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Research article

Blood biochemistry and haematology values of juvenile Eurasian cranes (*Grus grus*) raised in captivity for reintroduction

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Abstract
The Eurasian crane (*Grus grus*) is currently held in over 50 zoological collections worldwide and present in the wild in a number of countries across Europe and Asia. Normal ranges have not previously been published for a number of haematological and biochemical parameters in the species and this study is the first to provide biochemical parameters in captive individuals of this species. Blood samples were collected from 90 juvenile Eurasian cranes with an average age of 70 days, across five consecutive years (2010-14), as part of health screening prior to reintroduction in the south-western UK. Haematology and biochemistry values were determined for 40 parameters. Statistical analyses were carried out to determine the effect of age, sex and year on these values. Mean values of many haematological and biochemical parameters differed between years: haemoglobin, mean corpuscular haemoglobin concentration, white blood cell count, heterophil percentage and number, lymphocyte percentage and number, monocyte percentage and number, eosinophil percentage and number, basophil percentage, total protein, albumin, globulin, albumin/globulin ratio, sodium, potassium, total calcium, ionised calcium, phosphorous, calcium/phosphorous ratio, alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transferase, bile acids, creatinine kinase, cholesterol, and sodium/potassium ratio. Calcium/phosphorous ratio, uric acid, bile acids, creatinine kinase, cholesterol and triglycerides decreased with age, while red blood cell count, haemoglobin, haematocrit, basophil number, potassium, total calcium and phosphorus increased with age. Females had higher values of red blood cell count, haemoglobin, lymphocyte number, basophil number, total protein, albumin, alkaline phosphatase, and creatinine kinase than males. A comparison with previously published values of captive and wild cranes is presented: the young age of the birds in our study was likely to have led to some of the observed differences to previous studies.

Introduction

The Eurasian (or common) crane (*Grus grus*) was once widespread in the UK but was extirpated in the 1600s as a result of hunting and wetland drainage. There has been a small naturally recolonising breeding population in eastern England since 1979 and more recent small-scale recolonisation in Scotland. Although chicks have been produced in recent years the populations remain small (Stanbury et al. 2011).

In 2009 the Wildfowl & Wetlands Trust (WWT), the Royal Society for the Protection of Birds (RSPB), Pensthorpe Conservation Trust (PCT) and Viridor Environmental Credits embarked on the Great Crane Project (GCP) – a collaborative attempt to reintroduce Eurasian cranes to the Somerset Levels and Moors in the South West of the UK.

Wild crane eggs collected in Germany were hand-reared in a purpose-built biosecure facility at WWT Slimbridge in Gloucestershire, England. At three-four months old the birds were transported to Somerset and released after a short stay in a pre-release enclosure. Between 2010 and 2014, 93 birds were captive reared and released into the wild; over 70 still survive in the wild in February 2016, with successful breeding taking place for the first time in 2015.

A full health screen of the birds for disease conditions was carried out before the move to Somerset, including swabs and blood samples.

The Eurasian crane is present in the wild in a number of countries across Europe and Asia and the species is held in over 50 zoological collections worldwide (International Species Information system (ISIS), 2015). Normal blood values are not

currently available for this species on the ISIS database. Previous studies in this species have either looked only at haematological values in relatively small numbers of captive adult birds (Hawkey et al. 1983) or relatively restricted panels of biochemistry in wild birds (Abelenda et al. 1993, Puerta et al. 1990). This study will be the first to provide values for biochemical parameters in captive individuals of this species. As their numbers increase in the wild in the UK, the publication of normal ranges of both haematology and biochemistry for individuals of this species has increased in importance.

The birds in the GCP were fed and housed in very similar conditions to birds reared for captivity at WWT (O'Brien et al. 2011) and other zoological collections, therefore the range of values in this paper are most probably typical of captive individuals.

Values from healthy individuals are of use in establishing reference ranges which can subsequently be used in health assessment of both captive and wild individuals (Kaminski et al. 2014), in particular, in interpretation of clinical cases and responses of Eurasian cranes to specific pathological conditions.

Methods

Study population and husbandry

Blood samples were taken from Eurasian cranes hatched and reared in captivity at WWT Slimbridge, Gloucestershire, UK as part of the GCP, from 2010 to 2014.

Housing

All birds were housed in the same rearing facility in each year. Eggs were artificially incubated and, once hatched, chicks were moved into fibreglass rearing tanks. Four purpose-built rearing sheds held six individual rooms, comprising an indoor area of 1.8m x 2m annexed to outdoor runs of 1.8m x 4m. After 2-5 days in the rearing tanks, chicks were transferred into the larger rooms. Hard wearing carpet matting covered the indoor concrete floor. A "pop-hole" hatch gave access to turfed outdoor runs. Barrier fences comprised weldmesh lined with tough woven plastic sheeting, preventing birds damaging themselves on the mesh or causing injury to birds in adjacent runs, whilst still allowing visualisation of their neighbours. A netted aviary measuring 50m x 30m, was accessible to the chicks via their outside runs.

Exercise

From 3-5 days after hatching the crane chicks were exercised outside daily (the onset varied depending on individual progress). They were exercised individually in the larger aviary for a minimum of 20 minutes twice daily, gradually increasing to a minimum of 30 minutes' twice daily from two to four weeks old. Before the cranes were old enough to socialise, exercise took place with one chick per aviculturalist (costumed to prevent imprinting). As they progressed the chicks were exercised in small groups, then larger cohorts until all the birds could remain together.

Diet

Immediately post hatch, chicks were fed an artificial diet of crushed Crane Grower Mini Pellet (Charnwood). Except in the final year when no starter diet was used, Lundi 35 (Lundi) was provided as a chick starter diet, removed once weight gain began at approximately three days old. Throughout rearing, all chicks received the coccidiostat monensin in-feed at 90 ppm (Elancoban G200 premix) to reduce the risks from coccidial infection leading to disseminated visceral coccidiosis which had previously caused mortality in crane chicks at WWT (O'Brien et al. 2011). Calcium and Vitamin D3 supplement (Nutrobal, Vetark professional) was added to the crane grower pellet throughout rearing. The amount of food provided depended on the individuals' growth rate and general development.

Growth charts were produced showing a predicted rate of growth, using normal growth rates of similar crane species (Wellington et al. 1996), and estimated using the individuals' dry hatch weights. Chick weights were kept just under the standard growth line, where possible, to lessen the chance of long bone growth abnormalities (Kelley and Hartup 2008).

Health management

Biosecurity

Rigorous biosecurity protocols were followed at the rearing facility. When no cranes were present, turf and gravel were replaced in the individual runs to reduce infection build-up and the exercise enclosure was limed to reduce survival of *Mycobacterium avium* (Pavlik et al. 2009). Vegetation management was carried out, to reduce risks from, for example, aspergillosis, and pest control measures were in place.

Prophylactic treatment

Chicks were treated prophylactically with oral fenbendazole (Panacur 2.5%, Intervet) at 20 mg/kg during the rearing process. Anthelmintic treatment frequency increased over the course of the project, occurring every two weeks in 2014 with three doses preceding health screening, the last given 16 days prior. This was necessary due to increased parasitic contamination of the rearing area over time despite biosecurity. All birds were also treated with ivermectin (Ivomec Super injection, Merial) at 0.2 mg/kg by intramuscular injection prior to moving to Somerset.

