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Hematologic, Plasma Biochemical, Protein Electrophoretic, and Total Solid Values of Captive Oriental Turtle Doves (*Streptopelia orientalis*)

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I-Ting Tsai, Chau-Hwa Chi, and Pin-Huan Yu (2018) As part of ongoing studies on the blood profiles of Taiwanese avian species, hematology, plasma biochemistry, and serum protein electrophoresis values and hand-held refractometer readings of total solids were determined in Oriental turtle doves (*Streptopelia orientalis*). Blood samples were collected and analyzed from 44 healthy captive adult and sub-adult Oriental turtle doves. There were no significant gender differences in any of the parameters studied. However, adults had significantly higher values of total protein, albumin, globulin, cholesterol, sodium, chloride, and alpha$_2$ globulin, while sub-adults had higher values of phosphorus and potassium. The refractometer readings were highly correlated with values of total protein obtained by the biuret method. The results of the current study provide valuable data for clinically evaluating Oriental turtle doves and will be beneficial for the conservation of this species.

Key words: Oriental turtle doves, Hematology, Biochemistry, Refractometer, Protein electrophoresis.

BACKGROUND

The Oriental turtle dove (*Streptopelia orientalis*) belongs to the family Columbidae (Ramesh et al. 2011). Its conservation status is "least concern" and it lives throughout low altitude areas of Taiwan. *Streptopelia orientalis orii* is the subspecies endemic to Taiwan (Ding and Fang 2010). In our rescue center, the number of Oriental turtle dove cases increases each year, and these wild casualties urgently require medical support. This demand highlights the importance of establishing species-specific diagnostic references.

Plasma biochemistry and hematology are the cornerstone for medical diagnosis of disease (Harr 2002). In avian patients, the assessment of hematologic and biochemical parameters is important because birds often present with subtle or non-specific clinical signs such as anorexia, fluffy feathers and tail bobbing (Le Souëf et al. 2013). The evaluation of these parameters can provide information on metabolic disorders, nutrient deficiencies, and organopathies to support the correct diagnosis (Hochleithner 1994; Aina and Ajibade 2014; Al-Gamal 2014). Protein electrophoresis is also a sensitive method of detecting nephritis, hepatitis, mycotic diseases, chronic inflammatory diseases, and chlamydiosis, as well as being an excellent prognostic indicator (Cray and Tatum 1998; Melillo 2013). Determination of serum protein values from total solid values measured using refractometry is a simple procedure used in clinical practice (Melillo 2013). Additionally, avian studies have reported inconsistencies between the results measured by biuret and refractometer. While some studies

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report good correlation, others present major discrepancies (George 2001). Owing to its convenience and usefulness in clinical practice, especially in small birds providing low-volume blood samples, we examined the correlation between these two methods in the present study.

The aim of the current study was to establish reference intervals for hematology, plasma biochemistry analytes, plasma protein electrophoresis, and total solid values measured by refractometry of Oriental turtle doves for conservation purposes. In addition, we analyzed the influence of age and gender on these values, and compared these results to those from other avian species.

MATERIALS AND METHODS

There were 36 adult and 8 sub-adult Oriental turtle doves (25 female, 19 male) included in this study. The sub-adult doves were distinguished by the spotted pattern on their necks. Males and females were determined by endoscopic observation, since they are monomorphic species. Average body weights were 186.9 g, 255.7 g, and 229.1 g for sub-adult, male, and female doves, respectively. The birds were rescued orphans and wild casualties rescued from northern Taiwan that were unfit for release. They were kept in individual cages measuring 60 × 45 × 40 cm at the National Taiwan University Veterinary Hospital (Taipei, Taiwan). The semi-outdoor space measured 10 m^2 and was well ventilated. Photoperiod was identical to natural photoperiod and temperature was kept at 24-28°C using heating devices. All doves were fed commercial food for pigeons and doves (Versele-Laga Youngsters, S-Jaeger-Son Co., Tainan, Taiwan), and water and mineral supplement (Versele Colombine Ideal-Bloc, Versele Laga, Deinze, Belgium) were given *ad libitum* and changed daily.

