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RESEARCH ARTICLE

Determination of hematological and serum biochemical values and detection of *Chlamydophila psittaci* antibodies in captive hornbills at the Ninoy Aquino Parks and Wildlife Nature Center

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ABSTRACT

Background: This study aimed to provide an initial report on hematologic and serum biochemical values and to detect an intracellular bacterium, *Chlamydophila psittaci* on Tarictic Hornbill (*Penelopides panini*), Rufous Hornbill (*Buceros hydrocorax*), and Palawan Hornbill (*Anthracoceros marchei*) held captive at the Ninoy Aquino parks and wildlife nature center. **Aims and Objectives:** Being indigenous to the Philippines, the initial reports of hematological values and the common diseases of this subspecies are significant to be determined and studied. This study was conducted to provide an initial report on hematological and serum biochemical values and the detection of antibodies against *C. psittaci* in the blood serum of Tarictic, Rufuos, and Palawan hornbill at the Ninoy Aquino Parks and Wildlife Nature Center. **Materials and Methods:** Captive birds at the Ninoy Aquino Parks and Wildlife Nature Center in the Philippines were used as samples. Blood was collected and subjected to hematology and serum analysis. **Results:** The obtained parameters for hematology are as follows: Hematocrit is 44% ± 3; total red blood cell (RBC) count is 10.24×10^6 cells/mm^3 ± 0.98; hemoglobin is 13.47 g/dL ± 0.73; mean corpuscular volume is 130.07 femtoliter ± 13.52; mean corpuscular hemoglobin (MCH) is 13.31 picogram (pg) ± 1.85; MCH concentration is 30.68% ± 2.88; total white blood cell (WBC) is 17.53×10^3 cells/mm^3 ± 2.89; total heterophil is 10.97×10^3 cells/mm^3 ± 2.68; total basophil is 1.16×10^3 cells/mm^3 ± 1.00; total eosinophil is 0.54×10^3 cells/mm^3 ± 0.40; total lymphocyte is 3.27×10^3 cells/mm^3 ± 1.33; total monocyte is 1.61×10^3 cells/mm^3 ± 0.75; and total thrombocyte is 236667×10^3 cells/mm^3 ± 6264.98. The obtained parameters for serum chemistry are as follows: Glucose is 237.34 mg/dl ± 27.33; cholesterol is 171.09 mg/dl ± 23.80; triglyceride is 116.26 mg/dl ± 17.67; high-density lipoprotein is 70.12 mg/dl ± 11.20; low-density lipoprotein (LDL) is 77.72 mg/dl ± 14.71; very-LDL is 23.25 mg/dl ± 3.53; blood urea nitrogen is 4.07 mg/dl ± 0.47; creatinine is 0.26 mg/dl ± 0.07; BUA is 21.49 mg/dl ± 1.97; SGPT is 50.90 Ul/L ± 15.99; and serum glutamic oxaloacetic transaminase is 310.69 Ul/L ± 38.10. The blood collected from the hornbills was then subjected to enzyme-linked immunosorbent assay test using the ImmunoComb® Avian *C. psittaci* Antibody Test Kit to detect the immunoglobulin G antibodies against *C. psittaci.* **Conclusion:** The resulting hematological and serum biochemical values do not differ much from the close relatives of the avians. Moreover, the results of the ImmunoComb® presented positive results for all nine samples indicating the high occurrence that the birds were infected before and have now recovered.

KEY WORDS: *Chlamydophila psittaci*; Enzyme-linked Immunosorbent Assay; Hematology; Hornbills; Philippines; Serum Chemistry

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INTRODUCTION

There are different species of hornbills and among those are the Palawan, Rufous, and Tarictic. These hornbills are classified under Kingdom Animalia, Phylum Chordata, Subphylum Vertebrata, Class Aves, Order Bucerotiformes, and Family Bucerotidae.[1] Although they are similar in

taxonomy until the family classification, they still possess characteristics that make them different from one another. The lack of standard hematological and serum biochemical values of different hornbills in the Philippines makes it difficult for clinicians to have a basis for diagnosis of diseases. Simply known as tarictic, the *Penelopides panini* is native to the islands of Guimaras, Negros, Panay, and Masbate which are located in the Philippines. Similarly, the *Buceros hydrocorax* and *Anthracoceros marchei* commonly known as Kalaw and Talusi, respectively, are also endemic to the Philippine islands. The Palawan hornbill or Talusi are endemic to the island of Palawan, but only a few sightings in small offshore islands have been recorded in the past years.[1] The Rufous hornbill or Kalaw, on the other hand, is also endemic in the Philippines specifically in Marinduque and Sierra Madre mountains but reports have confirmed that sightings have declined drastically.[2] Studies and observations of these birds also revealed that they are social creatures that emit loud calls and sounds to communicate in the rainforests. Most observations stated that the presence of the mating calls of these avians could be biological indicators of a healthy ecological system since hornbills are sensitive to changes in the environment.[3] The IUCN classified the Tarictic hornbills as endangered species, while the Palawan and Rufous hornbills are already vulnerable species. They are classified as such mainly because of the destruction of their natural habitat, poaching for their meat, and being sold at the live bird trade.[1] Researchers such as hematologic and blood chemistry values have already been performed for the Bucerotidae family including the genera of *Aceros*, *Anthracoceros*, and *Ceratogymna* that was captive in the Ornithological Park in Málaga, Spain. According to Villegas *et al.*,[4] the results they have obtained in their study was the first data available on reference ranges on blood values in individuals on the Bucerotidae family and may be useful as a diagnostic tool in the veterinary care of individuals of closely related species in captivity.

