MITCHELL BUSH, D.V.M
Department of Animal Health
and
ELIZABETH E. SMITH, B.S., M.T (A.S.C.P.)
Department of Pathology
National Zoological Park
Smithsonian Institution
Washington, D. C. 20008

Clinical Chemistry and Hematology as Diagnostic Aids in Zoological Medicine

ABSTRACT

Clinical pathology is a diagnostic aid in zoological medicine but it has not yet reached its full potential. A basic understanding of it is necessary to determine both "normal" values, and how selected stimuli (infectious, metabolic, psychological) alter the individual hematological response. This information is necessary on the wide variety of species encountered in a zoological environment.

We have used clinical pathology as a diagnostic adjunct for a screening procedure to identify ill animals. The investigation of clinical problems should also include clinical pathology data.

Introduction

Clinical procedures in zoological medicine have several inherent difficulties including the wide variety of species encountered and the uncooperative and potentially dangerous nature of some of the patients. This usually negates or severely curtails some diagnostic procedures, such as physical examination. Another problem is early recognition of the ill animal because most exotic species are capable of masking clinical signs of illness which otherwise might attract a predator. This adaptation is necessary in the wild but makes early disease recognition and therapy difficult. It is, therefore, necessary to utilize diagnostic aids, such as hematologic and clinical chemical procedures, to aid in diagnosis and to monitor the health status of a collection.

In man and most domestic species, many controlled studies have been performed and evaluated to establish normal values. Further studies were necessary to understand the hematologic response of man and domestic animals to various alterations from these normals (biologic, metabolic, physiologic, and environmental). The current literature continues to contain newer information which updates the "normal" and hematologic responses of man and domestic animals despite the extensive preexisting studies.

In using hematologic data from a zoological species, there are inherent problems which lessen their diagnostic use—the lack of normal values, for example, and the failure to understand the hematologic response of that particular species. Many times the patient is bled only when ill and, therefore, no reference data are available. If data were available, they may be from a different subspecies in a different environment, performed by a different technique. Therefore, a concerted effort is needed to continue to establish "comparison values" (rather than normal values which will be difficult to obtain and substantiate in a zoological environment). Another acknowledged problem is the quantity of species and subspecies encountered; ideally, each of these should have its own set of comparison values. In gathering these data, the following variables should be defined: methodology of collecting, processing, and analyzing blood samples, and the biological and environmental situation of the patient. The collection of these data is essential for the utilization of clinical pathologic values. After comparison values are substantiated, it will be possible to monitor hematologic changes when the homeostasis of the individual is altered either biologically or environmentally. As this information becomes available, it will allow improved clinical use of the hematologic data in zoological medicine.

Clinical pathology laboratories, performing works for zoological specimens, vary in their scope. They can be a human diagnostic laboratory, a research-oriented laboratory, or a diagnostic laboratory with a zoo. The majority of current data on zoo animals probably originate from human laboratories which are limited by their being unfamiliar with hematologic and chemical variances, especially occurring in birds and reptiles. The research laboratories may only be interested, for the comparative medical implications, in a very specific and sophisticated test on certain species, and they may not be equipped to perform more routine studies of clinical value.

We are fortunate at the National Zoological Park (NZP) in having an in-house clinical pathology laboratory and technical staff, which allow us to perform diagnostic studies, screening procedures, and selected clinical research.

To maximize the use of our clinical pathology laboratory, we conduct numerous screening programs (defined later) to monitor the health status of the collection by checking for ill animals that may be masking clinical signs; this approach also enables us to establish comparison values on normal individuals. It would be ideal if each zoological collection could gather its own comparison values as the data would be available and specifically applicable to that collection. We collect blood samples from all quarantined mammals and birds and all individual animals that are anesthetized for manipulative or management procedures. The hematologic data generated serve as guidelines for this species and the particular individual in question; these are updated by obtaining successive blood samples when a new opportunity arises. This initial information is important if that individual becomes ill in the future, and blood studies are obtained as diagnostic aids.

Methodology

Blood samples are taken in ethylenediamine tetraacetic acid (EDTA) from most mammals and in heparinized syringes from very small mammals, birds, and reptiles. Blood smears are made, and a hematocrit (PCV) is processed by the standard microhematocrit method. The plasma in the microhematocrit tube is delivered into a refractomer[a] for the total protein (TP) determination. The white blood-cell count (WBC) and red blood-cell count (RBC) are determined by an electronic counter.[b] Because of a possible reduction in count values, due to coincident passage of cells, a

[a] T. S. Meter, American Optical Corporation, Scientific Instruments Division, Buffalo, New York.