Prior to entering the study, doves were treated for parasites using metronidazole (Funa Tablet, Curie Chem & Pharm Co., Ltd., Taiwan), trimethoprim/sulfamethoxazole (BAKTAR, Taiwan Shionogi & Co., Ltd, Taiwan) or fenbendazole (Panacur, Coopers Animal Health, Bendigo East, Australia), based on the results of fecal and crop examinations; medications ceased after two consecutive negative fecal and crop samples. Physical examinations were conducted, including cardiac, lung, and air sac auscultation; body and head palpation; plumage, cloacal, and oral examinations; and imaging studies if needed. Birds were held in captivity and monitored for activity, appetite, defecation, urination, body condition, and body weight for 3 months before the study commenced. At the time of study, none of the doves had medical issues.

Between February 2014 and February 2015, 1 mL blood samples were drawn from the basilic vein of physically restrained birds using 1 mL insulin syringes (Insulin Syringe U-100, TERU- MOt, Terumo Corporation, Hatagaya, Shibuya-ku, Tokyo, 151-0072, Japan) between 10:00 and 13:00. Blood films were made from blood containing no anticoagulant and stained with Wright-Giemsa stain (Hematek® Stain Pak, Siemens Healthcare Diagnostics Inc., NY, USA) for manual differential leukocyte counts (Hochleithner 1994; Clark et al. 2009). Samples were then divided and placed into ethylenediaminetetraacetic acid (EDTA) coated tubes (BD Microtainer® tube with $_{k2}$EDTA, Becton, Dickinson and Company, USA) for hematology, and lithium heparin coated tubes (Greiner Bio-One Co, VACUETTE®, USA) for biochemical and protein electrophoretic analysis (Hochleithner 1994).

Packed cell volume (PCV) was measured using standard hematocrit methods after centrifugation for 3 min at 12000 rpm. The plasma collected from the capillary tube was transferred to the refractometer for total solid reading. Red blood cell (RBC) and white blood cell (WBC) counts were obtained using a hemocytometer and Natt and Herrick's solution (Hochleithner 1994; Clark et al. 2009). Hemoglobin concentration (Hb) was determined by cyanmethemoglobin methods (TOA Medical Electronic Co., Sysmex K-1000, Japan) (Jain 1993). Erythrocyte indices - including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) - were calculated from RBC count, Hb concentration, and PCV values using standard formulas (Hochleithner 1994).

Plasma was separated and immediately removed from heparin tubes after centrifugation at 8000 rpm for 1 min. Biochemical analysis was performed with an autoanalyzer (VITROS® 350 Chemistry System, Ortho Clinical Diagnostics, Johnson & Johnson, Melbourne, Australia) within 5 min of obtaining plasma. The parameters measured were uric acid, total protein, glucose, cholesterol, triglyceride, aspartate aminotransferase (AST), urea, creatine kinase (CK), gamma glutamyltransferase (GGT), calcium,

and inorganic phosphorus. Sodium, potassium, and chloride ions were assayed with an automatic analyzer (Vetlyte® Electrolyte Analyzer, IDEXX Laboratories, Inc., USA).

Agarose gel electrophoreses were performed on plasma samples in a SPIFE unit (SPIFE® 3000, Helena Laboratories, USA) with two applications at an electric voltage of 100 V for 25 min using an agarose gel (TITAN® III Cellulose Acetate Plate, Helena Laboratories). Staining was performed using Ponceau S Stain (Cat. No. 5526, Helena Laboratories). The gel images were scanned and read with platinum gel analysis software (QuickScan 2000, Helena Laboratories).

In some cases, the amount of plasma obtained was insufficient to assay all the parameters, therefore the sample sizes are not uniform. The gender of each dove was determined by endoscopy under general anesthesia through Hopkins telescope (64301BA, Karl Storz, Tuttlingen, Germany) using previously described techniques (Joyner 1994).