Disease screening

To comply with the import and release licences, Balai regulations and health management plans produced prior to the start of the project, various types of disease screening were carried out, for example avian influenza (AI) and Newcastle disease virus (NDV) screening within the first 10 days post-hatch. Faecal samples were taken monthly from individual cranes for parasitological and bacteriological screening. Each year, one set of pooled faecal samples was screened by PCR for *Chlamydia psittaci* infection. Responding to the Disease Risk Assessment produced by the Zoological Society of London (Sainsbury and Vaughn-Higgins 2012), pre-release testing involved blood sampling for signs of avian tuberculosis, haematazoa and West Nile virus, in addition to standard haematology and biochemistry (see below). Buccal and cloacal swabs were taken to screen for inclusion body disease of cranes virus (IBDCV), NDV and AI. The age of the birds sampled at this pre-release screening ranged from 55 to 82 (average 70) days old.

Blood sampling procedure and analysis

Blood samples were taken from either the medial metatarsal (2010–2013) or basilic vein (2014). Up to 3 ml of blood was taken into 1.3ml tubes (Micro Tubes – Plasma Lithium-Heparin, Starstedt) using a 23-gauge needle, 2.5 ml or 5 ml syringe. Samples were stored at 4° C and sent to the laboratory on the same day at ambient temperature together with individual fresh blood smears. Analysis was performed within 48 hours of sample collection.

Birds were manually restrained on a padded table, with position determined by blood sampling site. All birds were hooded to reduce stress and handling time was kept to a minimum, with birds bled less than 10 minutes after capture.

Blood sample analysis

From 2010 to 2012, blood samples were analysed by Carmichael Torrance Diagnostic services in Garforth, W. Yorkshire. Biochemistry was carried out using an IL650 random access biochemical analyser. Electrolytes and ionised calcium were determined using

a separate IL analyser with pH compensated ionised calcium and ion specific electrodes. Haemoglobin (Hb) was assayed using a Hemacue (Gustavsson 2015). Red cell counts (RBC) were produced using an ABC analyser and haematocrit (Hct) was produced using spun technique (Campbell and Ellis 2007). White cell count (WBC) was produced using a manual oxalic acid and standard chamber technique and differentials were created manually based on the blood smear (Samour 2006).

In 2013 and 2014, samples were analysed by CAPL laboratories, Staffordshire, UK. Biochemistry was carried out using the same analyser and chemistry methods as CTDS. Hb was assayed using a Siemens Advia 120 haematology analyser. RBC were produced using a manual standard chamber technique using a Neubauer counting chamber and Hct was produced as above. WBC was produced using Rees and Ecker solution and a Neubauer counting chamber (Campbell and Ellis 2007). Differentials were created as above.

Statistical analyses

We examined the effect of bird age (days), rearing cohort (hatch year) and sex (M, F) on haematological and biochemical parameters. Statistical analyses were conducted with the R statistical package (version 3.20) (R Development Core Team 2010) using packages stats (R Core Team 2013), nlme (Pinheiro et al. 2013) and lmtest (Zeileis and Hothorn 2002). Following initial data exploration, we developed linear models (lm command) with the parameter of interest as the response variable, *age* as a covariate and *sex* as a factor. The *year* effect was confounded by *laboratory*, because one laboratory was used for all samples in 2010–12 and a different one in 2013–14. We therefore used *year* nested within *laboratory* as a factor. No interaction terms were examined, due to small sample sizes.

We initially assumed a normal distribution of parameter values. Where model diagnostics indicated poorly fitting linear models, e.g. due to variances that differed between years, we fitted generalised least squares models (GLS) using REML estimation, which allows variance to differ between years (Zuur et al. 2009). In GLS models it was not possible to specify nested fixed effects, so we used *year* as an explanatory factor, and assessed parameter estimates for each year to determine whether there was evidence of a *laboratory* rather than a *year* effect.

We conducted step-wise removal of non-significant variables ($P \geq 0.05$) from the models using F-statistics (linear models) and likelihood ratio tests (GLS), until a final model was attained in which all explanatory variables were significant.

Means and 95% confidence limits for each parameter were calculated from the final models. Where *sex* was included in the final model, we report separate modelled values for each sex. Where *year* was included, we estimated mean and SE (standard error) for each year from the model, and report a mean value and combined 95% CI across all years. Where age was included, we report mean and 95% CI for a bird of mean age in the data-set (70 days). Infection with parasites or bacteria was also assessed using a variation of these models.

Results

Inclusion criteria

Blood samples from 90 birds were included in this study: 22 from 2010, 14 from 2011, 14 from 2012, 20 from 2013, and 20 from 2014. Coincidentally 45 of these were from females and 45 from males. Seven samples were excluded due to clots or significant haemolysis in the samples. Birds which displayed a degree of injury or lameness post-catch were included since this occurred post blood sampling. Some parameters were analysed in different numbers of samples due to changes in the laboratory panels offered between years or where some were prioritised if insufficient sample was available for all tests.

The most recent faecal samples taken prior to blood sampling were positive for nematodes in 26 cases, *Campylobacter* sp. in 49 cases and in 10 cases for both *Campylobacter* sp. and nematodes. These individuals were still considered to be in normal health for the purposes of the study, as the great majority of both captive and wild cranes will be carrying some form of parasite burden (WWT, unpublished data). Three birds suffering from cataracts, of undetermined cause, but considered unlikely to have an effect on blood haematology and biochemistry values, were included in this study.

Statistical analysis

Blood haematological and biochemical values together with significance levels for year, age and sex are presented in Tables 1 and 2.

The values of many haematological and biochemical parameters differed between years, both in variance and mean. In statistical models, the assumption of constant variance between years was clearly violated for a number of parameters, indicating that the spread of values differed between years.

The *year* effect was significant ($P<0.05$) in Hb, mean corpuscular haemoglobin concentration (MCHC), WBC, heterophil percentage and number, lymphocyte percentage and number, monocyte percentage and number, eosinophil percentage and number, basophil percentage, TP, albumin, globulin, albumin/globulin ratio, sodium, potassium, total calcium, ionised calcium, phosphorous, calcium/phosphorous (Ca:P) ratio, alkaline phosphatase (ALKP), aspartate aminotransferase (ALT), gamma-glutamyl transferase (GGT), bile acids, creatinine kinase (CK), cholesterol, and sodium/potassium ratio.

Total calcium showed a significant year effect, increasing across years from 2012–2014 and eosinophil number and percentage was noticeably higher in the first and last years of the project than in the three years between. Other year effects did not show any discernible pattern.

Ca:P ratio, uric acid (UA), bile acids, CK, cholesterol and triglycerides decreased with age, while RBC, Hb, Hct, basophil number, potassium, total calcium and phosphorus increased with age ($P<0.05$).

Females had significantly ($P<0.05$) higher values of RBC, Hb, lymphocyte number, basophil number, total protein (TP), albumin, ALKP, and CK levels than males.

Laboratory effects appear to have occurred in WBC, heterophil and lymphocyte number, sodium, phosphorus, Ca:P ratio, bile acids and cholesterol.

Comparison to other published values

Published values are presented in Tables 1 and 2 alongside results from the current study. Analytical methods may differ between this study and those published previously, but comparison is still considered worthwhile as they are the only reference values available.

The ranges of RBC, Hct and Hb found in this study of recently fledged juveniles are lower than those shown previously in adult captive (Hawkey et al. 1983; Isaacks et al. 1987) and adult and juvenile wild Eurasian cranes (Puerta et al. 1990) (See Table 1). Total WBC was lower in our study than reported in others (Table 1), in contrast to Abelenda et al. (1993) where juvenile wild Eurasian cranes, during their first winter, had a higher WBC than adults.