Even if mankind is the primary cause of the decrease in a number of the hornbills in the Philippines, intracellular bacteria known *Chlamydophila psittaci* greatly contributes to deaths of avians. According to West,[5] this organism depends on the host cell for energy and some amino acids like tryptophan. This is the reason why *Chlamydophila* is hard to culture in laboratories since it does not grow on a cell-free medium. This bacterium can cause diarrhea and respiratory syndrome to birds of all sizes that can eventually cause death.[6] Since *C. psittaci* is zoonotic and has a wide host spectrum in birds it can infect avian species with the disease avian psittacosis. Humans, on the other hand, can be affected with psittacosis or chlamydiosis. Having this kind of infections are hard to detect since psittacosis will present as non-specific clinical signs such as depression, anorexia, and bright green urates in birds.[7]

There is a specific concern with these particular bacteria because it has a zoonotic potential, which can be transferred into humans. According to a study conducted by Gaede *et al.*,[8] 24 individuals that came in close contact with infected poultry flocks during a *C. psittaci* outbreak in Germany were diagnosis with chlamydiosis after showing severe clinical symptoms of such as diarrhea, interstitial pneumonia, and rhinitis. This outbreak also caused a death of a person after several weeks of intensive medical treatment. There are published studies regarding the presence of *C. psittaci* in avian samples at the Ninoy Aquino Parks and Wildlife Nature Center so there is a need to conduct this study, especially on endangered or vulnerable birds to help preserve the species. Recent studies about detection of antibodies in the Philippines were conducted by Maluping *et al.*[9] and Perez[10] at the Ninoy Aquino Parks and Wildlife Center. Maluping *et al.*[9] reported that 9 avian samples, including 6 psittacines and 3 raptors, had antibodies against *C. psittaci* out of the 36 sample population. In the study of Perez,[10] all avian samples consisting of 12 eagle-owl tested negative for *C. psittaci*. Although Perez[10] reported negative results, the *C. psittaci* Avian ImmunoComb® kit showed suspicious marking according to the ImmunoComb® chart.

The values obtained in this study can thus be used by veterinarians in wildlife facilities and in the wild, for research and diagnosis that can be used in curing or treating closely related species in the genus *Penelopides, Buceros,* and *Anthraceros*. Being endangered and vulnerable species, determination of the hematologic and serum biochemical values can greatly advance the diagnostic capacity of veterinarians. The detection of *C. psittaci* antibodies was also performed to assure that there is no current infection in the hornbills to make the initial reports of hematological and serum biochemical values valid. Furthermore, since some of the hornbills are located in the viewing area of the Wildlife Rescue Center, there is still a chance of human infection.

MATERIALS AND METHODS

Sample Population

The sample population of this study was 4 captive *P. panini*, 4 *B. hydrocorax,* and 1 *A. marchei* that came from Ninoy Aquino Parks and Wildlife Nature Center, Philippines. The birds which were used in the study were clinically normal birds free from wounds and were not in current treatment for any illnesses.

The birds are housed together in a permanent holding cage. The cage for hornbills is 2 × 4 × 8 meters and constructed with wire mesh that is adjacent to each other. They were given chopped fruits and vegetables once daily. A floor-type manner of feeding was practiced, and water was provided in either a large basin and replaced daily. The birds were given dietary supplements of vitamins, minerals, and electrolytes if supply is available. Ectoparasiticidal dipping of birds was

done once a year. However, some birds that were found to have heavy ectoparasite load were dusted with gamma powder.

All procedures described below have been approved by the De La Salle University Institutional Animal Care and Use Committee and the Biodiversity Management Bureau, Department of Environmental Resources Management.

Blood Collection and Preparation

The collection of blood took place during the early morning to avoid heat and the people visiting the park thus preventing stress in the birds. To capture the subjects, in preparation for blood collection, each bird was first, cast by approaching the bird from behind and folding the wings in normal position. The wings were then held against its body while the feet were held firmly as it was wrapped in cloth to be able to control its movement. This type of handling followed by the park's zookeepers is from the Basic Zookeeping Handbook by the International Congress of Zookeepers.

Drawing of blood was done by the park veterinarians through the cutaneous ulnar vein of the birds. Using a 1 mL disposable syringe and a 25-gauge needle, approximately 0.5 mL of blood was taken from each bird. The blood was transferred into EDTA hematology tubes, shaken gently and placed in an ice chest maintained at approximately 4°C to minimize cellular degeneration. The blood samples were then analyzed within 6–8 h of blood collection.