All statistical analyses were performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Preparation and analysis of data follow the guidelines for the determination of reference intervals from International Federation of Clinical Chemistry and American Society for Veterinary Clinical Pathology. Outliers were detected and eliminated on the basis of Horn's algorithm using Tukey's interquartile fences. The normality of the data was determined by Kolmogorov-Smirnov test. Data that had, or transformed to have, Gaussian distributions were analyzed by parametric methods and upper and lower reference limits were established as the mean plus and minus two standard deviations (SD), respectively. Parametric methods were used to determine 90% confidence intervals (CI) of the reference limits. Data that could not be transformed to Gaussian distributions were analyzed by non-parametric methods. For sample sizes of fewer than 40 animals, reference intervals were determined using value ranges (minimum to maximum), rather than the mean ± 2 SD. When sample sizes were between 10 and 20, all values were listed in ascending order (Friederich et al. 2012; Conover 1980).

Potential differences were evaluated between males and females, as well as adults and sub-adults, doves using Mann-Whitney tests for nonparametric data. Statistical significance was set at $p < 0.05$. Correlation of total solids measured by refractometry and total proteins by the biuret method was analyzed by Pearson's correlation coefficient. Statistical significance was set at $p < 0.05$. Correlations between total solids and blood glucose, cholesterol, triglyceride, sodium, and chloride were also examined.

RESULTS

Hematology

All 44 birds underwent hematological examination. Table 1 lists the mean, SD, ranges of values, and 90% CI of the reference limits. There were no significant gender or age differences for any of the parameters studied (Table 2).

Clinical chemistry

Due to the small size of the turtle doves, blood samples were not always of sufficient volume to perform all tests. Given these practical considerations, cholesterol, triglyceride, GGT, and electrolytes received lower priority when testing small samples. There were 11 doves (8 adults, 3 sub-adults) without cholesterol and triglyceride results; one without sodium, potassium, and chloride; and only 11 birds in which the sample was sufficient to test GGT concentrations. Table 3 presents the mean, SD, ranges of values, and 90% CI of the reference limits for 14 parameters. There were no significant differences between female and male doves for any of the parameters studied. Significant differences existed between the sub-adults ($n = 8$) and adults ($n = 36$). Sub-adults had higher values of phosphorus ($p = 0$) and potassium ($p = 0$). Adults had higher values for total protein ($p = 0.003$), cholesterol ($p = 0.025$), sodium ($p < 0.001$), and chloride ($p = 0.001$) (Table 4).

Protein electrophoresis

Protein electrophoresis was performed for all 44 doves. The mean, SD, ranges of values, and 90% CI of the reference limits are listed in table 5. Graphical representations of each fraction are presented in figure 1. There were no significant gender differences for any of the parameters studied. Significant differences existed between the sub-adults ($n = 8$) and adults ($n = 36$). Adults had higher values of albumin ($p = 0.012$), globulin ($p < 0.001$), and alpha$_2$ globulin concentrations ($p = 0.033$) (Table 6).

Table 1. Hematology values of 36 adult and eight sub-adult captive Oriental turtle doves (*Streptopelia orientalis*)