A number of studies (Puerta et al.1989, 1990; Samour et al. 1994; D'Aloia et al. 1995, 1996b) report higher lymphocyte count in young birds compared with adults whereas in our study levels appear similar or lower than those reported for adults. The higher number and percentage of heterophils compared to lymphocytes

Table 1. Haematology values for captive juvenile Eurasian cranes (*Grus grus*) and comparison with results from previous studies.

Parameter	n	Mean (Range)	SE	*Grus grus* Captive Hawkey et al. 1983	*Grus grus (adult)* Wild Puerta et al. 1990	*Grus grus (juv)* Wild Puerta et al. 1990	*Grus grus* Captive Isaacks et al. 1987	*Grus antigone sharpii* Captive Thiptara et al. 1998	*Grus vipio (adults)* Captive Rayhel et al. 2015	*Grus vipio (juv)* Captive Rayhel et al. 2015	*Grus canadensis pratensis* Captive Olsen et al. 2001	*Grus canadensis* Captive Olsen et al. 2001	*Grus americana* Captive Olsen et al. 2001
RBC (10x12/L) M	45	1.79 (1.39–2.18)[f, g]	0.03	2.7(2.4–3.0)	2.4 +/- 0.08	2.5 +/- 0.1	2.26	2.38+/-0.48	2.3 (1.8–2.8)	2.22 (1.7–2.7)			
RBC (10x12/L) F	45	1.88 (1.49–2.27)	0.03										
Hb (g/dl) M	45	9.90 (7.92–11.88)[c, f, g]	0.26	16.7(16.5–17.1)	14.6 +/- 0.6	15.0 +/- 0.6	15.8+/-0.8	12.8+/-2.14					
Hb (g/dl) F	45	10.33 (8.35–12.31)	0.26										
HCT %	90	31.20 (25.66–36.73)[f]	0.30	48 (46–52)	42.7 +/- 1.2	43.1 +/- 1.3	43.2+/-2.1	41+/-3	46 (34–52)	37 (29–46)	36.9	39.1	39.6
MCV (fl)	68	171.49 (148.68–194.29)	1.38	177(173–186)	176 + 5	172 +/- 6	230		197 (152–261)	169 (132–200)			
MCH (pg)	68	54.10 (47.37–60.83)	0.80	62.1(55.0–67.6)	60.4 +/- 1.8	60.1 +/- 2.5							
MCHC %	68	31.52 (28.66–34.37)[c]	0.34	34.9(31.7–36.3)	34.4 +/- 1.1	35.1 +/- 1.7							
WBC (10x9/L)	90	6.56 (5.98–7.14)[c]	0.30	10.2(5.1–13.8)	21.9 +/- 1.3	28.1 +/- 1.5		15.16+/-6.62	8.5 (4.3–14)	8.9 (4.0–14.9)	12	9.6	14.5
Het%	90	61.99 (57.45–66.54)[a]	2.32	48(31–66)	55.4 +/- 2.6	47.3 +/- 3.7		41.73+/-12.22	60 (34–79)	58 (33–85)	66.4	64.1	63.7
Het no. (10x9/L)	90	4.20 (3.68–4.71)[c]	0.26	4.98(2.24–7.18)					5.1 (2.2–8.4)	5.3 (2.4–10.0)			
Lym%	90	32.63 (32.11–33.14)[b]	0.26	24(14–29)	35.8 +/- 2.8	47.4 +/- 3.4		46.58+/-12.23	31 (10–65)	37 (13–62)	31.1	34.2	32.3
Lym no. (10x9/L) M	45	1.89 (0.80–2.97)[c, g]	0.14	2.36(1.42–3.31)					2.7 (0.6–5.1)	3 (1.2–5.8)			
Lym no. (10x9/L) F	45	2.15 (1.06–3.23)	0.14										
Mono%	90	1.90 (1.38 – 2.41)[c]	0.26	4(3–5)	3.4 +/- 0.5	2.5 +/- 0.4		6.69+/-2.28	8 (1–15)	4 (0–11)	1	0.5	1.2
Mono no. (10x9/L)	90	0.13 (0.00–0.64)[c]	0.26	0.40(0.15–0.55)					0.6 (0.1–1.1)	0.4 (0–1.4)			
Eos%	90	2.39 (1.68–3.10)[c]	0.36	21 (16–27)	3.7 +/- 0.6	3.0 +/- 0.6		5.0+/-3.24	0	0	1.3	1.2	2.6
Eos no. (10x9/L)	90	0.18 (0.00–0.70)[c]	0.26	2.08(1.12–2.99)					0	0			
Baso%	50	1.76 (0.76–2.76)[c]	0.51	3.5(1–8)	1.9 +/- 0.2	1.1 +/- 0.3			1 (0–3)	1 (0–3)			
Baso no. (10x9/L) M	27	0.04 (0.02–0.07)[e, h]	0.01	0.36(0.11–0.89)					0.1 (0–0.3)	0.1 (0–0.4)			
Baso no. (10x9/L) F	23	0.05 (0.01–0.08)	0.02										

Key: [a] year significant P<0.05, [b] year significant P<0.01, [c] year significant P<0.001, [d] age significant P<0.05, [e] age significant P<0.01, [f] age significant P<0.001, [g] sex significant P<0.05, [h] sex significant P<0.01, [i] sex significant P<0.001

Table 2. Biochemistry values for captive juvenile Eurasian cranes (*Grus grus*) and comparison with results from previous studies.

				Grus grus (adult)	*Grus grus (juv)*	*Grus antigone sharpii*	*Grus vipio (adults)*	*Grus vipio (juvs)*	*Grus canadensis pratensis*	*Grus canadensis*	*Grus americana*
Parameter	n	Mean (Range)	SE	Puerta et al. 1990	Puerta et al. 1990	Thiptara et al. 1998	Rayhel et al. 2015	Rayhel et al. 2015	Olsen et al. 2001	Olsen et al. 2001	Olsen et al. 2001
TP (g/L) M	90	27.52 (22.31–32.73)[c, g]	0.66	49 +/- 0.2	48 +/- 0.1	33.53+/-6.38	40 (36–46)	32 (27–39)	30	30	33
TP (g/L) F	90	28.91(23.69–34.12)	0.67								
ALB (g/L) M	45	11.57 (9.33–13.81)[c, h]	0.29				18 (13–29)	13 (10–17)	14	14	15
ALB (g/L) F	45	12.26 (10.02–14.50)	0.29								
Glob (g/L)	90	16.29 (12.49–20.09)[c]	0.44						17	16	18
A:G ratio	90	0.74 (0.57–0.91)[c]	0.02						0.85	0.94	0.89
Na (mmol/L)	88	141.33 (137.13–145.53)[c]	0.49				147 (137–153)	144 (140–148)			
K (mmol/L)	88	3.06 (2.67–3.44)[c, d]	0.20				3.8 (2.8–4.7)	4.1 (3.2–5.9)			
Calcium (mmol/L) Total	90	2.61 (2.33–2.89)[c, d]	0.03			1.95+/-0.20	0.57 (0.52–0.72)	0.59 (0.53–0.73)	0.55	0.51	0.51
Ca ionised	48	1.17 (0.96–1.37)[c]	0.03								
Phosphorus (mmol/l)	54	2.12 (1.92–2.32)[c, f]	0.10			1.04+/-0.25	0.22 (0.1–0.49)	0.31 (0.13–0.44)	0.23	0.2	0.2
Ca:P ratio	54	1.38 (1.22–1.54)[c, f]	0.08								
Uric acid (umol/L)	90	248.12 (95.88 – 400.37)[e]	8.38	256.36 +/- 35.36	388.96 +/- 44.2	690+/-260	654.16 (256.36–1096.16)	822.12 (406.64–1440.92)	838.9	541.9	502.11
Alk phos (U/L) M	45	805.09 (425.54–1184.63)[c, h]	48.40				112 (53–303)	563 (108–1176)	479	371	169
Alk phos (U/L) F	45	931.11 (551.26–1310.96)	48.76								
AST (U/L)	89	222.40 (123.66–321.14)[b]	11.58			183.41+/-20.92	239 (142–570)	(154–488)	196	195	294
GLDH (U/L)	90	3.54 (3.18–3.91)	0.19								
Gamma GT (U/L)	50	11.21 (6.96–15.45)[c]	0.51								
Bile acids (umol/L)	90	27.83 (20.18–35.49)[c, d]	3.91								
Glucose (mmol/l)	14	11.38 (10.79–11.99)	0.30			11.04+/-1.52	13.21 (11.54–15.21)	14.6 (11.77–17.15)	14	13.76	13.38
CK (U/L) M	37	793.13 (607.15–979.12)[c, f, i]	94.89				206 (77–574)	1352 (145–4030)	1784	284	375
CK (U/L) F	33	883.37 (698.31–1068.43)	94.42								
Cholest (mmol/l)	90	5.05 (3.07–7.02)[b, d]	0.24	11.27 +/- 0.61	10.82 +/- 0.83	3.50+/-1.17					
Sodium:potassium ratio	40	47.95 (45.24–50.65)[c]	1.38								
Triglyc (mmol/l)	50	2.23 (1.88–2.60)[d]	0.18	4.5 + 0.28	6.11 + 0.44						
Chloride (mmol/L)	40	102.61 (101.96–103.25)	0.33			106.96+/-3.39	108 (105–112)	107 (101–111)			
Vit D (nmol/l) M	2	10.25 (3.12–17.38)	3.64								
Vit D (nmol/l) F	2	28.15 (21.02–35.28)	3.64								