Hematological Value Determination

The total erythrocyte, leukocyte, and thrombocyte counts were determined using the Natt and Herrick's method as described by Thrall *et al.*[11] and Harrison and Lightfoot.[12] Duplicate readings were made for each sample, and the mean was taken and recorded.

The PCV was measured using the microhematocrit method described by Coles[13] while the hemoglobin concentration was determined using the cyanmethemoglobin method. The RBC indices were calculated and expressed as femtoliter (fL) for mean corpuscular volume (MCV), pg for mean corpuscular hemoglobin (MCH), and percent (%) for MCH concentration (MCHC) as described by Ritchie *et al.*[14]

Blood smears were prepared and stained using the Giemsa staining technique. These smears were used for the differential leukocyte count, and the mean was taken after duplicated readings for each sample following the description of Coles.[13]

Serum Biochemical Value Determination

Blood samples collected were allowed to coagulate, and serum was sent to a diagnostic laboratory with fully-automated equipment while the low-density lipoprotein (LDL) and very-LDL (VLDL) were calculated.

Detection of *C. psittaci* Antibody

The enzyme-linked immunosorbent assay (ELISA) test kit for avian *C. psittaci* (ImmunoComb®) used detects immunoglobulin G-antibodies (IgG) against *C. psittaci.* IgG is produced during the later parts of the immune process and is the most abundant Ig. The ELISA test kit uses solid phase immunoassay containing antigen principle. In the process, when an antigen attaches to a solid phase, there is a direct reaction with an enzyme-linked antiserum. With this method, an estimation of an enzyme-labeled antibody, specific for an antigen is done.

The procedure provided by the manufacturer was followed when the ELISA test was conducted. The results of the hornbills were read by comparing the shade of gray of the rest result with the CombScale card. After which, the results were also read using the CombScan.

Data Analysis

The mean ± standard deviation was computed for the hematological values reported as a range. For the ELISA results, the relative absorbance was computed. These results were translated into clinical results. The clinical results were evaluated based on the variability of response of parrots to the ImmunoComb® Avian *C. psittaci* antibody test kit.

RESULTS

The blood samples obtained from the avian samples were tested for total RBC, packed cell volume, hemoglobin, erythrocytic indices, total WBC, differential WBC, thrombocyte, serum chemistry, and ELISA using an antibody test kit. Results of the hematological values are shown in Tables 1 and 2, while those of the different biochemical tests are shown in Tables 3 and 4.

From a sample of nine captive birds, a total of nine avian demonstrated antibodies against *C. psittaci* [Table 5]. Three *P. panini*, four *B. hydrocorax*, and one *A. marchei* have a CombScale value of one (1=suspicious). One *P. panini* showed a CmbScale value of 2 (2=low positive).

DISCUSSION

There have been no studies regarding the hematology for these specific genera of hornbills yet. This is mainly because the birds are endemic to the Philippines, which limit other researchers from conducting studies, and the birds are under the category of endangered and vulnerable according to the IUCN.[1]

Table 1: Hematological values of captive Tarictic hornbill (*P. panini*), Rufous hornbill (*B. hydrocorax*), and Palawan hornbill (*A. marchei*)

Parameters	Unit	Tarictic hornbill (mean±SD [range])	Rufous hornbill (mean±SD [range])	Palawan hornbill
Hematocrit	%	44±0.03 (43.97–44.03)	44±0.05 (43.95–44.05)	45
Total RBC	10^6 cells/mm^3	10.38±1.12 (9.26–11.5)	10.09±1.12 (8.97–11.21)	10.29
Hgb	g/dL	13.2±0.88 (12.32–14.08)	13.7±0.68 (13.02–14.38)	13.6
MCV	fL	129.47±20.12 (109.35–149.59)	130.39±9.04 (121.35–139.43)	131.20
MCH	pg	12.88±2.11 (10.77–14.99)	13.75±2.03 (11.72–15.78)	13.22
MCHC	%	29.82±0.36 (29.46–30.18)	31.66±4.44 (27.22–36.1)	30.22
Total WBC	10^3 cells/mm^3	18.78±3.42 (15.36–22.2)	16.74±2.56 (14.18–19.3)	15.67
Total heterophil	10^3cells/mm^3	12.51±2.98 (9.53–15.49)	10.53±1.81 (8.72–12.34)	6.58
Total basophil	10^3cells/mm^3	0.37±0.21 (0.16–0.58)	1.69±0.44 (1.25–2.13)	2.19
Total eosinophil	10^3 cells/mm^3	0.23±0.41 (−0.18–0.64)	0.74±0.41 (0.33–1.15)	0.94
Total lymphocyte	10^3 cells/mm^3	4.33±0.84 (3.49–5.17)	2.00±1.65 (0.35–3.65)	4.07
Total monocyte	10^3 cells/mm^3	1.4±0.22 (1.18–1.62)	1.75±0.50 (1.25–2.25)	1.99
Thrombocyte	10^3 cells/mm^3	25750.0±4784.14 (20965.86–30534.14)	21750.0±8421.20 (13328.8–30171.2)	230000.0

