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RESEARCH ARTICLE

Determination of the hematological values and detection of *Chlamydophila psittaci* antibodies in captive Palawan Hill Mynah at the Ninoy Aquino Parks and Wildlife Nature Center

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ABSTRACT

Background: *Gracula religiosa palawanensis* is a subspecies of the avian species *Gracula religiosa* under the order Passeriformes. Birds belonging to this order are famously and distinctly known for their unique ability to imitate sounds. The particular subspecies is endemic to the Philippines, solely found in the elevated points of Balabac, Busuanga, Culion, and the Mainland Palawan. **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. The study aimed to provide an initial report on the hematological values and to detect the presence of antibodies against *Chlamydophila psittaci* in the blood serum of Palawan hill mynah held captive 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 packed cell volume (PCV), total red blood cell (RBC) count, RBC indices, hemoglobin concentration, total and differential white blood counts, and total thrombocyte counts were accounted for the hematological values of the three sampled *G. religiosa palawanensis*. Values of 45.00 ± 3.00%, 3.61 ± 0.20 × 10^6 cells/mm^3, 13.47 ± 0.78 g/dL, and 14.86 ± 2.05 × 10^3 cells/mm^3 were, respectively, observed for the PCV, total RBC counts, hemoglobin concentration, and total white blood cell (WBC) count. The mean corpuscular hemoglobin (MCH), MCH concentration, and mean cell volume values, the RBC indices, were found to be 37.35 ± 2.40 pg, 29.77 ± 2.08%, and 126.05 ± 14.76 femtoliter, respectively. The following showed the differential WBC count with their mean relative values and their mean absolute values enclosed in parentheses: 60.00 ± 8.00 % (8.95 ± 2.19 × 10^3 cells/mm^3), 1.00 ± 1.00 % (0.15 ± 0.16 × 10^3 cells/mm^3), 1.00 ± 1.00 % (0.20 ± 0.10 × 10^3 cells/mm^3), 37.00 ± 8.00% (5.33 ± 0.60 × 10^3 cells/mm^3), and 0.00 ± 1.00 % (0.05 ± 0.09 × 10^3 cells/mm^3). The values correspond to the heterophil, eosinophil basophil, lymphocyte, and monocyte counts accordingly. The presence of *C. psittaci* was observed from the avian specimens but was merely observed at low concentrations, having a low positive ImmunoComb® reading. **Conclusion:** The blood values of the Palawan Hill Mynah do not differ from their close relatives. Furthermore, the presence of *Chlamydophila psittaci* may show the previous infection to the bacteria.

KEY WORDS: *Chlamydophila psittaci*; ELISA; Hematology; Palawan hill Mynah; Philippines

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INTRODUCTION

Gracula religiosa palawanensis is of the class Aves, subclass Neognathae, order Passeriformes, and family Sturnidae. Also known as the Palawan Hill Mynah, it is one of the 10 known subspecies of Hill Mynahs and is the only one endemic to the Philippines.[1] The *G. religiosa* species

inhabits semi-evergreen or moist forests in hills, lowlands and mountains of Sri Lanka, India, and the Philippines; mainly in Palawan. It is known for its ability to mimic sound, including human speech. This is the reason why this species is one of the sought after avian pets. Hence, trade, along with habitat loss due to deforestation has resulted in significant population decline. The main threat to the survival of this species is exploitation and uncontrolled collection of juveniles and eggs due to the great commercial demand for this species.[2] Due to this regard, the *G. religiosa palawanensis* was included in the CITES Appendix II in 1997,[1,3] which is a list of species that are not in danger of extinction but will become so if the trade is not monitored. Along with exploitation, forest destruction also decreases Hill mynah population.[4]