Key: [a] year significant P<0.05, [b] year significant P<0.01, [c] year significant P<0.001, [d] age significant P<0.05, [e] age significant P<0.01, [f] age significant P<0.001, [g] sex significant P<0.05, [h] sex significant P<0.01, [i] sex significant P<0.001.

in our chicks has also been seen in adult birds (Hawkey et al. 1983; Puerta et al. 1990) but not in the juveniles sampled by Puerta et al. (1990) where numbers were similar.

Of particular note are our values of ALKP and CK which appear high in comparison to most previous studies.

Discussion

Year, age and sex had notable effects on both the haematology and biochemistry values in this study and need to be taken into consideration in inter-study comparisons. The age range in this study is small which must also be noted when comparing age effects reported in other studies.

The majority of parameters differed between years. This could be due to a very large number of variables including weather, genetics, nutrition, amount of exercise and parasite burden – these all, inevitably, varied between individuals within years, but also differed between cohorts of birds due to changes made to husbandry to reduce growth related disease and increased contamination of the environment with parasites over time.

In our project, the year effect was somewhat confounded by a change in laboratory in mid-study. In some cases therefore we are unable to distinguish a genuine difference between years from an artefact due to laboratory effect.

Levels of infection with parasites and bacteria may also have contributed to a year effect although no statistical difference was seen between parameters in chicks carrying infections and those shown to be clear of them at sampling. The different blood sampling site used in 2014 compared to previous years could also be a factor. Sample site has been shown to affect haematological values in samples in reptiles (Lopez-Olvera et al. 2003) and rodents (Abatan et al. 2008) but in quail (Arora 2010) no difference was shown between two peripheral blood sample sites. Further work could be carried out to investigate this putative effect.

Age and sex have also been identified as major factors that can influence blood parameters in this study, and others, such as time of year or stage of the breeding cycle, may also be important (Kostelecka-Myrcha et al.1985; Pastor et al. 2001a, b; Campo and Davila 2002; Gayathri et al. 2004; Villegas et al. 2004; Baos et al. 2006; Kasprzak et al. 2006; Jakubas et al. 2008).

The increase of RBC, Hct and Hb with age in this study is a pattern previously reported in a large number of avian species including egrets (Celdran et al. 1994), storks (Puerta et al. 1989b; Alonso et al. 1991; Montesinos et al. 1997), flamingos (Hawkey et al. 1984a, b), Masai ostrich (*Struthio camelus*) (Levi et al. 1989; Palomeque et al. 1991; Fudge 1996), poultry (Ferguson et al. 1964; Priya and Gomarthy 2008), domestic fowl, geese, and quail (Hodges 1977), various psittacines (Clubb et al. 1990, 1991a, b; Vaz et al. 2015,), bustards (Alonso et al. 1990; D'Aloia et al. 1995; Howlett et al. 1998) and Canada geese (*Branta canadensis*) (Charles-Smith et al. 2014). This increase has previously been reported in both free-living (Campbell and Dein 1984; Kostelecka-Myrcha et al. 1985; Gessaman et al.1986; Puerta et al.1989b) and captive birds (Fallow et al. 1976), with levels increasing as chicks develop to adulthood (Samour et al. 1994; D'Aloia et al. 1995, 1996a).

The increase in calcium with age seen in this study was also seen in houbara (*Chlamydotis undulata macqueenii)* (Bailey et al. 1999) and kori *(Ardeotis kori)* (D'Aloia 1996) bustards. Low circulating calcium levels in juvenile birds may be due to the requirement of calcium for skeletal growth. However, this was not found by Olsen et al. (2001) where calcium continued to decrease with age over a number of years, in two species of crane. The increase in phosphorus with age was also shown in ostriches (Palomeque et al. 1991) although it decreases in the majority of species (de le Court 1995). Further comparison with adult values would be required before this trend could be confirmed in Eurasian cranes.

The higher RBC and Hb in females in this study has also been found in white storks (*Ciconia ciconia)*, where male chicks had significantly lower levels of RBC, Hct, Hb and MCHC (Kaminski et al. 2014). Suggested reasons included changes in activity of sex steroid hormones in chicks during development or the hierarchy of chicks in particular broods (Kaminski et al. 2014).

As in this study, ALKP was also higher in females than males in flamingos (Eren et al. 2006) and black stork (*Ciconia nigra*) nestlings (Lanzarot et al. 2005) which is opposite to reported values in bald ibises (*Geronticus eremita*) (Dutton et al. 2002). TP was also greater in females than in males, as previously found in Lanzarot et al. (2005) and Hochleitner (1994).

Increasing Hb with age may be caused by the decrease in blood volume per unit of weight with age (Palomeque and Planas 1978; Celdran et al. 1994). Also, since flight increases the oxygen demands of birds (Lasiewski 1972; Berstein et al. 1973) and since RBC, Hct and Hb have been shown to be higher in good fliers than in flightless species (Balasch et al. 1974; Viscor et al. 1985), it is not surprising that the young birds in this study, not yet fully flighted, had levels that were likely still increasing. This has also previously been shown in white storks (Puerta et al. 1989b). Puerta et al. (1990) found juvenile and adult Eurasian cranes had similar RBC, Hct and Hb, however the juvenile birds were already fully flighted and part of a migratory population. Although the oxygen carrying capacity of adult and young Eurasian crane blood has not been measured, this hypothesis seems to fit with the available data. As free-living chicks in some species have been shown to have lower RBC and a lower Hct than captive ones (Puerta et al. 1992), comparing the levels to wild Eurasian crane juveniles of the same age would be worthwhile.