A. marchei: *Anthracoceros marchei, P. panini: Penelopides Panini, B. hydrocorax*: Buceros hydrocorax, fL: Femtoliter, SD: Standard deviation, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell

The hematocrit value of Palawan hornbill is best compared with the values of *Anthracoceros* from the study of Villegas *et al.*[4] since they belong to the same genus. Their study was only limited to hematocrit value and serum chemistry. The other hematological values of Tarictic hornbill and Palawan hornbill, however, are compared to hornbill species from Fowler and Miller[15] since no hematological studies have been conducted yet for the genus of *Penelopides* and *Anthracoceros*. The hornbills from their study belong in the same order as Tarictic hornbill. Rufous hornbill is compared with the values of *Buceros rhinoceros* coming from the same study since they are the same in genus and only differ in species. The species of comparison for secondary blood indices are from *Bucorvus abyssinicus* and *Buceros bicornis* also obtained by Fowler and Miller[15] since no studies have been made yet for these indices. The PCV value obtained from Tarictic hornbill, Rufous hornbill, and Palawan hornbill is 44% ± 0.3, 44% ± 0.5, and 45% with ranges of 42–46%, 39–49%, and 45%, respectively. These values are comparable to the range of closely-related hornbills from the study conducted by Villegas *et al.*[4] A normal PCV value is important because it means that the birds are in the correct environmental condition. Sufficient oxygen is in the surrounding air since low oxygen could lead to hypoxia which will cause an increase in RBC production.[16] The total RBC counts obtained from the hornbills are 10.38×10^6 cells/mm^3 ± 1.12, 10.09×10^6 cells/mm^3 ± 1.12, and 10.29×10^6 cells/mm^3, with ranges of 9.33–11.94 × 10^6 cells/mm^3, 9.06–11.67 × 10^6 cells/mm^3, and 10.29 × 10^6 cells/mm^3. These values are comparable with the range of total RBC counts from *Aceros corrugatus, Buceros rhinoceros,* and *Rhyticeros undulatus* obtained by Fowler and Miller.[15] The hemoglobin counts of the captive hornbills are 13.2 g/dL ± 0.88, 13.7 g/dL ± 0.68, and 13.6 g/dL with ranges of 12.30–14.10 g/dL, 12.70–14.20 g/dL, and 13.60 g/dL. These values are within the range gathered by Fowler and Miller.[15] Low counts of hemoglobin would indicate that the birds have anemia.[17] The secondary blood indices involve MCV, MCH, and mean corpuscular hemoglobin concentration. The obtained MCV from Tarictic hornbill, Rufous hornbill, and Palawan hornbill are 130.07 fL ± 13.52 having a range of 116.54–143.59 fL. This did not fall within the range of MCV obtained by Fowler and Miller[15] for *B. abyssinicus* and *B. bicornis* having a range of 157.41–239.8 fL. Having low MCV values is a sign

Table 2: Mean hematological values for all captive Tarictic hornbill (*P. panini*), Rufous hornbill (*B. hydrocorax*), and Palawan hornbill (*A. marchei*)

Parameter	Unit	All (mean±SD [range])
Hematocrit	%	44±3 (41–47)
Total RBC	10^6 cells/mm^3	10.24±0.98 (9.26–11.22)
Hgb	g/dL	13.47±0.73 (12.74–14.19)
MCV	fL	130.07±13.52 (116.54–143.59)
MCH	pg	13.31±1.85 (11.46–15.15)
MCHC	%	30.68±2.88 (27.80–33.57)
Total WBC	10^3 cells/mm^3	17.53±2.89 (14.63–20.42)
Total heterophil	10^3cells/mm^3	10.97±2.68 (8.29–13.65)
Total basophil	10^3cells/mm^3	1.16±1.00 (0.16–2.16)
Total eosinophil	10^3 cells/mm^3	0.54±0.40 (0.14–0.93)
Total lymphocyte	10^3 cells/mm^3	3.27±1.33 (1.94–4.60)
Total monocyte	10^3 cells/mm^3	1.61±0.75 (0.86–2.37)
Thrombocyte	10^3 cells/mm^3	23666.67±6264.98 (17401.68–29931.65)