The baseline hematological values of hill mynahs in Thailand that have been established by Archawaranon[5] are the closest available reference for the hematological values of *G. religiosa*. Since the *G. religiosa palawanensis* is a subspecies of the *G. religiosa*, the values established for it are the best possible reference to use. Initial reports on the hematological values of the Palawan Hill Mynah will greatly aid when curing this animal for any type of ailment. Knowing this data would increase the diagnostic capability of veterinarians significantly. Getting rid of disease as a possible threat for the survival of this species is evidently beneficial.[1]

Avian chlamydiosis is a zoonotic disease caused by *Chlamydophila psittaci*. This bacteria has been isolated from over 460 birds, mostly psittacine birds, such as parrots. The bacteria are also frequently found in pigeons and doves. However, all species of birds are susceptible to avian chlamydiosis. Infected avians spread the bacteria through feces and nasal discharges. Signs of infection are lethargy, ruffled feathers, anorexia, mucopurulent ocular, serous, nasal discharge, conjunctivitis, excretion of yellow-green to green urates, and diarrhea. It must be noted that *C. psittaci* can be transmitted from birds to humans.[6] Vanrompay *et al.*[7] found that in 39 breeding facilities for Psittaciformes that often utilized antimicrobial drugs, 14.9% of humans in those facilities were positive for *C. psittaci* genotypes. In another study, Dickx *et al.*[8] found that 12.5% of pigeon fanciers that work with homing pigeons in Belgium were positive for *C. psittaci*. Maluping *et al.*[9] were able to detect the presence of *Chlamydophila psittaci* antibodies in captive birds at the Wildlife Rescue Nature Center, Philippines. Their study consisted of 36 captive birds, 25% of which were positive for antibodies against *C. psittaci*. Perez[10] conducted a study to determine the presence of *C. psittaci* antibodies in 12 eagle owls. The results were negative, however, suspicious markings were observed on the ImmunoComb® after calibration.

The standard hematological values of this animal are still unknown, and this is an obstacle for those who want to pursue studies concerning the Palawan Hill Mynah. Not knowing the hematological values makes it difficult to determine if a certain Palawan Hill Mynah is healthy or not. Providing preliminary values for *G. religiosa palawanensis* will have a significant impact on the preservation and protection of the species and having initial values will aid in determining an individual's health status, thus resulting in improved health care for the species. Since the samples obtained came from Ninoy Aquino Parks and Wildlife Nature Center, which houses a number of avian species, it is imperative that the presence of *C. psittaci* antibodies are to be determined within the samples. Previous reports have confirmed that *C. psittaci* is indeed present in the park. Hence, detection for *C. psittaci* antibodies in the sampled Palawan hill mynahs is essential to determine whether the sampled birds are diseased or not. Finally, since the park is planning to rehabilitate and release subsequently the animals, knowing its status of infection will be beneficial so as not to release and infect the animals in the wild with *Chlamydophila psitacci*.

MATERIALS AND METHODS

Sample Population

All of the 3 captive Palawan hill mynah (*G. religiosa palawanensis*) from the 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 used in current treatment for any illnesses.

The birds are housed together in a permanent holding cage. They were given fruits or vegetables once daily. A floor-type manner of feeding was practiced and water was provided in either a large basin and replaced daily. Only sick or weak and newly rescued birds were given dietary supplements of vitamins, minerals, and electrolytes. Ectoparasiticide 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 diurnal fluctuations and to prevent stress in the birds. To capture the subjects, in preparation for blood collection, each bird was first, casted 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.

Drawing of blood, which was done by Dr. Rizza A. Salinas and Dr. Oscar Jhan E. Cabanayan, was done 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 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 packed cell volume (PCV) was measured using the microhematocrit method described by Coles[13] while the hemoglobin concentration was determined using the cyanmethemoglobin method (Appendix D). The red blood cell (RBC) indices were calculated and expressed as femtoliter (fL) for mean cell volume (MCV), picogram (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]

Detection of *Chlamydophila psittaci* Antibody

The 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 an indirect 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 Palawan hill mynah 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 *Chlamydophila psittaci* antibody test kit.