Puerta et al. (1990) suggested that captivity may reduce exposure to infection and therefore WBC in captive birds. This is supported by the lower values in our chicks compared with the wild juveniles and adults in that study. However, Puerta et al. (1992) found higher WBC numbers in captive, compared to wild, flamingo chicks. In captive kori bustards, initial low WBC values were attributed to the fact that birds had been reared indoors in hygienic conditions (Howlett et al. 1998). The ensuing months showed an increase when the birds were moved into an outdoor aviary previously inhabited by other koris and kept together in larger numbers. The relatively low WBC in our cranes may have arisen for similar reasons, but the single blood sampling did not allow for longitudinal analysis of the effects on WBC as their environment became more natural through the rearing process. Sequential sampling would be a useful future study.

The higher number and percentage of heterophils compared to lymphocytes in our chicks, which differs from the wild juveniles in Puerta et al. (1990), is likely to be due to inter-species variation in age-related differences in total and differential WBC counts (Hodges 1977). Differential leukocyte counts also differ among avian species. Abelenda et al. (1993) found significant temporal variation in the relative proportions of heterophils and lymphocytes, with eosinophils, basophils and monocytes relatively constant over time in Eurasian cranes. This temporal variation was only measured over the winter/spring months; therefore further variation is likely and could be a factor in the differing proportions of leucocytes seen in our study as samples were collected in July.

In white-naped cranes (*Antigone vipio*), monocyte number and percentage increased with age – potentially due to increased exposure to antigens and infectious agents (Rayhel et al. 2015). Puerta et al. (1990), and our study, suggest this may also occur in Eurasian cranes.

The eosinophil percentages measured here are similar to those in wild juveniles (Puerta et al. 1990) and lower than in captive adults (Hawkey et al. 1983), in contrast to white storks where the number and percentage of eosinophils in adults is only 20–25% of

that encountered in chicks (Alonso et al. 1991). As our juveniles were hand-reared in a captive environment it is likely that they encountered fewer parasites and bacteria than wild chicks, and prophylactic medication may have reduced the level of infection. They may also have suffered fewer physiological stressors than wild chicks. 26/90 chicks had nematodes in faecal samples but the presence of nematodes did not appear to have a significant effect on eosinophil number, a similar result was also shown in white stork chicks (Montesinos et al. 1997). However, the higher eosinophil levels encountered in the first and last years of this study may be due to minimal prophylactic treatment in the first year and a greater challenge from parasite build-up in the enclosure by the final year. In Hawkey et al. (1983) and Puerta et al. (1992) high eosinophil counts were thought to be due to sub-clinical intestinal nematode infection.

Although toxicity to fenbendazole has been shown to have an effect on haematologic values in some species (Howard et al. 2002; Weber et al. 2002) no adverse effect has been documented to the authors' knowledge in Grus species. It is therefore unlikely to have had a significant effect on haematologic values in this study. Further research could be carried out to confirm this.

A smaller number of studies have presented blood biochemistry values compared to haematology values in birds, and the blood biochemistry data that has been published for Eurasian cranes is limited (Puerta et al. 1990), making interpretation of biochemistry values in this study complex. Multiple parameters were included in this study to create a biochemical profile that can be used as a baseline against which abnormal values can be judged in juvenile cranes.

De le Court et al. (1995) suggested that higher triglyceride level in white spoonbill chicks *(Platalea leucorodia)* could be related to the intense mobilization of endogenous lipid metabolism prior to becoming independent (Griminger and Scanes 1986). Although triglyceride decreased with age in our chicks, the mean value was lower than that in Puerta et al. (1990), so further study would be needed to determine whether this is also true in Eurasian cranes. Bile acids in Olsen et al. (1996) were reported as <80 umol/L and the values in our study also fall below this level.

The low TP in our study is consistent with previous studies in young psittacines (Joyner and Duarte 1994), white storks (Montesinos et al. 1997), houbara (Bailey et al. 1999) and kori (D'Aloia 1996) bustards. Protein is essential to growth being a major constituent of tissues and may also increase with age due to increasing immunological response (Bailey et al. 1998a, b; Puerta et al. 1989a).

UA levels in this study were similar to those in wild adult Eurasian cranes, although with a wider range, but were lower than those in juveniles in another study and other species (Table 2). Since the blood level of this compound diminishes during starvation (Sykes 1971) it was suggested that the low UA may have been indicative of feeding restraint. However, the low level in our chicks where food was regularly provided suggests that this may not be the only cause.

Only four biochemical parameters have previously been assessed in Eurasian cranes so many of the parameters in this paper should provide useful information for further studies and diagnostics. Vitamin D level was analysed in four birds only, and although this is a very small sample size it is worth reporting as it may be useful when assessing long bone abnormalities in juvenile birds in conjunction with calcium and phosphorus levels. The finding of higher levels in females would need to be confirmed by studies in greater numbers of birds. Long bone abnormalities can occur commonly in captive long-legged birds such as cranes (Kelley and Hartup 2008).

Our juveniles show higher values for ALKP and calcium than other crane species, but without ranges for these parameters in adult Eurasian cranes we are currently unable to assess whether these levels are higher in juveniles, although this is the case in other species (Table 2). Elevated levels of ALKP in other precocial species have been associated with calcium deposition in the skeleton, as a function of intensive bone ossification and elongation during the rapid growth phase (Calabuig et al. 2010) and the high levels of ALKP, calcium and phosphorus seen in our juvenile birds may reflect the intense osteoblastic activity needed for somatic growth (Vinuela et al. 1991, Hochleithner 1994, Lanzarot et al. 2005). Young psittacines and bustards have also been shown to have higher ALKP than adults (Clubb et al. 1990; D'Aloia 1996; Bailey et al. 1998a, b). Qin and Klandorf (1993) have suggested that pubertal increase of plasma 17 beta-oestradiol can affect calcium absorption from the intestine by increasing the activity of ALKP in immature chicks. Our low Ca:P ratio (which should be 2:1 for growing birds (Olsen et al. 1996)) may be skewed since values were particularly low during one year where a number of factors, including severe weather, may have influenced calcium metabolism. Further work is needed to assess the reasons behind these low values.

Creatinine kinase (CK) values appear similar to those in Rayhel et al. (2015), which are six times higher in juvenile white-naped cranes compared with adults. CK levels declined with age in our study, but comparison with adult levels would help determine if this is also the case in Eurasian cranes. Levels also decreased with age in sandhill cranes (*Antigone canadensis*) (Olsen et al. 2001). Differences in CK between adult and juvenile houbara bustards (Bailey et al. 1999) were considered to be related to bird temperament and/or sampling time after capture, rather than being an age-related change. Patient excitement, handling, trauma, and irritating injections can elevate CK (Bollinger et al. 1989; Fudge 1996; Bailey et al. 1997). Younger birds may be more prone to struggle during handling, or as bone and muscle structure develops there may be reduced likelihood of injury during handling.

Plasma protein electrophoresis has been carried out on biochemistry samples from Whooping cranes (*Grus americana*) (Hausmann et al. 2015) and white-naped cranes (Rayhel et al. 2015) and this technique could be used to gain further insight into samples from Eurasian cranes in future work.

The birds in this study were captive and hand reared and therefore sampling from cranes hatched and reared in the wild would be useful as comparison. Artificial pelleted food and reduced exercise compared with wild chicks could affect certain aspects of their physiology leading to differences in blood values. As our birds are recently fledged juveniles it is expected that there will be differences between their blood values and those of adult captive Eurasian cranes for whom biochemical reference values still need to be published.

Normal values for blood constituents vary widely for different species (Wyk et al. 1998). Thus, to assess the physiologic and pathologic condition of birds it is of paramount importance to know normal blood values for individual species. In wildlife conservation programs, blood parameters are increasingly used as physiological indicators (Maceda-Vega et al. 2015). With the Eurasian crane already present in numerous zoological collections, and with the wild population also expanding in the UK, the production of these values should be of use in the health assessment of both wild and captive birds.