A. marchei: Anthracoceros marchei, P. panini: Penelopides *Panini, B. hydrocorax: Buceros hydrocorax*, fL: femtoliter, SD: Standard deviation, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, pg: Picogram, WBC: White blood cell

of microcytic anemia.[16] Another reason is a defect in the synthesis of the heme group which could cause microcytic anemia.[18] High MCV values could be due to newly synthesized erythrocytes. The values of Fowler and Miller[15] came from the species *Bucorvus abyssinicus* which is one of the two largest hornbill species, and this can be attributed for the differences in the values obtained. The MCH value obtained from the sample is 13.31 pg ± 1.85 having a range of 11.46–15.15 pg. This is again lower than the values from of Fowler and Miller[15] having a range of 49.5–75.3 pg. It is possible that the difference in MCH count is due physiological difference.[19] This could affect the blood cell characteristics of avians like cell size. The mean corpuscular hemoglobin concentration values of the sample are 30.68%±2.88 having a range of 27.80–33.57% which is within the values of MCHC from Fowler and Miller.[15] MCHC is the measure of hemoglobin concentration in a definite volume of blood. This means that the experimental value obtained is normal. Both MCH and MCHC values can be affected by age, gender, and environmental condition differences.[20]

The WBC counts of Tarictic hornbill, Rufous hornbill, and Palawan hornbill are 18.78 × 10^3 cells/mm^3 ± 3.42, 16.74 × 10^3 cells/mm^3 ± 2.56, and 15.67 × 10^3 cells/mm^3, respectively, which is within the range of the values from Fowler and Miller.[15] Environmental stressors could increase the WBC including temperature, nutritional deprivation, and psychological distress.[21] The heterophil counts Tarictic hornbill, Rufous hornbill, and Palawan hornbill are 12.51 × 10^3 cells/mm^3 ± 2.98, 10.53 × 10^3 cells/mm^3 ± 1.81, and 6.58 × 10^3 cells/mm^3, respectively, which fall within the range of values from closely related birds established by Fowler and Miller.[15] Heterophil count of sampled birds is within 2.5–21.64 × 10^3 cells/mm^3. Possible reason for an increase in heterophil is due to the response to stressors during the handling and collection of blood samples.[22] Heterophil counts can also be affected by seasonal changes, gender, and diet.[20] The hormonal response of the birds is linked to the leukocyte counts. The mean eosinophil counts of the birds are 0.23 × 10^3 cells/mm^3 0.41, 0.74 × 10^3 cells/mm^3 ± 0.41, and 0.94 × 10^3 cells/mm^3, respectively. These values are within the established baseline by Fowler and Miller.[15] Little is known about the eosinophils of avians but being within the established values suggests that the eosinophils of the sampled birds are normal. Mammalian eosinophils are for defense against helminthic infections; however, the functions for avian eosinophils are still not understood. The basophil counts of the sampled birds are 0.37 × 10^3 cells/mm^3 ± 0.21, 1.69 × 10^3 cells/mm^3 ± 0.44, and 2.19 × 10^3 cells/mm^3, respectively. These values lie within the range of closely related hornbill species obtained by Fowler and Miller.[15] Basophils are involved in medication allergic reactions.[16] It is possible that allergies could occur due to the enclosure of the birds, however, the hornbills were not tested for allergies. The lymphocyte counts of the hornbills are 4.3310^3 cells/mm^3 ± 0.84, 2.0010^3 cells/mm^3 ± 1.65, and 4.0710^3 cells/mm^3 with ranges of 3.49–5.17 × 10^3 cells/mm^3, 0.35–3.65 × 10^3 cells/mm^3, and 4.07 × 10^3 cells/mm^3. The values fall within the range of lymphocyte counts done by Fowler and Miller[15] for closely related hornbill species. The monocyte counts of the hornbills are 1.4 × 10^3 cells/mm^3 ± 0.22, 1.7510^3 cells/mm^3, and 1.75 × 10^3 cells/mm ± 0.50 with ranges 1.18–1.6210^3 cells/mm^3, 1.18–1.62 × 10^3 cells/mm^3, and 1.99 × 10^3 cells/mm^3. These values are within the range of established baseline by Fowler and Miller.[15] Monocytes can be affected by nutritional deficiencies, which could cause an increase in monocyte count.[20] This would depend on the diet of the bird if the nutritional needs are met.

The thrombocyte counts for Tarictic Hornbill, Rufous Hornbill, and Palawan hornbill are 25750.0 × 10^3 cells/mm^3 ± 4784.14, 21750.0 × 10^3 cells/mm^3 ± 8421.20, and 230000.0 × 10^3 cells/mm^3, respectively. Normal thrombocyte counts for avians range from 20,000 to 300,000 × 10^3 cells/mm^3.[15]

All hematological parameters are comparable with the values from closely-related species with the exception of MCV

Table 3: Serum biochemical values of captive Taractic hornbill (*P. panini*), Rufous hornbill (*B. hydrocorax*), and Palawan hornbill (*A. marchei*)