Analyte	Adult					Sub-adult	
	n	Mean	SD	Minimum Maximum[a]	90% CI of reference limits	*n*	All measured values[b]
PCV (%)	36	51.03	1.41	45-57	48.69-53.36	8	44, 48, 48, 49.5, 50, 51, 54.5, 56
Hb (g/dL)	35	16.33	1.27	13-23.6	14.23-18.43	8	12.5, 14.5, 14.8, 14.9, 15.9, 16.6, 17.6, 20
RBC (10^6/uL)	36	3.19	0.90	1.81-4.88	1.70-4.68	8	2.36, 3.01, 3.09, 3.26, 3.36, 3.49, 3.83, 4.18
MCV (fL)	36	165.75	54.71	112.70-254.14	75.48-256.02	8	122.00, 130.55, 137.54, 151.84, 159.47, 162.20, 181.23, 186.44
MCH (pg)	35	52.48	23.36	38.01-75.23	13.94-91.02	8	35.65, 15.40, 47.32, 47.56, 48.17, 52.22, 52.97, 56.96
MCHC (g/dL)	35	31.88	3.42	28.26-42.91	26.23-37.52	8	28.41, 29.17, 29.22, 29.90, 30.21, 31.43, 34.58, 40
WBC (uL)	35	12396.89	4548.12	5772-22866	4889.50-19898.27	8	8880, 9990, 10212, 11100, 17538, 18426, 22200, 24864
Heterophils (%)	36	32.78	0	15-55	32.78-32.78	8	15, 17, 18, 22, 28, 34, 51, 53
Lymphocytes (%)	36	63.03	2.83	34-80	58.36-67.69	8	44, 48, 62, 65, 73, 78, 78, 82,
Basophils (%)	31	0.42	0	0-2	0.419-0.419	8	0, 0, 0, 0, 0, 1, 2, 3
Eosinophils (%)	34	0.00	0	0-0	0-0	8	0, 0, 0, 0, 0, 0, 0, 0
Monocytes (%)	36	3.31	2.83	0-7	0-7.97	8	1, 2, 3, 3, 3, 4, 5, 5
Heterophils (/uL)	35	4009.26	1182.51	1531.8-7692.3	2058.12-5960.40	8	1531.8, 1698.3, 2442, 2486.4, 4475.52, 6264.84, 8944.38, 11766
Lymphocytes (/uL)	35	7758.51	3577.73	3574.2-14065.9	1855.25-13661.77	8	5772, 7792.2, 8103, 8373.84,8418.24, 9768, 11424.12, 19393.92
Basophils (/uL)	32	50.44	0	0-253.08	50.44-50.44	8	0, 0, 0, 0, 0, 102.12, 177.6, 333
Eosinophils (/uL)	35	241.72	0	0-0	0-0	8	0, 0, 0, 0, 0, 0, 0, 0
Monocytes (/uL)	35	390.03	212.13	0-892.44	40.01-740.05	8	175.38, 204.24, 333, 444, 499.5, 666, 737.04, 745.92

[a]When the sample size was less than 40 animals, reference intervals were determined with a range of values (minimum to maximum), rather than mean ± 2SD. [b]When sample size was between 10 and 20, all values were listed in ascending order.

Table 2. Sex and age differences in hematology values of captive Oriental turtle doves (*Streptopelia orientalis*)

Analyte	Sex differences			Age differences		
	Male	Female	*P*-value	Adult	Sub-adult	*p*-value
PCV (%)	-	-	0.830	-	-	0.435
Hb (g/dL)	-	-	0.934	-	-	0.533
RBC (106/uL)	-	-	0.934	-	-	0.584
WBC (/uL)	-	-	0.785	-	-	0.315
Heterophils (%)	-	-	0.953	-	-	0.512
Lymphocytes (%)	-	-	0.981	-	-	0.513
Basophils (%)	-	-	0.368	-	-	0.838
Eosinophils (%)	-	-	0.212	-	-	0.500
Monocytes (%)	-	-	0.259	-	-	0.988
Heterophils (/uL)	-	-	0.687	-	-	0.831
Lymphocytes (/uL)	-	-	0.644	-	-	0.212
Basophils (/uL)	-	-	0.432	-	-	0.801
Eosinophils (/uL)	-	-	0.212	-	-	0.500
Monocytes (/uL)	-	-	0.381	-	-	0.353

Refractometer

In seven doves (5 adults, 2 sub-adults), not enough plasma was collected for total solids evaluation. Refractometry readings from 37 doves were compared with total protein values measured by the biuret method (Table 3). Total solid values ranged from 4.2-7, with a 90% confidence interval of 4.76-6.63. The mean total solid value was 5.69, with a standard deviation of 0.57. The Pearson

Table 3. Biochemistry values of 36 adult and eight sub-adult captive Oriental turtle doves (*Streptopelia orientalis*)

