Experiences in avian clinical pathology

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Introduction

140 avian biochemistry and/or haematology submissions to the Sydney IDEXX laboratory were analysed in regard to signalment, presenting signs and results of the biochemical and haematological assays performed. Repeated analysis from the same bird were excluded where it was possible to identify them.

As a result of this, and from experiences in the laboratory, a list of problems in the collection and evaluation of avian biochemical and haematology data was developed.

Data Review

Signalment

Species information was provided in all cases. The list of species is shown below (Table 1). The most common 6 species (sulphur crested cockatoos, galahs, cockatiels, eclectus, budgies and lorikeets) composed nearly 75% of the submissions.

Table 1: Accessions per species

Sulphur crested	22	Major Mitchell	4	African Grey	2
Galah	21	Corella	3	Kelp Gull	1
Cockatiel	20	Lovebird	3	Chicken	1
Eclectus	16	Ringneck	2	Penguin	1
Budgie	14	King Parrot	2	Princess	1
Lorikeet	10	Albatross	2	Tawny Frogmouth	1
Black cockatoo	5	Quaker	2	Wedge Tail	1
Macaw	4	Duck	2	Total	140

Gender was recorded in 92 cases (66%) with 58% males and 42% females. Age was recoded in 89 cases (64%), and ranged from 6 months to 52 years, averaging 7.5 years. Average ages for the most common species were:

sulphur crested cockatoos	18.5 years,
galahs	11.4 years
cockatiels	3.5 years
eclectus	2.7 years
budgies	3.2 years
lorikeets	1.4 years.

Renal Parameters

Renal parameters (urea, uric acid) were evaluated in 90% of cases. Urea was low in approximately 10% of cases, and elevated in 12% of cases. In the cases with urea elevation, total protein was never elevated. Does this mean that urea is a more sensitive indicator of dehydration than total protein?

Uric acid was high in 9 cases, of which 5 cases had concurrent elevated urea, reflecting a pre-renal cause of uric acid elevation. Thus 4/123 cases (3%) where renal function was evaluated had clear metabolic evidence of renal insufficiency.

"Hepatic" parameters

At least one hepatic indicator was measured in all cases submitted. AST was high in 46/140 cases, but in only 17 of these was CK not significantly elevated ie in 12% of cases there was convincing metabolic evidence of hepatocellular disease. In some of the 29 cases with elevated AST and CK, other factors such as concurrent elevation of GLDH suggested a component of hepatocellular disease.

GLDH was assayed in 123 cases and was high in 64 cases. Of these, 28 were <5 IU/L, and hence reflect possible dilution artifact. 17 cases had concurrent significant elevation of AST.

Bile acids were measured in 114 cases and were high in 10% of these cases, all of which had concurrent evidence of hepatic disease. Unlike in mammals, elevated bile acids were rarely associated with other evidence of a functional hepatopathy (low urea, low albumin, low cholesterol etc).

Other biochemistry

Total serum protein, albumin and globulin were low in approximately 30% of cases. This may reflect differences in methodologies between published reference ranges (often serum protein electrophoresis) and biochemical methods, as well as pathological processes.

Cholesterol was high in 60% of 114 assays. Cholesterol was elevated in >70% of galahs, budgies, lorikeets and major mitchell cockatoos. As the most common cause of elevated cholesterol may be a high fat diet, the high percentage of cases with elevated values in these species may reflect a disease of captivity.

Ca was usually normal or artifactually low in association with low albumin. Occasional high values were usually from females with a recent history of egg laying.

Amylase was high in 20% of 104 assays, being >1000 IU/L in 16% of assays. Such elevations may reflect pancreatic disease, including zinc associated pancreatic disease and pancreatitis. Other differentials include enteric disease, possibly renal disease, or non-specific elevations with other primary coelomic diseases.