Parameter	Unit	Tarictic hornbill (mean±SD [range])	Rufous hornbill (mean±SD [range])	Palawan hornbill
Glucose	mg/dl	235.58±8.81 (226.77–244.39)	222.89±15.10 (207.79–237.99)	302.22
Cholesterol	mg/dl	165.41±19.72 (145.69–185.13)	180.68±29.58 (151.1–210.26)	155.45
Triglyceride	mg/dl	107.10±21.75 (85.35–128.85)	119.07±4.61 (114.46–123.68)	141.71
HDL	mg/dl	66.80±10.41 (56.39–77.21)	73.76±13.90 (59.86–87.66)	68.83
LDL	mg/dl	77.19±10.71 (66.48–87.9)	83.11±17.25 (65.86–100.36)	58.28
VLDL	mg/dl	21.42±4.35 (17.07–25.77)	23.81±0.92 (22.89–24.73)	28.34
BUN	mg/dl	4.01±0.18 (3.83–4.19)	4.01±0.68 (3.33–4.69)	4.54
Crea	mg/dl	0.25±0.09 (0.16–0.34)	0.25±0.04 (0.21–0.29)	0.33
BUA	mg/dl	20.83±2.32 (18.6–23.06)	22.51±1.51 (21–24.02)	19.99
SGPT	Ul/L	54.73±8.44 (46.29–63.17)	50.63±22.89 (27.74–73.52)	36.70
SGOT	Ul/L	303.73±55.98 (247.75–359.71)	321.70±20.57 (301.13–342.27)	294.50

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, *A. marchei: Anthracoceros marchei, P. panini: Penelopides Panini, B. hydrocorax*: Buceros hydrocorax, BUN: Blood urea nitrogen, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SD: Standard deviation

and MCH which was below the range from the hornbill of comparison. This could be attributed to age, gender, and environmental differences.[20] The age and gender of the sampled birds were not determined by the researchers. Stress and handling of the birds could also play a factor for the heterophil counts.[22] Environmental conditions that are favorable to the birds also help in reducing stress.[21,23-25] The difference in the climate and environment of the birds used as a reference could also contribute the values obtained. Hematological parameters are also attributed to the measure of adrenal hormones, and it is also linked to leukocyte responses. Hormonal stress responses are indications for leukocyte count changes.[26] This would also affect the differential leukocyte counts of the birds. Stress is an important factor for all hematological parameters and should be taken into consideration because handling and blood collection of the birds increases stress. Environmental conditions should also be noted as it also contributes to the stress levels of the birds.

In relation to the study conducted by Villegas *et al*.[4] in Malaga, Spain, using the genera *Aceros, Anthracoceros*, and *Ceratogymna*, which are of different species utilized in this study, the species *P. panini* (Taractic hornbill)*, B. hydrocorax* (Rufous hornbill)*, and A. marchei* (Palawan hornbill) unfortunately have no distinct standard values for blood serum chemistry and were studied mainly to set normal values for researchers who will conduct studies on birds endemic to the Philippines. These endemic birds are considered to be endangered and vulnerable according to the IUCN.[1] The values that were used to compare the serum biochemical results of *P. panini* (Taractic hornbill)*, B. hydrocorax* (Rufous hornbill)*, and A. marchei* (Palawan hornbill) came from its closely-related species, namely, *Aceros, Ceratogymna*, and *Anthracoceros*. Since the lack of a closer-related species, these genera are the only ones with established and published serum biochemical values that could be of comparison to the birds being studied. Furthermore, *Aceros, Ceratogymna*, and *Anthracoceros* are also captive hornbills just like the birds that were used in this study. The mean for the 3 species was obtained mainly because the establishment of values for the Palawan hornbill (*A. marchei*) is not enough.

Glucose concentrations of 235.58 mg/dl, 222.89 mg/dl, and 302.22 mg/dl from Taractic Hornbill, Rufous Hornbill, and Palawan Hornbill, respectively, when compared with the standard range of glucose concentration of 238.0–356.0 mg/dl and 291.0–381.0 mg/dl, can be deduced that the Taractic Hornbill with a value of 235.58 mg/dl and the Rufous Hornbill with a value of 222.89 mg/dl fell short from the range of values while the Palawan Hornbill fell in place with 302.22 mg/L for the *Aceros*, while no experimental value lying between the range of values of *Anthracoceros*.[4] Furthermore, a plausible explanation is that different species of hornbills may have varying normal glucose concentrations

in their bloodstream and does not necessarily indicate poor sugar diet or disease.[4] Another reason of possible difference in blood glucose concentration is the occurrence of husbandry practices, which can cause shifts in blood glucose concentration.[16] The level of activeness or exercise the hornbills go through can also cause glucose concentration difference.[20]