	Adult					Sub-adult	
Analyte	*n*	Mean	SD	Minimum-Maximum[a]/ All measured values[b]	90% CI of reference limits	*n*	All measured values[b]
AST (U/L)	35	135.54	10.61	70-207[a]	118.04-153.04	8	84, 99, 103, 105, 117, 125, 136, 141
CK (U/L)	35	707.74	29.70	408-1085[a]	658.74-756.75	8	472, 544, 682, 788, 870, 985, 1554, 1700
Uric acid (mg/dL)	36	6.50	2.33	2.3-9.8[a]	2.65-10.35	8	2.8, 3.2, 3.9, 5.1, 5.5, 8.3, 8.5, 14.4
TP (g/dL)	36	3.16	0.71	2.3-4.0[a]	1.99-4.32	8	2.2, 2.2, 2.2, 2.6, 2.6, 2.7, 2.8, 3.5
Ca (mg/dL)	36	8.61	0.50	6.4-9.4[a]	7.80-9.43	8	6.1, 8.5, 8.7, 8.7, 8.8, 8.8, 9.4, 9.7
P (mg/dL)	33	3.44	0.14	2.0-5.8[a]	3.21-3.67	8	4.6, 5.4, 5.6, 6.4, 6.5, 6.8, 7.0, 8.6
Glucose (mg/dL)	35	322.69	34.65	245-390[a]	265.52-379.86	8	286, 298, 302, 308, 316, 317, 322, 346
Cholesterol (mg/dL)	29	268.48	94.05	172-352[a]	113.31-423.66	4	122, 176, 192, 258
Triglyceride (mg/dL)	29	198.17	18.38	144-281[a]	167.84-228.51	4	124, 184, 198, 288
GGT (U/L)	7	-	-	< 5, < 5, < 5, < 5, < 5, 5, 8[b]	-	4	< 5, < 5, < 5, 6
BUN (mg/dL)	36	< 1	-	< 1[a]		8	< 1, < 1, < 1, < 1, < 1, < 1, < 1, < 1
Na (mmol/L)	33	155.00	4.24	147-164[a]	148.00-162.00	8	144, 145, 147, 147, 149, 149, 154, 15
K (mmol/L)	35	2.97	1.20	2.0-4.1[a]	0.99-4.95	8	3.1, 3.5, 4.0, 4.2, 4.5, 4.7, 4.7, 5.2
Cl (mmol/L)	35	118.89	3.54	112-128[a]	113.05-124.72	8	109, 112, 112, 114, 114, 115, 116, 119

[a]When the sample size was less than 40 animals, reference intervals were determined with a range of values (minimum to maximum), rather than mean ± 2SD. [b]When sample size was between 10 and 20, all values were listed in ascending order.

Table 4. Sex and age differences in plasma biochemistry values of captive Oriental turtle doves (*Streptopelia orientalis*)

Analyte	Sex differences			Age differences		
	Male	Female	*p*-value	Adult	Sub-adult	*p*-value
AST (U/L)	-	-	0.741	-	-	0.057
CK (U/L)	-	-	0.864	-	-	0.212
Uric acid (mg/dL)	-	-	0.173	-	-	0.402
TP (g/dL)	-	-	0.643	H*	-	0.004
Ca (mg/dL)	-	-	0.191	-	-	0.725
P (mg/dL)	-	-	0.213	-	H***	< 0.001
Glucose (mg/dL)	-	-	0.061	-	-	0.241
Cholesterol (mg/dL)	-	-	0.957	H*	-	0.027
Triglyceride (mg/dL)	-	-	0.368	-	-	0.738
GGT (U/L)	-	-	a	-	-	a
BUN (mg/dL)	-	-	a	-	-	a
Na (mmol/L)	-	-	0.314	H***	-	0.001
K (mmol/L)	-	-	0.540	-	H***	< 0.001
Cl (mmol/L)	-	-	0.561	H***	-	0.001
Total solid	-	-	0.357	H**	-	0.003

H means significantly higher than for the other sex or age. a means concentrations were too low to be of value for statistical analysis. Asterisks indicate levels of significance: a single asterisk (*) for $p \leq 0.05$, double asterisks (**) for $p \leq 0.01$, and triple asterisks (***) for $p \leq 0.001$).

correlation coefficient was 0.832, meaning the results from the two methods were highly correlated ($p < 0.05$).

There was no significant correlation between total solids and blood glucose ($p = 0.055$), cholesterol ($p = 0.836$), triglyceride ($p = 0.171$), sodium ($p = 0.811$), or chloride ($p = 0.693$).