RBC parameters

At least some assay of RBC parameters was performed in 41% of accessions, with PCV the most common assay. In 30% of cases, anaemia was present. This was usually mild, non-regenerative, and in most cases, associated with an inflammatory leukogram. Mild anaemia appears to be a common finding in birds with inflammatory disease and may be part of the avian response to

inflammation. Regenerative anaemias were less common, and were never associated with identifiable RBC parasites, and may reflect haemolytic or haemorrhagic disease.

WBC parameters

White cell counts were performed in 51% of accessions, the majority of these also receiving a differential count. Leukocytosis was present in 65% of cases. Absolute and relative WBC differential values were available in 39 cases. Immature heterophils and toxic changes were uncommon, and when present were usually associated with severe clinical disease. Differential data is shown below (Table 2).

Table 2: Comparison of relative and absolute WBC differentials

Heterophils	59% relative heterophilia 20% absolute heterophilia
Lymphocytes	41% relative lymphopenia 0% absolute lymphopenia
Monocytes	11% relative monocytosis 5% absolute monocytosis
Eosinophils	no significant changes
Basophils	13% relative basophilia 3% absolute basophilia

Based on this data, it appears that for these cases, use of relative WBC differential data may result in over-estimation of inflammatory or stress leukograms. The use of relative WBC differential data for interpretation of leukograms in domestic mammals is generally frowned upon, as inaccuracies, including over- and under- interpretation of the data may occur. The use of absolute data "corrects" for variations in white cell count as shown below.

For example, two cockatoos may have the data below (data outside of the reference ranges in **bold**):

			Relative	**Absolute (x 10^9)**
Bird 1	WCC = 6.3	Hetrophils	75	4.72
		Lymphocytes	13	0.82
		Monocytes	**8**	0.50
		Eosinophils	2	0.13
		Basophils	**2**	0.13
Bird 2	WCC = **34.6**	Hetrophils	75	**25.95**
		Lymphocytes	13	4.50
		Monocytes	**8**	**2.77**
		Eosinophils	2	0.69
		Basophils	**2**	**0.69**

Both birds have the same relative white cell differential, and this would result in a diagnosis of a monocytosis and basophilia. When the absolute values are evaluated, Bird 1 has a normal leukogram, while Bird 2 has a significant heterophilia, monocytosis and mild basophilia. These findings are significantly different, and would likely lead to different diagnostic paths for further evaluation.

Differentials / clinical presentation

A major differential diagnosis or a clinical history was provided in 110 cases. Note this means that in over 20% of cases, no history was submitted. The histories submitted are listed in Table 3 below.

Table 3: Differentials / histories

Hepatic	23 (21%)	Circovirus	10 (9%)	Heavy metal	2 (2%)
Feather picking or self-mutilation	19 (17%)	Health check	10 (9%)	Endocrine	2 (2%)
Sick bird look	19 (17%)	Renal	7 (6%)	Behaviour	1 (1%)
Gastrointestinal	13 (12%)	Female reproductive	3 (3%)	Respiratory	1 (1%)

Evaluating the laboratory results against the 6 most common differentials (excluding circovirus) in the 6 most common species, the following results were determined (Table 4). The laboratory findings may total greater or less than 100%, as not all cases provided definitive data, and some cases exhibited multiple changes. Inflammation is listed separately where the inflammatory process was not localised to a specific organ system.

Table 4: Comparison of differentials and the laboratory findings

Differential	Laboratory findings
Hepatic	35% hepatic disease
Feather picking or self-mutilation	57% high cholesterol, 14% hepatic disease
Sick bird look	46% inflammation, 31% hepatic disease, 23% elevated amylase
Gastrointestinal	56% high cholesterol, 11% high amylase
Health check	43% hepatic disease, 43% high cholesterol
Renal	57% hepatic disease, 71% high cholesterol

Overall, in these species, the following major findings were made:

- 37% high cholesterol
- 25% hepatic disease
- 21% NSAD
- 7% non-localised inflammation
- 6% high amylase
- 3% functional hepatopathy
- 1% anaemia

In comparison to the major differentials, these results reflected a slightly more common biochemical diagnosis of hepatic disease (including functional hepatopathies) and lower diagnosis of renal disease than the clinical history / findings suggested. In over 20% of birds, no significant abnormalities were identified.