Cholesterol concentrations for Tarictic Hornbill, Rufous Hornbill, and Palawan Hornbill all fell in the range of values set at 109.0–468.0 mg/dl from *Aceros* giving the impression that cholesterol concentrations of the hornbills are at par with a study conducted by Villegas *et al*.[4] from Malaga Spain, while no experimental value fell between the standard range of values from *Anthracoceros* of 188.0 to 305.0 mg/dl.[27] Triglyceride concentrations for all hornbills lie within the standard range of value of 38.0–211.0 mg/dl for *Aceros* and 73.0–178.0 mg/dl for *Anthracoceros* that gives the researchers a good idea about the balance between the *Aceros* and *Anthracoceros'* bi-directional transference of adipose and blood glucose that excludes the Hornbill from the risk of coronary artery diseases and stroke.[28] High-density lipoprotein (HDL) concentrations in the bloodstream of the hornbills had values ranging from 56.39 to 77.21 mg/dl, 59.86–87.66 mg/dl, and 68.83 mg/dl for Tarictic hornbill, Rufous hornbill, and Palawan hornbill, respectively. Normal concentrations of HDL would result in a better LDL or bad cholesterol scavenging that could help the avians in keeping endothelial walls clean from plaques and reprocessing bad cholesterol in the liver.[29] LDL concentrations had values ranging from 66.48 to 87.9 mg/dl, 65.86–100.36 mg/dl, 58.28 mg/dl for Tarictic hornbill, Rufous hornbill, and Palawan hornbill, respectively. High concentrations of LDL in the bloodstream could cause plaque build-up and block blood vessels from performing its function in blood circulation.[15] VLDL concentrations had values ranging from 17.07 to 25.77 mg/dl, 22.89–24.73 mg/dl, and 28.34 mg/dl for Tarictic hornill, Rufous hornbill, and Palawan hornbill, respectively. High concentrations of VLDL in the bloodstream would permit free flow of cholesterol and fats and could cause plaque build-up in endothelial walls.[30]

Table 4: Meam serum biochemical values for all captive Tarictic hornbill (*P. panini*), Rufous hornbill (*B. hydrocorax*), and Palawan hornbill (*A. marchei*)

Parameter	Unit	All three species mean±SD (range)
Glucose	mg/dl	237.34±27.33 (210.01–264.67)
Cholesterol	mg/dl	171.09±23.80 (147.29–194.90)
Triglyceride	mg/dl	116.26±17.67 (98.59–133.94)
HDL	mg/dl	70.12±11.20 (58.92–81.32)
LDL	mg/dl	77.72±14.71 (63.0–92.43)
VLDL	mg/dl	23.25±3.53 (19.72–26.79)
BUN	mg/dl	4.07±0.47 (3.60–4.54)
Crea	mg/dl	0.26±0.07 (0.20–0.33)
BUA	mg/dl	21.49±1.97 (19.51–23.46)
SGPT	Ul/L	50.90±15.99 (34.91–66.89)
SGOT	Ul/L	310.69±38.10 (272.59–348.79)

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, *A. marchei: Anthracoceros marchei, P. panini: Penelopides Panini, B. hydrocorax*: Buceros hydrocorax, BUN: Blood urea nitrogen, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SD: Standard deviation

Blood Urea concentration for all hornbills lies within the standard range of value of 2.0–12.0 mg/dl for *Aceros* and 3.0–8.0 for *Anthracoceros*.[4] With normal concentrations of blood urea in the bloodstream, the kidneys of the hornbills are optimally functioning because it is able to filter urea from the blood properly.[29] Creatinine concentrations had values with a range of 0.16–0.34 mg/dl, 0.21–0.29 mg/dl, and 0.33 mg/dl for Tarictic hornbill, Rufous hornbill, and Palawan hornbill, respectively. High creatinine content in the body of avians

Table 5: Comb scan reading of blood samples from Tarictic, Rufous, and Palawan hornbills tested for *C. psittaci*

Comb value	IgG (MAT) Titer	Tarictic hornbill	Rufous hornbill	Palawan hornbill	Total
0	0	-	-	-	0
1	<1:50	3	4	1	8
2	1:50	1	-	-	0
3	1:100	-	-	-	0
4	1:200	-	-	-	0
5	1:400	-	-	-	0
6	1:800	-	-	-	0
>6	>1:800	-	-	-	0
	Total	4	4	1	9

C. psittaci: Chlamydophila psittaci, IgG: Immunoglobulin G

could indicate reduced blood flow to the kidney.[31] Blood Uric Acid concentrations for Tarictic Hornbills and Palawan Hornbill fell within the range of value of 3.0–20.8 mg/dl for *Aceros*.[4] Rufous Hornbill for *Aceros* and all Hornbills correlated with *Anthracoceros*, on the other hand, has high blood uric acid concentration compared to a standard range of 2.3–12.2 mg/dl for *Anthrococeros* and a diet high in foods with high uric acid content such as nuts, beans, and the like may be applicable to the Hornbill.[4]