RESULTS

The obtained blood samples were tested for total RBC, white blood cell (WBC), thrombocytes, indices, and differential WBC count [Table 1] and indirect ELISA [Table 2]. Overall, the results for the Palawan Hill Mynah had similarities with the captive Thailand Hill Mynah, thus making their values comparable. The mean values for total RBC count, hematocrit, hemoglobin, the RBC indices, and thrombocytes of the Palawan Hill Mynah are all within the range of the RBC indices of the Thailand Hill Mynah. The total WBC and differential counts were in range except for the heterophils, eosinophils, and monocytes.

The ELISA results [Table 2] indicate that the sampled *G. religiosa palawanensis* is positive for *Chlamydophila*

Table 1: Hematological values of the Palawan Hill Mynah *(G. religiosa palawanensis)*

Parameters	Units	Mean±SD (range)
Erythrocytes	10^6 cells/mm^3%	3.61±0.20 (3.41–3.81)
PCV	%	45±3.00 (42–49)
Hemoglobin	g/dl	13.47±0.78 (12.69–14.24)
MCV	µm^3	126.05±14.76 (111.29–140.81)
MCH	pg	37.35±2.40 (34.95–39.75)
MCHC	g/dl	29.77±2.08 (27.69–31.85)
Leukocytes	10^3 cells/mm^3	14.68±2.05 (12.63–16.73)
Heterophils	10^3 cells/mm^3	8.95±2.19 (6.76–11.14)
Lymphocytes	10^3 cells/mm^3	5.33±0.60 (4.73–5.94)
Eosinophils	10^3 cells/mm^3	0.15±0.16 (0.00–0.31)
Basophils	10^3 cells/mm^3	0.20±0.10 (0.10–0.30)
Monocytes	10^3 cells/mm^3	0.05±0.09 (0.00–0.14)
Thrombocytes	cells/mm^3	27333.33±3055.05 (24278.28–30388.38)

PCV: Packed cell volume, MCV: Mean cell volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration. *G. religiosa palawanensis: Gracula religiosa palawanensis*

Table 2: CombScan reading of blood samples from Palawan Hill Mynah tested for *Chlamydophila psitaccii*

Comb value	IgG (MAT) Titer	Palawan Hill Mynah
0	0	-
1	<1:50	3
2	1:50	-
3	1:100	-
4	1:200	-
5	1:400	-
6	1:800	-
>6	>1:800	-
Total		3

C. psittaci: Chlamydophila psittaci, IgG: Immunoglobulin G

psittaci antibodies. The CombScan resulted in figures that showed an IgG (MAT) titer (i.e., concentration of IgG that the samples produced) of <1:50 for the three birds sampled. This reading is associated with the ImmunoComb® value of S1, which means a low positive presence of antibodies.

DISCUSSION

Results of the hematological testing are within the normal values of the Thailand Hill Mynah, unless otherwise noted. The average total RBC count ($3.61 \pm 0.20 \times 10^6$ cells/mm^3) falls within the range that set for the Thailand Hill Mynah: 3.75 ± 0.44.[5] A normal total RBC count is to be expected when the subject is exposed to a healthy unpolluted environment, and given proper diet and nutrition to meet its physiological demands.[15-17] It is noteworthy to mention that, though the animals may be held captive is not the most ideal conditions, the needed care and management of the animals are being provided by the rescue center. Furthermore, regular veterinary care is being given as to ensure that animals may be suitable for release back into the wild. The mean PCV obtained from the three birds is 45.00 ± 3.00% falls within the range of hematocrit values of captive Hill Mynahs in Thailand.[5] Since hematocrit is a reflection of the total number of RBCs and hydration status of an animals,[18] this shows that the animals neither have problems of both. The average Hgb obtained is 13.47 ± 0.78 g/dL. This falls within the range established for Thailand Hill Mynahs (14.26 ± 1.18 g/dL).[5] The production of hemoglobin by the body heavily is dependent on the nutrition and supplementation of an individual. Insufficient vitamin B_{12}, folic acid, and iron deficiencies result in a decline in hemoglobin synthesis. Thus, reducing the hemoglobin levels of an individual.[15] The MCH, MCHC, and MCV of the Palawan Hill Mynah are 37.35 ± 2.40pg, 29.77 ± 2.08%, and 126.05 ± 14.76 fL, respectively. These values are within range of the red cell indices of the captive Thailand Hill Mynah, which are 38.36 ± 3.57pg, 30.06 ± 1.53%, and 126.04 ± 11.69 fL for MCH, MCHC, and MCV, respectively.[5] RBC indices are used to determine the average RBC (MCV), hemoglobin (MCH), and the hemoglobin concentration. These help determine whether the individual is anemic or normal. Results that fall within the normal range indicate that the cells are normocytic and normochromic.[19]