DISCUSSION

Comparative findings

Lashev et al. (2009) reported that three Columbidae species had higher hemoglobin content and RBC count than other birds. Considering that the Hb values measured in the present study were higher than in this previous report, it can be assumed that Oriental turtle dove Hb levels that are at the upper limits of the Columbidae species. On the other hand, Oriental turtle doves had fewer heterophils, more lymphocytes, and smaller proportions of basophils, eosinophils, and monocytes than other birds, similar to most other Columbidae species (Lashev et al. 2009).

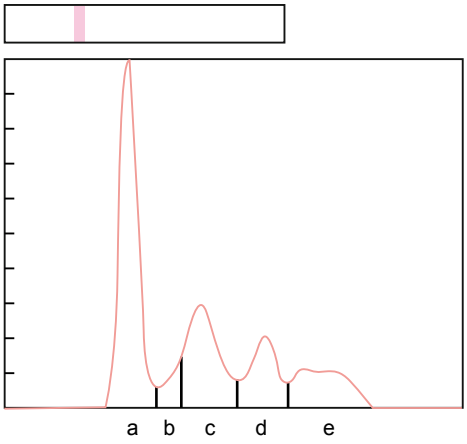

Fig. 1. Electrophoretic tracings and gels of representative samples from one clinically healthy turtle dove. a-e indicate each fraction: (a) albumin, (b) alpha$_1$ globulin, (c) alpha$_2$ globulin, (d) beta-globulin, and (e) gamma globulin, respectively.

Compared to pigeons (*Columba livia domestica*), Oriental turtle doves had higher AST, CK, glucose, sodium, and chloride concentrations. AST and CK are sensitive markers of muscular injury (Capitelli and Crosta 2013). Stress will cause elevated glucose concentrations (Harr 2002). Since they are not as tame as pigeons, the capture and physical restraint of Oriental turtle doves for blood sampling may lead to higher values of these three analytes. However, interspecies differences may also explain the results. While dehydration could cause increasing sodium and chloride concentrations (Capitelli and Crosta 2013), physical examination of these doves did not reveal any abnormalities related to dehydration. Thus, the higher sodium and chloride concentrations were because of interspecies differences.

Effect of gender

Generally, the total erythrocyte counts

Table 5. Protein electrophoresis fractions of 36 adult and eight sub-adult Oriental turtle doves (*Streptopelia orientalis*)

	Adult					Sub-adult	
Fraction/plasma concentrateon	*n*	Mean	SD	Minimun- Maximum[a]	90% CI of reference limits	*n*	All measured values[b]
Albumin (%)	36	40.96	4.67	31.63-50.30	33.26-48.66	8	31.1, 33.4, 34.2, 36.4, 39.5, 40.0, 44.5, 55.1
Alpha$_1$ globulin (%)	29	3.87	1.06	1.75-5.99	2.12-5.62	8	1.6, 1.6, 1.9, 2.7,3.5, 4.0, 6.2, 6.6
Alpha$_2$ globulin (%)	36	24.13	0.57	23.00-25.26	23.20-25.06	8	4.5, 21.0, 24.5, 25.0, 25.2, 28.5, 29.7, 29.8
Beta globulin (%)	36	13.22	4.10	5.02-21.42	6.45-19.99	8	13.0, 16.6, 18.2, 18.2, 20.5, 21.0, 22.7, 26.1
Gamma globulin (%)	28	13.84	1.06	11.72-15.96	12.09-15.59	8	11.9, 12.0, 12.8, 13.1, 13.3, 15.0, 15.0, 17.3
A/G ratio	36	0.7	0.07	0.56-0.84	0.59-0.82	8	0.5, 0.5, 0.5, 0.6, 0.7, 0.7, 0.8, 1.2
Albumin (g/dL)	36	1.3	0.13	1.04-1.55	1.09-1.51	8	0.68, 0.80, 0.88, 0.89, 1.07, 1.16, 1.17, 1.54
Alpha$_1$ globulin (g/dL)	29	0.31	0.05	0.02-0.21	0.04- 0.19	8	0.04, 0.04, 0.04, 0.07, 0.08, 0.14, 0.17, 0.17
Alpha$_2$ globulin (g/dL)	36	0.75	0.23	0.29-1.21	0.23-1.13	8	0.08, 0.13, 0.55, 0.62, 0.63, 0.65, 0.68, 0.77
Beta globulin (g/dL)	36	0.41	0.23	0-0.87	0.03-0.79	8	0.34, 0.40, 0.40, 0.45, 0.50, 0.53, 0.58, 0.91
Gamma globulin (g/dL)	27	0.41	0.07	0.27-0.55	0.30-0.53	8	0.08, 0.29, 0.30, 0.33, 0.33, 0.37, 0.42, 0.47

