{-1})	5	29.38 ± 33.61	6.70–87.90
Alkaline phosphatase (U l^{-1})	10	109.95 ± 51.62	52.00–221.00
Urea (mmol l^{-1})	10	9.65 ± 2.24	6.20–13.50
Creatinine (µmol l^{-1})	10	49.72 ± 11.49	29.00–66.00
Total bilirubin (µmol l^{-1})	10	9.83 ± 6.29	4.00–26.30
Direct bilirubin (µmol l^{-1})	5	7.22 ± 4.25	3.80–14.30
Calcium (mmol l^{-1})	6	2.37 ± 0.14	2.22–2.58
Phosphorous (mmol l^{-1})	1	2.26	–
Amylase (IU l^{-1})	6	678.00 ± 229.12	368.00–1 028.00
Serum glucose (mmol l^{-1})	8	5.56 ± 2.03	2.00–9.70
Sodium (mmol l^{-1})	9	140.58 ± 4.07	134.60–146.30
Potassium (mmol l^{-1})	9	8.32 ± 2.81	4.60–12.00

Results

The haematological parameters were reasonably consistent and the ranges overall were quite narrow (Table 1). The highest WBC count was recorded in the individual with an abscess (Supplementary Table S1), while the lowest lymphocyte value was recorded for the pregnant female (Supplementary Table S1). The ranges for calcium and sodium were narrow, while potassium showed a relatively wide range (Table 1; Supplementary Table S1).

There was a positive monotonic relationship between mass and haemoglobin (r_S = 0.833, n = 8, p = 0.015), mean corpuscular haemoglobin (r_S = 0.976, n = 8, p < 0.01) and albumin:globulin ratio (r_S = 0.943, n = 6, p = 0.017), while the correlation between mass and red blood cell count was near-significant (r_S = 0.699, n = 8, p = 0.054). There was a negative monotonic relationship between mass and alkaline phosphatase (r_S = −0.733, n = 10, p = 0.021) as well as between mass and amylase (r_S = −0.943, n = 6, p = 0.017). There were positive monotonic relationships between body condition and lymphocytes (r_S = 0.835, n = 8, p = 0.010) and monocytes (r_S = 0.931, n = 8, p < 0.001), and negative monotonic relationships between body condition and mean corpuscular volume (r_S = −0.712, n = 8, p = 0.048), alanine aminotransferase (r_S = −0.773, n = 10, p = 0.009) and total bilirubin (r_S = −0.772, n = 10, p = 0.009).

Discussion

The analyses suggest that mean corpuscular volume (MCV), lymphocyte, monocyte, alanine aminotransferase (ALT) and total bilirubin (TBil) concentrations are independent of mass (and as a proxy, probably age as well) and may therefore be useful indicators of the overall health and condition of Temminck's pangolins. These markers could therefore provide a fairly rapid and accurate assessment of a confiscated pangolin's physical and medical condition and guide subsequent treatments, although a larger sample size is required to evaluate whether these markers remain reliable and independent of mass using a larger dataset, and whether there are any sex-related differences.

The haematology and blood biochemistry values that we report are similar to the values recorded for Chinese pangolins (*Manis pentadactyla aurita* Hodgson, 1836) (Heath 1986), although our haemoglobin concentration (Hb) and MCV values are at the lower end of the Chinese pangolin spectrum. Mean corpuscular volume and mean corpuscular haemoglobin (MCH) were also lower than the values reported by Heath (1986). Our alkaline phosphatase

(ALP) values overlap with the previously reported values, although having a much higher maximum value, while our amylase values are also substantially higher than those recorded for Chinese pangolins (Heath 1986).

The blood values recorded in this study are similar to those recorded for Formosan pangolins (*Manis pentadactyla pentadactyla* Linnaeus, 1758) (Chin et al. 2015; Khatri-Chhetri et al. 2015), although the amylase values were higher, and a single aspartate aminotransferase (AST) value was substantially higher.

The packed cell volume (PCV), red blood cell count (RBC) and Hb concentration that we report are higher than those recorded for a single sick female Indian pangolin (*Manis crassicaudata* Geoffroy Saint-Hilaire) (Mohapatra et al. 2014), while our MCV, MCH and mean corpuscular haemoglobin concentration (MCHC) values are lower. The total protein concentration was similar to that reported by Mohapatra et al. (2014). These apparent differences should, however, be treated with caution considering the very small sample size for the Indian pangolin (n = 1), as well as the relatively small sample size in the current study.

Our results are also similar to those obtained by Oyewale et al. (1997, 1998) for the white-bellied pangolin (*Phataginus tricuspis* [Rafinesque, 1821]), with the exception that our PCV, MCV and MCH values are lower. This may indicate that the animals in Oyewale et al.'s (1997) study were slightly dehydrated, although this possibility needs to be investigated with additional analyses of the blood biochemistry of that species. Our monocyte values are higher than those reported for white-bellied pangolins (Oyewale et al. 1997), while our total leucocyte counts are similar.

Collecting haematology and biochemistry data are crucial for Temminck's pangolins, and all threatened species, as they provide baseline health values that can be used for comparison during clinical examination of individuals confiscated from trade or which otherwise require extended periods of veterinary care. This study provides initial values for Temminck's pangolin and future studies should aim to expand on this by collecting baseline haematological values from across this species' geographic range and from more individuals to assess whether there are any regional, sex or ontogenetic differences. Future studies should also endeavour to ascertain what effects, if any, season and prevailing climatic conditions have on haematological values for Temminck's pangolin, especially as both of these factors can be viewed as natural stressors for this species.

Acknowledgements

We are indebted to the Zimbabwe Parks and Wildlife Services for entrusting us with the care and rehabilitation of these individuals. Dr Gareth Tate (Endangered Wildlife Trust, Cape Town, South Africa) and Sean Heighton (University of Toulouse III Paul Sabatier, France) are thanked for their assistance with statistical analyses. Diagnopath Veterinary Laboratory, Harare, Zimbabwe and Victoria Falls Wildlife Trust Laboratory, Victoria Falls, Zimbabwe are thanked for performing the analyses. Four anonymous reviewers and the editors are thanked for improving this manuscript. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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