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References

Abatan O.I., Welch K.B. and Nemzek J.A. (2008) Evaluation of saphenous venipuncture and modified tail-clip blood collection in mice. *Journal of the American Association of Laboratory Animal Science* 47: 8–15.

Abelenda M., Nava M.P., Fernandez A., Alonso J.A., Alonso J.C., Muñoz-Pulido R., Bautista L.M. and Puerta M.L. (1993) Blood values of common cranes (*Grus grus*) by age and season. *Comparative Biochemistry and Physiology Part A: Physiology* 104: 575–578.

Alonso J. A., Alonso J.C., Muñoz-Pulido R., Naveso M.A., Abelenda M., Huscas V. and Puerta M.L.(1990) Hematology and blood chemistry of free-living young great bustards (*Otis tarda*).
Comparative Biochemistry and Physiology 97A: 611–613.

Alonso J.C., Huecas V., Alonso J. A., Abelenda M., Muñoz-Pulido R. and Puerta M. L. (1991) Hematology and blood chemistry of adult white storks (*Ciconia ciconia).Comparative Biochemistry and Physiology* 98A: 395–397.

Arora K.L. (2010) Differences in haemoglobin and packed cell volume in blood collected from different sites in Japanese quail (*Coturnix japonica*). *International Journal of Poultry Science* 9: 828–830.

Bailey T.A., Wernery U., Naldo J. and Samour J. H. (1997) Plasma concentrations of creatine kinase and lactate dehydrogenase in houbara bustards (*Chlamydotis undulata macqueenii*) immediately following capture. *Comparative Haematology International* 7: 113–116.

Bailey T.A., Wernery U., Naldo J., Howlett J. and Samour J.H. (1998a) Normal blood chemistry and age-related changes in the white-bellied bustard *(Eupodotis senegalensis),* with some clinical observations. *Comparative Haematology International* 8: 61–65.

Bailey T.A, Wernery H., Howlett J., Naldo J. and Samour J.H. (1998b) Age-related plasma chemistry changes in the buff-crested bustard (*Eupodotis ruficrista gindiana*). *Journal of Veterinary Medicine* 45: 635–640.

Bailey T.A., Wernery U., Howlett J., Naldo J. and Samour J.H. (1999) Age-related plasma chemistry changes in Houbara and Kori bustards in the United Arab Emirates. *Journal of Wildlife Diseases* 35: 31–37.

Balash J., Palomeque J., Palacios L., Musquera S. and Jimenez M. (1974) Haematological values of some great flying and aquatic-diving birds. *Comparative Biochemistry and Physiology* 49A: 137–145**.**

Baos R., Jovani R., Pastor N., Tella J.L., Jimenez B., Gomez G., Gonzalez M.J. and Hiraldo F. (2006) Evaluation of genotoxic effects of heavy metals and arsenic in wild nestling white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) from southwestern Spain after a mining accident. *Environmental Toxicology and Chemistry* 25: 2794–2803.

Berstein M.H., Thomas S.P. and Schmidt-Nielsen K.(1973) Power input during flight of the fish crow *Corvus ossifragus. Journal of Experimental Biology* 58: 401–410.

Bollinger T., Wobeser G., Clark R.G., Nieman D.J. and Smith J.R. (1989) Concentration of creatine kinase and aspartate aminotransferase in the blood of wild mallards following capture by three methods for handling. *Journal of Wildlife Diseases* 25: 225–231.

Calabuig C.P., Ferrer M., Muriel R. and Tilgar B. V. (2010) Plasma alkaline phosphatase as a sensitive indicator of age and skeletal development in wild coscoroba swans. *Wildlife Research* 37: 504–511.

Campbell T.W. and Dein F.J. (1984) Avian hematology: the basics. *Veterinary Clinics of North America Small Animal Practice* 14: 223–248.

Campbell T.W. and Ellis C.K. (2007) Hematology of birds. In: Campbell T.W. and Ellis C.K. *Avian and Exotic Animal Hematology & Cytology* 3rd edn. Oxford: Blackwell Publishing, 3–50.

Campo J.L. and Davila S.G. (2002) Estimation of heritability for heterophil: lymphocyte ratio in chickens by restricted maximum likelihood. Effects of age, sex, and crossing. *Poultry Science* 81: 1448–1453.

Celdran J.F., Polo F.J., Peinado V.I., Viscor G. and Palomeque J. (1994) Hematology of captive herons, egrets, spoonbill, ibis and gallinule. *Comparative Biochemistry and Physiology* 107A: 337–34.1

Charles-Smith L.E., Rutledge M.E., Meek C.J., Baine K., Massey E., Ellsaesser L.N., DePerno C.S., Moorman C.E. and Degernes L.A. (2014) Hematologic parameters and hemoparasites of nonmigratory Canada geese (*Branta canadensis*) from Greensboro, North Carolina, USA. *Journal of Avian Medicine and Surgery* 28: 16–23.

Clubb S.L., Schubot R.M., Joyner K., Zinkl J. G., Wolf S., Escobar J. and Clubb K.J. (1990) Hematologic and serum biochemical reference ranges in juvenile macaws, cockatoos and eclectus parrots. In: *Proceedings of the American Association of Avian Veterinarians*. Lake Worth, Florida: AAAV, 58–59.

Clubb S.L., Schubot R.M., Joyner K., Zinkl J.G., Wolf S., Escobar J. and Kabbur M.B. (1991a)
Hematologic and serum biochemical reference intervals in juvenile macaws (*Ara* sp.). *Journal of the*
Association of Avian Veterinarians 5: 154–162.

Clubb S.L., Schubot R.M., Joyner K., Zinkl J.G., Wolf S., Escobar J. and Kabbur M.B. (1991b) Hematologic and serum biochemical reference intervals in juvenile cockatoos. *Journal of the Association of Avian Veterinarians* 5: 16–26.

D'Aloia M.A., Howlett J.C., Samour J.H., Bailey T.A. and Naldo J. (1995) Normal haematology
and age-related findings in the rufous-crested bustard (*Eupodotis ruficrista*). *Comparative*
Haematology International 5: 10–12.

D'Aloia M.A. (1996) Preliminary studies on age-related plasma chemistry changes in the kori bustard
(*Ardeotis kori*). *Comparative Haematology International* 6: 96–101.

D'Aloia M.A., Samour J.H., Bailey T.A., Naldo J. and Howlett J.C. (1996a) Normal blood chemistry of the kori bustard (*Ardeotis kori*). *Avian Pathology* 25: 161–165.

D'Aloia M.A., Samour J.H., Howlett J.C., Bailey T.A. and Naldo J. (1996b) Normal blood chemistry of the houbara bustard (*Chlamydotis undulata macqueenii*). *Avian Pathology* 25: 167–173.

De le Court C., Aguilera E. and Recio F. (1995) Plasma chemistry values of free-living white
spoonbills *(Platalea leucorodia). Comparative Biochemistry and Physiology Part A: Physiology* 112: 137–141.

Dutton J.C., Allchurch A.F. and Cooper J.E.(2002) Comparison of hematologic and biochemical
reference ranges between captive populations of northern bald ibises (*Geronticus eremita*).
Journal of Wildlife Diseases 38: 583–588.

Eren M., Çam Y., Uyaniki F. and Atalay Ö. (2006) Some blood biochemical parameters in flamingos
Revue Médicine Véterinaire 157: 277–279.