[b] Coulter Counter ZB1$_6$, Coulter Electronics, Hialeah, Florida.

correction chart is used for RBC counts and WBC counts over 10,000. Our procedure for counting the very small RBCs of goats, gazelles, and deer is modified. A 1:200,000 dilution is prepared and this amplification is increased for samples from these species so that the electronic counter is within its appropriate range for accuracy.

The nucleated erythrocyte of birds and reptiles precludes WBC and RBC counts by the above methods. Therefore, we have developed a unopette method[c] using Natt-Herrick solution (3) as a diluent. The Natt-Herrick stain is prepared in advance and paraffin-sealed in unopette containers. When a blood count is to be done, 20 μl of whole blood, using an unopette pipette, is delivered to the reservoir containing 1.98 ml of the Natt-Herrick diluent. The unopette assembly is then immediately placed on an aliquot mixer for 10–15 minutes, delivered to a hemocytometer and the RBCs and WBCs counted. The methyl violet in the Natt-Herrick solution differentially stains the cellular components and facilitates differential counting of the RBCs and WBCs. The nucleus of the erythrocytes stains violet with slightly lighter cytoplasm. The more rounded leukocytes stain a uniformly dark violet. The nucleus of the smaller elliptical thrombocyte stains a light violet with a very faint cytoplasm.

The hemoglobin determination for birds and reptiles is a modification of the standard cyanmethemoglobin method using 20 μl of whole blood in 5 ml of cyanmethemoglobin reagent. The addition of 5 ml of distilled H_2O allows complete lysing of the RBC to eliminate the cloudiness of the solution which otherwise creates a false reading. The solution is then centrifuged for five minutes to make sediment of the cellular and protein debris. The supernatant is read at 540 mμ wavelength on a spectrophotometer, and the results are multiplied by 2. Human hemoglobin controls are run concurrently.

Results and Discussion

Most of the patients handled because of suspected illnesses are bled, and the data are evaluated. The amount and method of blood collecting requires clinical judgment so the sampling technique is safe to the animal and personnel. Many times it is necessary to chemically immobilize an animal for bleeding, while other times physical restraint may be used. The amount required varies with the species and individual temperament of the animal and the proficiency of the veterinarian. Consideration must be made as to how the blood sample was obtained, since immobilizing drugs and extreme physical exertion of the animal during manual restraint can alter hematological values. But since few, if any, samples can be obtained without some stress, we must recognize, define, and evaluate these effects on an individual's hematological profile.

During routine blood sampling of our Bactrian camels (*Camelus bactrianus*), we noticed alterations in various components of the hematologic profiles (2), depending on whether the camel was immobilized with xylazine[d] manually restrained for the procedure. The xylazine-restrained camels had statistically lower RBC, PCV, and hemoglobin and elevated blood glucose levels than those restrained by hand. There was also a higher WBC noted, but this was not statistically significant. These alterations must, therefore, be recognized when evaluating hematologic data from exylazine-immobilized Bactrian camels.

In the interim, while the guideline values are being established, the hematologic data collected from clinically ill patients are evaluated in light of the existing knowledge. We can first extrapolate information from man and domestic species, or, if available, from data reported on the particular species or closely related species. When extrapolating data, we assume similar insults elicit similar hematologic responses in the different species, a rationale that may prove invalid, but offers a workable interim method of data evaluation.

This paper discusses our utilization of a clinical pathology laboratory in zoological medicine. We use the data to aid in diagnosis, for screening procedures, to monitor the clinical course and treatment of patients, and to study clinical problems that arise. The discussion will focus on screening procedures and some of the diagnostic challenges encountered, and how certain investigational studies were applied to these problems.

Hematological Screening Procedures in Birds

In compiling comparison values for various species of birds, we found some variation in the hematological profiles (Table 1). For critical evaluation of clinical values, it is necessary to recognize these species' differences.

Total protein determination has been useful in evaluating the nutritional status of individuals. During routine bleeding of Eastern crowned cranes (*Balearica pavonina*), low total protein values (below 2.4 gm %) in certain individuals indicated a problem. We first eliminated the possibility of internal parasites by fecal

[c] UNOPETTE Disposable Diluting Pipette, Becton-Dickinson, Rutherford, New Jersey.

[d] Rompun, Haver Lockhart Laboratories, Division of Bayvet Corp., Shawnee, Kansas.

examination and minimized the chance of it being an infectious disease, determining that WBCs were normal. The evaluation of the husbandry practices indicated that a single feeding station was present in the enclosure, and the dominant pair of cranes were preventing subordinate cranes from feeding. This was corrected by adding more feeding stations, and on subsequent bleedings the total proteins of the affected cranes returned to the normal range.