[a]When the sample size was less than 40 animals, reference intervals were determined with a range of values (minimum to maximum), rather than mean ± 2SD. [b]When sample size was between 10 and 20, all values were listed in ascending order.

and PCV tended to be lower in female birds compared with males. This difference may be a hormonal effect because estrogen depresses erythropoiesis (Campbell 2015). Several studies have shown sexual variation in some plasma biochemical parameters. In reproductively active female birds, plasma cholesterol and calcium can be significantly elevated due to vitellogenesis and egg production (Harr 2002). Plasma levels of sodium, potassium, total protein, albumin, and uric acid are higher in male than female pigeons, while female spotted doves (*Streptopelia chinensis*) had higher potassium levels than males (Gayathri and Hegde 1994). During our study period, neither egg production nor reproductive behavior were observed. Nor was there any follicular development detected when performing endoscopic sex determinations. These might partly explain why there were no differences in either hematology or plasma biochemistry between male and female Oriental turtle doves in the present study. Similar findings have also been reported in king eiders (*Somateria spectabilis*) and California condors (*Gymnogyps californianus*) (Milani 2009; Ferrer 1990).

Effect of age

Adult birds generally have higher PCV, Hb concentration, and RBC count than young birds (Le Souëf et al. 2013; Al-Gamal 2014). However, a study of Japanese quails revealed no significant differences between adults and sub-adults (Aina and Ajibade 2014). On the other hand, a study analyzing the correlation between blood components and age in sexually mature pigeons demonstrated that increasing age was associated with decreasing values of hematocrit and hemoglobin (Prinzinger and Misovic 2010). In the present study, there was no significant difference between adult and sub-adult doves. However, because age is difficult to assess in birds past sexual maturity (Le Souëf et al. 2013), we were not able to precisely age each individual, and age-correlated variation, as described above, may be one of the factors affecting our results.

Young birds generally have higher concentrations of alkaline phosphatase, phosphorus, and potassium, while having lower albumin, globulin, total protein, calcium, sodium, and chloride concentrations than adults, because of differences in growth hormone regulation, organ development, nutritional status, and antigen stimulation (Hochleithner 1994; Harr 2006; Naidoo et al. 2008). In pigeons, adults also have higher albumin, globulin, total protein, uric acid, sodium, and chloride values, while having lower cholesterol concentrations than young birds (Al-Gamal 2014). The present study demonstrated that age has similar influences on phosphorus, potassium, total protein, albumin, globulin, sodium, and chloride in Oriental turtle doves. However, cholesterol values were higher in adult Oriental turtle doves than pigeons, but similar to the northern bald ibis (*Feronticus eremita*) (Villegas et al. 2004).

Protein electrophoresis

Significantly higher total protein, albumin, and globulin values were observed in adult pigeons than young ones (Al-Gamal 2014). Similar findings were also observed in *Phasianus colchicus*, in which the globulin fraction increased with age. We observed a similar trend in Oriental turtle doves, a

Table 6. Sex and age differences in protein electrophoresis fractions of captive Oriental turtle doves (*Streptopelia orientalis*)

Analyte	Sex differences			Age differences		
	Male	Female	*p*-value	Adult	Sub-adult	*p*-value
Albumin (%)	-	-	0.420	H*	-	0.012
Globulin (%)	-	-	0.380	H***	-	0.001
$Alpha_1$ globulin (%)	-	-	0.123	-	-	0.110
$Alpha_2$ globulin (%)	-	-	0.934	H*	-	0.033
Beta globulin (%)	-	-	0.822	-	-	0.085
Gamma globulin (%)	-	-	0.394	-	-	0.196
A/G ratio	-	-	0.961	-	-	0.408