Serum glutamic pyruvic transaminase (SGPT) concentration for all hornbills lie within the standard range of values of 4.1–144.0 UI/L for the genera *Aceros* while the experimental values fell short of the range of values from *Anthrococeros* at 291.0 to 381.0 UI/L.[4] Liver function for all the hornbills is in optimal condition. The liver enzyme alanine aminotransferase (ALT) is working optimally and remains in the liver and not out of the bloodstream giving the impression that the liver is not damaged, inflamed, or diseased, while the standard range of values correlated from *Anthracoceros* suggests that there are limited amounts of ALT or SGPT concentrations in the blood leaving the conclusion that the hornbills took too much fluids.[32] Serum glutamic oxaloacetic transaminase (SGOT) concentrations for Tarictic hornbill and Palawan hornbill lie within the standard range of values of 91.3–304.6 UI/L, which indicates the optimal function of the kidney, liver, heart, brain, and muscle.[32] While the Rufous hornbill lies above the standard range of values with a value of 321.70 UI/L, which can indicate that AST (aspartate transaminase) leaked out of the different organs into the bloodstream and indicates a possible damage in one of the organs.[29] SGOT concentrations for the correlation from *Anthracoceros* fall between the standard range of value of 182.6–391.8 UI/L.[4] In nature, captive birds have the tendency for lower cholesterol, AP, hematocrit and LDH levels with higher uric acid, calcium, total protein, AST, and phosphorus concentrations than their free-living counterparts. Activity level and muscle tone, diet composition, and quality or health status are factors that might be the reason their differences.[32]

All serum biochemical parameters are comparable to its closely related species. The deviations were primarily caused by geographical differences, environmental factors, evolutionary differences, and not by sickness or disease.[11] Factors that could affect serum biochemical parameters would be the level of activeness of the birds. Low activity or exercise could pave the way for unhealthiness with increasing blood glucose, cholesterol, LDL, VLDL, and triglyceride, which can cause the plaque build-up in the hornbills' blood vessels and could, therefore, lead to inefficient blood circulation and liver function because of its burden to recycle and reprocess LDL in high concentrations.[32] Another factor is diet and aviary dimension. Food that is given to the hornbills might be high in fat, or food comes too regularly, not compensating with the hornbills' small aviary wherein they are limited from maximizing husbandry practices and exercise.[16]

The presence of the IgG antibodies could mean that they were exposed to the bacteria *C. psittaci* in the past and immunoglobuin G is an antibody that is known to bind with specific antigens.[33] This type of antibodies are long-lasting, and it may still have traces in the bird's immune system. Based on a study conducted in the same wildlife park by Maluping *et al.,*[9] their results showed that the *A. marchei* and *B. hydrocorax* were negative for *C. psittaci*. The results of the ELISA test just showed that the animals might have been infected as there is the presence of an antibody for *C. psittaci* but it is not safe to say that hornbills are infected at the present. *C. psittaci* bacteria are avian strains that can be transmitted to humans. However, it may or may not manifest itself in infected individuals.[34] The bacteria pose a threat to humans, most especially for the people of the Philippines since not enough information is made known to the public. Thus, it is important to detect its incidence in the avian population.[6] The presence of antibodies against *C. psittaci* can also be linked to the arrangement of the enclosure in the wildlife rescue center. Fellow researchers tested different avian species that is caged exactly beside each other such as the Palawan Hill Mynah and the Blue-naped parrot, and it also showed the presence of the specific antibody. It is also in very close proximity with the different species of hornbills. This suggests that previous infections of *C. psittaci* in the avian sample were due to transmission because of close contact. Furthermore, a previous study conducted by Perez,[10] showed negative results for *C. psittaci* since all avian samples consisting of 12 eagle-owls are isolated far from the enclosure of the hornbills, Palawan hill mynah, and Blue-naped parrot.

CONCLUSION

The study was specifically about providing initial reports of hematological and serum biochemical values of Tarictic hornbill (*P. panini*), Rufous hornbill (*B. hydrocorax*), and Palawan hornbill (*A. marchei*) that can be used in comparison for captive hornbills and closely related species. The detection of *C. psittaci* was to check and clarify initial studies conducted in the park to make sure the initial reports were invalid because of a bacterial infection. In general, the hematological values of all sampled hornbills are within the range of counts in comparison to other closely related species. The only outliers are the MCH and MCV values since the captive birds have lower values from the reference count. This could be an indication of microcytic anemia wherein the RBCs produced are smaller in volume than the normal size. It is recommended by the group that the birds are tested regularly and monitored more often and treated as soon as the diagnosis is confirmed. In general, all three species of hornbills, namely, Tarictic hornbill (*P. panini*), Rufous hornbill (*B. hydrocorax*), and Palawan hornbill (*A. marchei*) are healthy. Most serum biochemical test result fell in the range of values, which in turn caused the group to

deduce the overall healthiness and optimal functioning of the hornbills' vital organs are going well. Although some of the values for some specific serum biochemical test went higher or lower, the correlation of the values is well in tune, and the experimental value does not fall far from the range of standard values. The deviation of the few experimental values from the range of standard values was not primarily caused by sickness or disease. It could be caused by an imbalanced diet, environmental factors, geographical differences, and evolutionary differences.

The results suggest that nine samples or 100% of the samples collected from the Ninoy Aquino Parks and Wildlife Rescue Center have been exposed to the intracellular bacteria *C. psittaci* thus the presence of antibodies for it. It presented suspicious to low positive results meaning there is a possibility that it has already recovered from the exposure to the bacteria or it can infect other avian and humans with close contact to it.

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