Fallow S. A., Jones J. E. and Hughes B. L. (1976) Hematocrit, erythrocyte, and hemoglobin values for male and female guineas at various ages. *Poultry Science* 55: 814–816**.**

Ferguson T.M., Golan F.A., Trammell J., Smith, E., Omar E. and Couch J.R. (1964) Haematological data and blood pressure for male broad breasted bronze turkeys. *Poultry Science* 43: 1318.

Fudge A.M. (1996) Clinical haematology and chemistry of ratites. In: Tully T.N. and Shane M. (eds). *Ratite Management, Medicine and Surgery* Malabar, Florida: Krieger Publishing Company, 105–114.

Gayathri K.L., Shenoy K.B. and Hegde S.N. (2004) Blood profile of pigeons (*Columba livia*) during growth and breeding. *Comparative Biochemistry and Physiology A Molecular Integrated Physiology* 138: 187–192.

Gessaman J. A., Johnson J. A. and Hoffman S. W. (1986) Hematocrits and erythrocyte numbers for Cooper's and sharp-shinned hawks. *Condor* 88: 95–96.

Griminger P. and Scanes C.G. (1986) Protein metabolism. In: Sturkie P.D.(ed.). *Avian Physiology*, 4th edn. Berlin: Springer, 326–344.

Gustavsson F. (2015) Validation study: HemoCue Hb 201 + as a tool in comparative physiological field studies on avian blood. Department of Physics, Chemistry and Biology. Linkopings Universitet, Linkoping, Sweden. 1–19. http://www.diva-portal.org/smash/get/diva2:840616/FULLTEXT01.pdf (accessed 5 December 2016).

Hausmann J.C., Cray C. and Hartup B.K. (2015) Comparison of serum protein electrophoresis values in wild and captive whooping cranes (*Grus americana*). *Journal of Avian Medicine and Surgery* 29: 192–199.

Hawkey C., Samour J.H., Ashton D.G., Hart M.G., Cindery R.N., Finch J.M. and Jones D.M. (1983) Normal and clinical haematology of captive cranes (Gruiformes). *Avian Pathology* 12: 73–84.

Hawkey C., Hart M.G., Samour H.J., Knight J.A. and Hulton R.E. (1984a) Haematological findings in healthy and sick captive rosy flamingos *(Phoenicopterus ruber ruber). Avian Pathology* 13: 163–172.

Hawkey C., Hart M.G. and Samour H.J. (1984b) Age related haematological changes and haematopathological responses in Chilean flamingos *(Phoenicopferus chilensis). Avian Pathology* 13: 223–229.

Hochleitner M. (1994) Biochemistries. In: Ritchie B.W., Harrison G.J. and Harrison L.R. (eds). *Avian Medicine: Principles and Application* Lake Worth, Florida: Wingers Publishing, Inc, 223–245.

Hodges R.D. (1977). Normal avian haematology (poultry). In: Archer B.K. and Jeffcoat L.B. (eds). *Comparative Clinical Haematology.* Oxford, UK: Blackwell Scientific Publications, 483.

Howard L.L., Papendick R., Stalis I.H., Allen J.L., Sutherland-Smith M., Zuba J.R., Ward D.L. and Rideout B.A. (2002) Fenbendazole and albendazole toxicity in pigeons and doves. *Journal of Avian Medicine and Surgery* 16:203–210.

Howlett J. C., Samour J.H., Bailey T.A. and Naldo J.L. (1998) Age-related haematology changes in captive-reared kori bustards (*Ardeotis kori*). *Comparative Haematology International* 8: 26–30.

International Species Information System (2015) Physiological reference intervals for captive wildlife—2015. *International Species Inventory System; c2014–2015* https://zims.isis.org/Main.aspx, accessed 20 December 2015.

Isaacks R.E., Kim C.Y., Goldman P.H., Putman M.S., Minande C.M. and Harkness D.R. (1987) Studies on avian erythrocyte metabolism –XV. Relationship between the major phosphorylated metabolic intermediates and whole blood oxygen affinity in the Gruiformes (cranes). *Comparative Biochemistry and Physiology* 87: 165–169.

Jakubas D., Wojczulanis-Jakubas K. and Kreft R. (2008) Sex differences in body condition and hematological parameters in Little Auk *Alle alle* during the incubation period. *Ornis Fennica* 85: 90–97.

Joyner K.L. and Duarte J.P.S. (1994) Hematology and serum biochemistry values in captive juvenile

yellow-naped amazons (*Amazona auropalliata*).In: Kornelsen, M.J. (ed.). *Proceedings of the Annual Conference of the Association of Avian Veterinarians*. Lake Worth, Florida: AAV, 185–188.

Kaminski P., Jerzak L., Sparks T.H., Johnston A., Bochenski M., Kasprzak M., Wisniewska E, Mroczkowski S. and Tryjanowski P. (2014) Sex and other sources of variation in the haematological

parameters of White Stork *Ciconia ciconia* chicks*. Journal of Ornithology* 155: 307–314.

Kasprzak M., Hetmanski T. and Kulczykowska E. (2006) Changes in hematological parameters in free-living pigeons (*Columba livia f. urbana*) during the breeding cycle. *Journal of Ornithology* 147: 599–604.

Kelley C. and Hartup B.K. (2008) Risk factors associated with developmental limb abnormalities in captive whooping cranes. *North American Crane Workshop Proceedings* 176: 119–124.

Kirkpatrick R.L. (1980) Physiological indices in wildlife. Management. In: Schemnitz S.D. (ed.). *Wildlife Management Techniques Manual*, 4th edn. Washington: The Wildlife Society, 99–112.

Kostelecka-Myrcha A., Dabrowska G. and Wieczorek B. (1985) Variations in the red blood picture during the nestling development of the jackdaw. *Zeszyty Naukowe Filii U.W., Biology* 10: 81–90.

Lanzarot M.P., Barahona M.V., San Andrés M.I., Fernández-García M. and Rodríguez C. (2005) Hematologic, protein electrophoresis, biochemistry and cholinesterase values of free-living black stork nestlings (*Ciconia nigra*). *Journal of Wildlife Diseases* 41: 379–386.

Lasiewskri C. (1972) Respiratory function in birds. In: Farner D.S. and King J.R. (eds).

Avian Biology, *Vol. 2.* New York: Academic Press, 288–342.

Levi A., Perelman B., Waner T., Van Grevenbroek M. and Van Creveld C., Yagil R. (1989)

Haematological parameters of the ostrich (*Struthio camelus*). *Avian Pathology* 18: 321–327.

López-Olvera J.R., Montané J., Marco I., Martínez-Silvestre A., Soler J. and Lavín S. (2003) Effect of venipuncture site on hematologic and serum biochemical parameters in marginated tortoise (*Testudo marginata*). *Journal of Wildlife Diseases* 39: 830–836.

Maceda-Veiga A., Figuerola J., Martínez-Silvestre A., Viscor G., Ferrari N. and Pacheco M. (2015) Inside the Redbox: Applications of haematology in wildlife monitoring and ecosystem health assessment. *Science of the Total Environment* 514: 322–332.

Montsinos A., Sainz A., Pablos M. V., Mazzucchelli F. and Tesouro M.A. (1997) Hematological

and plasma biochemical reference intervals in young white storks. *Journal of Wildlife*

Diseases 33: 405–412.

O'Brien M.F., Brown M.J., Stidworthy M.F., Peirce M.A., Marshall R.N., Honma H. and Nakai Y. (2011) Disseminated visceral coccidiosis in Eurasian cranes (*Grus grus*) in the UK. *Veterinary Record* 168: 216.