Avian WBCs, while difficult to perform, provide valuable clinical information. Hematology, including WBCs, is routinely obtained on all ill birds. When an ill bird's WBC is elevated, systemic antibiotics are administered, and the bird is observed for clinical improvement. A second blood sample is obtained 3–5 days later. If the WBC is falling or normal, this treatment may be continued for several more days; but if the WBC is still elevated or rising, the bird's condition is reevaluated and the antibiotic is changed. If on subsequent evaluation and hematology no clinical improvement or lower WBC is found, then chronic diseases, such as aspergillosis or avian TB, both of which have a poor prognosis, are considered.

We have used hematology as a screening procedure in the control of avian tuberculosis (ATB) in our bird collection (1). The results of tuberculin skin testing have been invalid; therefore, hematologic and other procedures were used. Table 2 compares the hematologic profiles of 3 groups of birds with ATB. Group 1 are birds from the collection with clinical tuberculosis. They have advanced disease, and it is reflected by the markedly elevated WBC. Group 2 are experimentally infected quail (*Coturnix coturnix*). The hematologic changes are striking between infected and noninfected quail, but also of interest is the marked difference observed between birds with minimal lesions compared to the birds with severe lesions. Group 3 are high-risk birds, vulturine guinea fowl (*Acryllium vulturinum*), in a contaminated environment. The hematologic findings are not diagnostic in this group of birds because the birds were in the early stages of the disease when euthanatized. Some guinea fowl had no histological lesions, but positive diagnosis was obtained by cultures of the liver or spleen or both. Screening hematology was, therefore, of some value in the antemortem diagnosis of advanced cases of ATB but not the early cases. The classical appearance of a bird

Table 1. Hematology comparison values for various groups of birds

Family	*Hct*	*RBC* (10^6 µl)	*WBC* (10^3 µl)	*TP gm %*
Anatidae (geese)				
Anatidae (ducks)	44.0	3.2	15,860	4.5
Gruidae (cranes)	45.0	3.9	11,564	4.6
Raptors	42.0	2.9	16,500	4.0
Psittacidae	41.5	2.8	16,400	4.2
(psittacines)	45.5	3.6	7,465	4.3

Table 2. Comparison of hematologic profiles of the 3 groups of birds with and without avian tuberculosis (ATB)

	Number of birds	*Average WBC/mm³*	*Average PCV %*	*Average TP gm %*
Group 1				
Clinical cases of ATB	17	62,475	34.5	4.6
Group 2				
Experimental quail	91			
ATB negative	59	4,750	49.0	4.7
ATB positive	32	21,750	39.5	5.5
Severe	22/32	26,250	37.0	5.2
Minimal	10/32	8,650	40.0	6.8
Group 3				
Vulturine guinea fowl	36			
ATB negative	20	12,400	37.5	3.7
ATB positive	16	13,800	37.0	3.8

Table 3. Hematology of normal and ill Caribbean flamingos

	PCV	*WBC (10^3 µl)*	*Comments*
Caribbean flamingos	49	11,330	Average comparison values of 18 normal birds
Initially ill flamingos			
Bird's I. D. #			
204,841	8	49,720	Died 3 days; Alpha strep cultured at necropsy
204,833	10	28,380	Died 22 days; Alpha strep cultured at necropsy
Screening hematology on sick flamingos removed from flock for therapy			
Bird's I. D. #			
204,835	10.5	58,740	Died; Alpha strep blood culture
204,836	21	16,000	Died; Alpha strep blood culture
204,837	20.5	40.480	Died
204,831	20	69,630	Lived
204,832	19	44,880	Lived
204,840	43	33,770	Lived

affected with advanced ATB in our collection is one that is underweight with a leukocytosis that does not respond to antibiotic therapy.

Twelve Caribbean flamingos (*Phoenicopterus r. ruber*) arrived at the National Zoological Park in February 1977. After passing quarantine, they entered the collection and seemed to be doing well. Two months later, tenectomy of the extensor carpi radialis was performed and all birds tolerated the procedure well. On May 29, 6 weeks following tenectomy, 1 bird was noted to be weak, and hematological findings were severe anemia (PCV 10) and leukocytosis (28,380). This bird was placed on intensive supportive care which included intravenous (i.v.) fluids, steroids, and antibiotics. The bird was fed by stomach tube and supplemented with hematinics. Two blood transfusions were administered from donor flamingos with a positive clinical response for 3–5 days. Despite this therapy, the bird died 3½ weeks later. At necropsy, amyloidosis was the major lesion, and alpha streptococcus was cultured from the liver, spleen, and kidney. During the course of this bird's treatment, a second flamingo was found to be weak. The hematology was similar (PCV 8 and WBC 49,720). This bird died within three days despite a similar course of therapy. At necropsy, amyloidosis was found and alpha streptococcus was cultured from spleen and kidney.