The average total WBC count obtained is $14.86 \pm 2.05 \times 10^3$ cells/mm^3 is only slightly lower than the established value at low range (14.95×10^3 cells/mm^3).[5] It is not uncommon for avian leukograms to vary between normal birds of the same species. In addition, the normal total leukocyte reference intervals obtained from avians are generally broader than those that are obtained from domestic animals. Hence, for a diagnostic significance to be observed, the leukogram values must vary greatly from the established reference interval. Since, the obtained WBC count (14.68×10^3 cells/mm^3) is not very far off from the normal range ($20.77 \pm 5.82 \times 10^3$ cells/mm^3), it can be concluded that the difference is quite insignificant.[11]

The relative heterophil count was found at 60.00 ± 8.00% with a corresponding absolute value of $8.95 \pm 2.19 \times 10^3$ cells/mm^3. The figure collected for the heterophil count of the samples were evidently higher than what has been established for the said species.[5] During the blood collection, the Hill Mynahs may have been under stress, which may explain the higher values observed compared to the ones established.[20] No influential decrease, or increase in the lymphocyte count was recorded. The values observed in the Palawan Hill Mynah subjects were well within the range of figures as compared to the standard values noted in another study.[21] The mean relative count obtained for the lymphocytes is 37.00 ± 8.00%, which corresponds to a mean absolute count of $5.33 \pm 0.60 \times 10^3$ cells/mm^3. A significant decrease in the eosinophil count was found in the bird samples considered. The obtained mean relative eosinophil count was 1.00 ± 1.00% with a mean absolute value of $0.15 \pm 0.16 \times 10^3$ cells/mm^3. It is considered that the avian eosinophil function different as compared to mammalian eosinophil function. Studies assume that avian eosinophils are related to a type IV delayed hypersensitivity reaction. With this, the decreased count in eosinophils may mean that a secondary response was not anymore required as the antigen may have been eliminated by the initial immune response.[20] No significant difference was observed in the basophil count as compared to the established count for Hill Mynahs. The mean of the relative count was found to be at 1.00 ± 1.00 % with a mean absolute value of $0.20 \pm 0.10 \times 10^3$ cells/mm^3. The mean relative monocyte count was 0.00 ± 1.00%, which was associated to a mean absolute value of $0.05 \pm 0.09 \times 10^3$ cells/mm^3. Since heterophils and monocytes originate from an identical stem cell, the production of the cells may be regulated to compensate one another. In this case, the heterophils were aberrantly high, which resulted to monocytopenia or the lower count of monocytes.[20]

The normal range of thrombocyte concentration ranges between 20,000 and 30,000 cells/mm^3.[22] The obtained total thrombocyte count is 27,333.33 ± 3055.05 cells/mm^3, which is in range of the normal concentration value.