Problems in avian clinical pathology

Problems in the collection and interpretation of avian clinical pathology data can be loosely divided into two areas:

1. sample based issues
2. laboratory and interpretation based issues.

The first area covers submission of adequate samples and supporting information to allow adequate investigation of the problem. The second area covers issues based around scarcity of reference ranges and some issues with how the existing reference ranges have been developed.

Sample based issues:

1. Short samples
2. Failure to submit blood smears
3. Insufficient histories

No-one can quibble about a small sample volume collected from a small bird, but collection of an unnecessarily small sample from a larger bird causes significant problems in the laboratory, and can result in inaccuracies in the results. Short samples are a problem in the laboratory for two reasons. Firstly, they result in a need to limit the range of assays run, reducing the diagnostic value of the sample. These limited range of assays often need to be run in small groups of one or two analytes according to the priorities of the case. This results in increased time to perform the assay, and also an increased wastage of sample as the dead volume of the equipment needs to be filled more times than when a panel can be run directly through. This in its self can limit the number of analyses performed.

Secondly, dilution of the sample is not suitable for some analytes, notable protein, albumin and calcium. In other assays, dilution of the sample may push the concentration of the analyte below the linear range of the assay, results in errors in the analysis which are compounded when the results are multiplied up to allow for the dilution. This may result in significant under- or over-estimation of the final value. This particularly affects analytes where the normal avian ranges are already near the lower limit of the linear range, including urea, uric acid and GLDH. Bile acids also appear to be adversely affected by dilution.

Clearly, submission of adequate sample to perform all required assays in an undiluted sample is preferable where possible.

Submission of blood films made from non-anticoagulated blood is important when submitting haematology samples. The blood left in the needle or syringe hub after sample collection is usually adequate to make at least one smear. These smears usually have better white cell morphology than smears made from anticoagulated blood, and can be invaluable in undertaking white cell differential counts and assessment of white cell and erythrocyte morphology.

Submission of an adequate history can be vital when interpreting haematology or biochemical data. For instance, a high serum calcium in a female bird with a history of egg laying does not probably require further evaluation, which a similarly elevated calcium in a non-egg laying female or male may be much more significant.

Laboratory and interpretation based issues:

1. Lack of species specific reference ranges
2. Lack of published data defining reactions to well defined insults in a range of species (not just pigeons, chickens and cockatiels)
3. Apparent variation in species response to disease
4. Lack of absolute WBC differential reference ranges in many species
5. Protein reference ranges

The first three of these points are self evident, and reveal enormous gaps in our knowledge that can only be filled by continued basic research. The major problem in this area is not lack of enthusiasm to do the work, but a lack of funding to undertake studies on a sufficient scale to provide solid results.

The use of absolute reference ranges for interpretation of white blood cell data is more accurate than using relative (percentage) data. Many avian studies developing reference ranges, and many tables of published avian reference ranges include only relative data. Further research, or re-evaluation of the original data would allow development of absolute reference ranges. Absolute ranges have the advantage of allowing for changes in the white cell count, and this results in their greater accuracy (see example above).

The published protein reference ranges are most commonly developed from serum protein electrophoresis data. Results from electrophoresis in birds tend to be higher than those determined biochemically. Reassessment of protein reference ranges in common species using biochemical methods appears indicated.

In conclusion, submission of adequate samples and complete histories provides the best chance to gain useful information from the blood sample. Where possible, avoid the need to dilute samples as this can lead to inaccuracies. Further research is required to develop more species-specific reference ranges, using common diagnostic methods, and to assess responses to disease in a wider range of species.