Olsen G. H., Carpenter J.W. and Langenberg J.A. (1996) Medicine and surgery. In: Ellis D.H., Gee G.F. and Mirande C.M. (eds). *Cranes: Their Biology, Husbandry, and Conservation.* Washington, D.C: National Biological Service and Baraboo, Wisconsin, USA: International Crane Foundation, 137–174.

Olsen G.H., Hendricks M.M. and Dressler L.E. (2001) Hematological and serum chemistry norms for Sandhill and Whooping cranes. *North American Crane Workshop Proceedings* 17: 178–184.

Palomeque J. and Planas J. (1978) Blood volume in domestic pigeons. *Comparative Biochemistry and Physiology* 59A: 413–417.

Palomeque J., Pinto D. and Viscor G. (1991) Hematologic and blood chemistry values of the Masai ostrich *(Struthio camelus). Journal of Wildlife Diseases* 27: 34–40.

Pastor N., Lopez-Lazaro M., Tella J.L., Baos R., Forrero M.G., Hiraldo F. and Cortes F. (2001a) DNA damage in birds after the mining waste spill in southwestern Spain: a comet assay evaluation. *Journal of Environmental Pathology, Toxicology and Oncology* 20: 317–324.

Pastor N., Lopez-Lazaro M., Tella J.L., Baos R., Hiraldo F. and Cortes F. (2001b) Assessment of genotoxic damage by the comet assay in white storks (*Ciconia ciconia*) after the Donana ecological

disaster. *Mutagenesis* 16: 219–223.

Pavlik I., Falkinham III J.O. and Kazda J. (2009) Transmission of Mycobacteria from the environment to Susceptible Hosts. In: Kazda J., Pavlik I., Falkinham III J.O. and Hruska K.(eds). *The Ecology of Mycobacteria: Impact on Animal's and Human's Health.* New York, USA: Springer, 283–312.

Pinheiro J., Bates D., DebRoy S., Sarkar D. and R Development Core Team (2013) *nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–110*. http://www.R-project.org/. Accessed February 23rd 2016.

Priya M. and Gomathy V.S. (2008) Haematological and Blood Biochemicals in male and female turkeys of different age groups. *Tamilnadu Journal of Veterinary & Animal Sciences* 4: 60–68.

Puerta M.L., Huecas V. and Garcia de1 Campo A.L. (1989a) Hematology and blood chemistry of the Chilean flamingo. *Comparative Biochemistry and Physiology* 94A: 623–625.

Puerta M.L., Mufioz-Pulido R., Huecas V. and Abelenda M. (1989b) Hematology and blood chemistry of chicks of white and black storks *(Ciconia ciconia* and *Ciconia nigra). Comparative Biochemistry and Physiology* 94A: 201–204*.*

Puerta M.L., Alonso J.C., Huecas V., Alonso J.A., Abelenda M. and Mufioz-Pulido R. (1990) Hematology and blood chemistry of wintering common cranes. *Condor* 92: 210–214.

Puerta M.L., Garcia Del Campo A.L., Abelenda M., Fernandez A., Huecas V. and Nava M.P. (1992) Hematological trends in flamingos, *Phoenicopterus ruber. Comparative Biochemistry and Physiology Part A: Physiology* 102: 683–686.

Qin X. and Klandorf H. (1993) Effect of estrogen in relation to dietary vitamin D3 and calcium on activity of intestinal alkaline phosphatise and Ca-ATP-ase in immature chicks. *General and Comparative Endocrinology* 90: 318–327.

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org/, accessed 23 February 2016.

R Core Team (2013) *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org/, accessed 23 February 2016.

Rayhel L., Aitken-Palmer C., Joyner P., Cray C., Lizarraga C.A., Ackerman B. and Crowe C. (2015) Hematology and biochemistry in captive white-naped cranes (*Grus vipio). Journal of Zoo and Wildlife Medicine* 46: 747–754.

Sainsbury A.W. and Vaughn-Higgins R.J. (2012) Analyzing disease risks associated with translocations. *Conservation Biology* 26: 442–452.

Samour J.H., Howlett J.C., Hart M.G., Bailey T.A., Naldo J. and D'Aloia M.A.(1994) Normal haematology of the houbara bustard (*Chlamydotis undulata macqueenii*). *Comparative Hematology International* 4: 198–202.

Samour J.H. (2006) Diagnostic value of hematology. In: Harrison, G.J., and Lightfoot, T.L. (eds). *Clinical Avian Medicine Volume II*. Palm Beach, Florida: Spix Publishing Inc., 587–610.

Stanbury A. and the UK Crane Working Group (2011) The changing status of the common crane in the UK. *British Birds* 104: 432–447.

Sykes A.H. (1971) Formation and composition of urine. In Bell D.J. and Freeman B.M. (eds). *Physiology and Biochemistry of the Fowl*. New York, USA: Academic Press, 358–397.

Thiptara A., Schuamitri P., Ousavaplangchai L., Kiatipattanasakul W., Yongsiri S., Sarikaputi M. and Pattanarangsan R.(1998) Preliminary study on hematology and blood chemistry of eastern sarus cranes (*Grus antigone sharpii*) in captivity. *Proceedings of the 24th Annual Conference of the Thai Veterinary Medical Association (TVMA) and the 4th Conference of the Veterinary Practitioner Association of Thailand*, 344–353.

Vaz F.F., Locatelli-Dittrich R., Sipinski E.A.B., Abbud M.C., Sezerban R.M., Schmidt E.M.S., Dittrich J. and Cavalheiro M.L. (2015) Hematologic and total plasma protein values in free-living red-tailed Amazon parrot nestlings (*Amazona brasiliensis*) in Paraná State, Brazil. *Journal of Avian Medicine and Surgery* 29: 187–191.

Villegas A., Sanchez Guzman J.M., Corbacho C., Corbacho P. and Vargas J.M. (2004) Blood values of bald Ibis (*Geronticus eremita*) in captivity: comparative ranges and variability with age, sex and
physical condition. *Journal of Ornithology* 145: 98–104.

Vinuela J., Ferrer M. and Recio F. (1991) Age-related variations in the plasma levels of alkaline phosphatase, calcium and inorganic phosphorus in chicks of two species of raptors. *Comparative Biochemistry and Physiology* 99A: 49–54.

Viscor G., Marques M.S. and Palomeque J. (1985) Cardiovascular and organ weight adaptations as related to flight activity in birds. *Comparative Biochemistry and Physiology* 82A: 597–599.

Weber M.A., Terrell S.P., Neiffer D.L., Miller M.A. and Mangold B.J. (2002) Bone marrow hypoplasia and intestinal crypt cell necrosis associated with fenbendazole administration in five painted storks. *Journal of the American Medical Association* 221: 417–419.

Wellington M., Burke A., Nicolich J.M. and O'Malley K. (1996) Chick rearing. In: Ellis D.H., Gee G.F. and Mirande C.M. (eds). *Cranes: Their Biology, Husbandry, and Conservation.* Washington, D.C: National Biological Service and Baraboo, Wisconsin, USA: International Crane Foundation, 77–104.

Wyk E., Bank H. and Verdoon G. H. (1998) Dynamics of haematology and blood biochemistry
in free-living African white-backed vulture (*Pseudogyps africanus*) nestlings. *Comparative*
Biochemistry and Physiology Part A 120: 495–508.

Zeileis A. and Hothorn T. (2002) Diagnostic checking in regression relationships. *R News* 2:7–10. http://CRAN.R-project.org/doc/Rnews/, accessed 23 February 2016.

Zuur A.F., Ieno E.N., Walker N.J., Saveliev A.A. and Smith G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. New York, USA: Springer.