All the hematological values, mentioned above, that were obtained in this experiment were all within range of the normal values that were used as a reference. However, the heterophils, monocytes, and eosinophils are excluded as they have varied results from the established values. As mentioned earlier, factors such as climate, state of environment, stressors, degree of physiological activity, diet, age, sex, drug administration, and intake of vitamins are factors that affect hematological values and could be the reasons for such result. The countries Philippines and Thailand are both tropical countries. Hence, these countries have similar ecological parameters. This means that both countries

have the same temperature, humidity, amount of rainfall, and sunshine. One important fact to be considered is that the sampled Hill Mynahs were kept in captivity. Thus, the environmental situation of the sampled Hill Mynahs in this experiment is almost completely identical with those of tested in Thailand.[5] Collecting blood samples from avians induces stress, which causes changes in hematological values. This stressor, along with other possible stressors that come along with being in the environment that the samples were exposed to, is another condition that the sampled Hill Mynahs have in common.[22] Taking into consideration all the factors that can affect hematological values and observing that both samples had almost every factor in common explains why the hematological values of *G. religiosa palawanensis* are within range of the hematological values of the captive *G. religiosa* in Thailand.

Chlamydophila psittaci is a bacteria that is most prevalent in tropical and subtropical countries. Although no human-to-human transmission of this bacteria has been recorded, cases of bird-to-human have been observed. Psittacosis results from human inhalation of the organism, which is aerosolized from secretions that originated from the infected bird.[6,23] The certain bacteria are rampantly being transmitted from an infected bird to a healthy specimen, which results to chlamydiosis. In fact, the infection is prevalent among mynah birds.[24,25] Avian chlamydiosis may bring various diseases to such as anorexia, apathy, conjunctivitis, diarrhea, rhinorrhea, and slower hatching rates.[24,26] Antibodies against chlamydiosis were found in the three birds mainly because they are confined in a single enclosure. As mentioned earlier, a floor-type manner of feeding was employed as well as a single water basin. Weak or ill birds were not isolated from the group and were merely given vitamins and dietary supplements as medication. With this, the birds are in close proximity with each other, permitting a spread of diseases. These statements are supported by the previous statement regarding the mode of transmission of the bacteria *C. psittaci*.[6,8] Although the result showed a positive finding, the presence of antibodies is relatively low. This finding may correspond, not necessarily to an existing infection, but to an infection that has previously occurred. A study by Maluping *et al.*[9] and Perez[10] in the same park, together with the results of this investigation yielded positive presence of *C. psittaci* antibodies, it can be deduced that the bacteria are indeed still present in the park and are indeed being transmitted. With this better monitoring and surveillance of the disease is warranted particularly due to its zoonotic nature.

The study focused on trying to establish as baseline data for the hematological parameters for the Palawan Hill Mynah. Since the Palawan Hill Mynah are already considered under threat and is endemic in the Philippines, obtaining samples from the wild will be difficult. As such, obtaining blood from rescued birds from illegal trade is considered more ethical and sound. Furthermore, since the park is a rescue center, subsequent release of the animals are underway, and as such, there is a need to establish the baseline data that they will use to ensure that animals area health. Finally, with the zoonotic potential of *Chlamydophila psittaci*, it is warranted to know the infection status of the animals.

CONCLUSION

Hematological constituents and *Chlamydophila psittaci* proliferation were determined and examined for the captive Palawan Hill Mynah under the protection of the Ninoy Aquino Parks and Wildlife Nature Center. The means and standard deviations were used to compare the values to the established normal hematological values of the same species found in Thailand. Majority of the hematological values obtained for *G. religiosa palawanensis* were observed to be within the range though some of the components of the differential WBC count, specifically the heterophil, eosinophil, and monocyte, on the other hand, had slight variations. These variations may be associated with a lack of vitamins, stress, and inflammation within the subject's body. The ELISA results indicate the presence of antibodies for *Chlamydophila psittaci*. The possible explanation for the presence of the antibodies, however, is that the sampled birds might have had a previous *Chlamydophila psittaci* infection due to a low positive reading. A more specific test for the presence of the infection that could be done is a direct ELISA for detecting the presence of the *C. psittaci* antigen itself.

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