H means significantly higher than for the other sex or age. Asterisks indicate levels of significance: a single asterisk (*) for $p \leq 0.05$, double asterisks (**) for $p \leq 0.01$, and triple asterisks (***) for $p \leq 0.001$).

finding that may be related to increased antigenic exposure and immune response (Sibley and Johnsgard 1959). During the breeding season, reproductively active females have higher alpha globulin levels and a lower albumin to globulin ratio because of vitellogenesis (Harr 2002; Gayathri and Hegde 1994). Currently, there is no study describing Oriental turtle dove reproductive physiology and breeding requirements. During the study period, there was no reproductive behavior or egg production observed; therefore, there was no difference between adult male and female doves.

Analyzing the globulin fractions revealed that $alpha_2$ globulin concentrations in sub-adult Oriental turtle doves were lower than those in adults. A similar result - that alpha and gamma globulins increase with age - was reported for the chicken (Brandt et al. 1950). $Alpha_1$ and $alpha_2$ globulins are primarily components of plasma lipoproteins. The significant increase in $alpha_2$ globulin concentration in the adult Oriental turtle dove is most likely the consequence of increased transport of fatty substances to satisfy energy requirements and in-built into tissue structures (Filipović et al. 2007). Currently, species differences in protein electrophoresis are well recognized, and it is necessary to establish species-specific reference values (Werner and Reavill 1999). The reference values that this study established for Oriental turtle doves will benefit future medical and conservation actions.

Total solids and total protein

Factors that commonly affect refractometer readings include hemolysis, lipemia, and hyperglycemia (Harr 2002; Cray and Tatum 1998; Melillo 2013). However, when sample quality is maintained, some studies report good correlation while others present major discrepancies between total solids and plasma total protein (George 2001). Therefore, a species-specific study is warranted (Schmidt 2008). In the current study, we examined correlations between total solid and total protein readings, while considering that common analytes acting as artefacts to affect total solid readings include cholesterol, triglyceride, glucose, sodium, and chloride. However, only the total protein value is highly correlated with total solid readings. Therefore, with ideal sample quality, total solid values obtained by refractometry should be able to predict plasma total protein values in Oriental turtle doves.

Our study does succumb to certain limitations. Due to the size of the Oriental turtle dove, we had to use 29-gauge insulin needles for blood sampling. However, with care, we were able to avoid hemolysis during the procedure. Although we kept all of the captive doves in a uniform environment throughout the study, the lifestyle and diet of each individual were different before they entered the study. Such differences may manifest in variations in blood parameters, as previously demonstrated in wild and captive birds (Campbell 2015). In addition, because only 8 sub-adults were enrolled in the study, further study may be required to ensure these were representative of the entire population.

CONCLUSIONS

This study presents the first published reference ranges for Oriental turtle doves. The results of this study will provide baseline data for future investigations of free-living Oriental turtle doves and can be used in clinical veterinary practice and in the conservation of this species in the wild.

List of abbreviations

AST, aspartate aminotransferase.
CK, creatine kinase.
CI, confidence intervals.
EDTA, ethylenediaminetetraacetic acid.
GGT, gamma glutamyltransferase.
Hb, Hemoglobin concentration.
MCV, mean corpuscular volume.
MCH, mean corpuscular hemoglobin.
MCHC, mean corpuscular hemoglobin concentration.
NTUVH, National Taiwan University Veterinary Hospital.
PCV, Packed cell volume.
RBC, Red blood cell.
SD, standard deviation.
WBC, white blood cell.

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Author's contribution: ITT, PHY and CHC designed the study, ITT performed the sample and statistical analyses, and ITT and PHY wrote the manuscript.

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Ethics approval consent to participate: Our study was performed according to the regulation of Institutional Animal Care and Use Committee of National Taiwan University. Project number was 104-1.1-SB-23. Furthermore, our study was performed according to the international, national and institutional rules of animal experimentation.

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