In an attempt to determine the scope of the disease, since there was a lack of clinical signs, the remaining flamingos in the flock were bled. From the resultant hematology, six birds were removed from the flock because of low PCV, 21 or less, or elevated WBC of greater than 33,500 (Table 3). One bird had a normal PCV of 43 but a WBC of 33,770 while one bird with a PCV of 21 had a WBC of only 16,000. These six birds were separated, and blood cultures were obtained. Two were positive for an alpha streptococcus on blood culture. The birds were divided into three groups of two each. Each group received a different injectable antibiotic; i.e., lincomycin,[e] ampicillin,[f] or gentamicin sulfate.[g] After three days of treatment, the birds were re-bled, and the groups receiving the lincomycin and ampicillin had responded with an average decreased WBC of 24,000, while with the gentamicin-sulfate group the WBC continued to rise. With this finding, the antibiotics were changed so the birds were receiving both ampicillin and lincomycin plus supportive tube feeding. Three of the six birds died with amyloidosis, but three responded and were returned to the flock, following the two weeks of treatment. Hematology allowed us to identify ill birds and monitor their response to treatment.

As the result of apparent transfusion failures in the anemic flamingo, we began a clinical research study on the efficacy of blood transfusions in birds by using heterologous Cr^{51} labeled erythrocytes. The recipient

[e] Lincocin, Upjohn Corporation, Kalamazoo Michigan.
[f] Polyflex, Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, New York.
[g] Gentocin, Schering Corporation, Kenilworth, New Jersey.

bird destroyed 90% of the transfused cells within six days. Therefore, it appears necessary to cross-match birds before transfusions, and it may not be feasible to transfuse birds in a zoological environment.

We are evaluating the prognostic value of measuring blood glucose in sick birds. Clinically we measure the level by placing one drop of whole blood on a reagent dip stick.[h] Normal birds have glucose levels greater than 200 mg %. When a bird's level is less, we consider the bird in critical condition and begin treatment with i.v. 50% dextrose and oral glucose solutions every few hours. With levels of 100 mg % or below, we have been uniformly unsuccessful in saving the bird.

Screening Procedures in Hoofstock

Neonatal examinations are attempted on all new-born hoofstock within the first 24 hours of life. The examination includes navel care, eye care, physical examination, prophylactic antibiotics, and a blood sample. This procedure was initiated to decrease calf mortality within the first month of life. It was successful in decreasing the neonatal deaths due to navel infections, and provides additional information when a particular calf is not doing well. To further evaluate the individual calf's problem, a subsequent examination is given and blood sample obtained. Sequential TP values can determine if a calf received colostrum by the rise in the total protein. This is of clinical significance and will determine if antibiotic prophylaxis will be required until the calf begins to produce its own gamma globulins.

During neonatal screening procedures in the sable antelope (*Hippotragus niger*), we noted a drastic drop in the hematocrit within the first week of life, a count which was interpreted as being abnormal. Various theories were suggested and subsequent clinical studies, to measure blood volume and RBC mass, were performed to study this phenomenon of apparent loss of RBCs, both before and after the decline in the PCV. Our studies found this was apparently normal in sable antelopes, and the falling hematocrit was a result of hydration following nursing, and not a loss of erythrocytes. Sable antelopes have the lowest blood volume and a smaller red-cell mass per unit bodyweight than has been described previously for any mammalian species, and the subsequent drop in hematocrit is due to the hydration of the calf when it nurses, and is not a pathological process.

In zoological medicine, clinical problems may initially be unsolved because of the lack of understanding of disease mechanism in exotic species. It is, therefore, imperative that accurate medical records be taken and maintained so the data can be reviewed as newer information is made available. The clinical data includes hematologic data collected during the course of the disease.

The discipline of zoological medicine is expanding due to the social consciousness for our wild animal populations and the resultant need to provide them with the needed health care. The limiting factors for the growth of this branch of medicine are dependent on gaining basic information on the animals. This includes understanding environmental, physiological, other biological, and medical needs.

Clinical pathology in zoological medicine is just beginning and its limitations must be recognized. The pressing need is to understand the hematologic response of the various species in relation to the various stimuli around them. While these basic studies continue, the daily clinical data obtained on ill patients must be used in conjunction with the clinical findings and clinical judgment to aid in the animals' overall medical care.

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[h] Dextrostix, Ames Co., Division of Miles Laboratories, Inc., Elkhart, Indiana.