ASSESSMENT OF AVIAN ANAEMIA

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KATE ENGLISH discusses how anaemia affects the pet bird and explains the reasons behind the affliction

ANAEMIA is a reduction in red blood cell mass. It may be identified by decreased PCV (haematocrit), decreased erythrocyte numbers or decreased haemoglobin.

Broadly, the causes of anaemia may be blood loss, increased destruction of the erythrocytes or decreased production of them. The common presentations and differential lists for anaemia may be varied between species. There is less literature on the subject of anaemia in avians, compared to other domestic pets, and it is difficult to determine what the most common causes of anaemia are. Assessment of the erythrocytes may provide information that will help the clinician manage an anaemic patient.

Blood sampling

To assess the erythrocyte, it is necessary to obtain a blood sample. Care should be taken when handling and restraining birds. However, detailing the techniques for this is outside the scope of this article.

Similarly, several venous sites are suggested as suitable for sampling, although each may have specific caveats regarding technique. In general, the right jugular vein is likely to be the most accessible spot for the majority of avian species.

Blood samples obtained from claw clipping are not acceptable samples for assessment of
haematology. Samples from this site may not be representative of venous blood, and may also contain osteoclasts if the bone has been damaged during sampling.

The volume of blood to be collected must be carefully calculated beforehand. In smaller birds, it may only be feasible to obtain very small volumes. In an anaemic patient, it may also be sensible to reduce the volume of blood collected. On average, blood samples up to one per cent of the bird’s body weight may be collected, such as 0.4ml in a 40g bird.

Samples should be collected into an anticoagulant. Heparin is frequently the anticoagulant of choice in avians. EDTA has been shown to cause haemolysis in several avian species.

Heparin may affect staining properties, particularly in leukocytes, and is also reported to result in white blood cell clumping. A freshly prepared smear made at the time of sampling is, therefore, a valuable addition to a blood sample when performing haematological analysis.

An additional advantage to using heparin as an anticoagulant is that once haematological tests are completed, residual plasma may be separated off and used for biochemistry testing.

**Erythrocyte assessment**

The half-life of the avian erythrocyte varies between species, but is – on average – approximately 35 days, compared to the average half-life of a canine erythrocyte (110 days). Changes may be seen rapidly within the erythrogram, presumably as a reflection of the short half-life of the erythrocyte.

Assessment of anaemia in avians is most commonly performed via PCV measurement. This may be obtained in exactly the same fashion as a mammalian PCV, with the bottom layer of the tube reflecting the red cell mass, the middle layer representing leukocytes and thrombocytes, and the top layer representing plasma.

The expected limits for PCV and erythrocyte numbers may vary according to species, age, sex, and (possibly) calendar season. Adult males frequently have higher PCV measurements. On average, PCV results of more than 35 per cent are considered adequate, although some reference intervals report PCV as down to approximately 25 per cent in young birds. The PCV results are not expected to exceed 55 per cent.

Methods for obtaining an erythrocyte count using a Neubauer chamber, or a different cell counting method, are also available. Haemoglobin may be measured following lysis of the erythrocytes and removal of the nuclear material.

**Erythrocyte morphology**
Avian erythrocytes are oval cells, on average 12µm by 6µm, with a central, oval nucleus.

Anisocytosis, a variation in size between the erythrocytes, may be observed. Macrocytosis or microcytosis may be noted, although this may be less easy to assess compared to mammalian blood smears.

Hypochromasia may also be identified, where the erythrocytes may be pale or have uneven staining, with approximately half or more of the cytoplasm displaying significant pallor (Figure 1).

Polychromasia is commonly noted in smears on healthy avians. The differentiation of these cells compared to normal erythrocytes is similar to that of mammalian polychromatophils. Polychromatophils may appear slightly larger, with cytoplasm that stains darker (Figure 2). On average, one to five per cent polychromasia is expected in avian blood smears. If required, polychromatophil number confirmation by demonstrating reticulocytes on a new methylene blue-stained smear may be carried out. The reticulocytes are identified by the circular area of aggregated nucleic acid material around the nucleus. In regenerative responses, it may be expected to see levels of polychromasia increase above 10 per cent, depending on the degree of anaemia and timing of the response.

Erythroplastids may occasionally be noted on blood smears (Figure 3). Erythroplastids are erythrocytes that have lost their nucleus.

Binucleate erythrocytes or early erythroid stages often appear as round in shape, with a round nucleus. They may be noted in low numbers, with a strong regenerative response. Mitotic figures may also occasionally be seen in regeneration. High numbers of these types of cells may raise suspicions of dyserythropoiesis, an abnormality in the erythrocyte lineage. Basophilic stippling may also be noted, with a strong regenerative response.

**Causes and changes**

- **Blood loss**

Acute blood loss (due to trauma, for example) may be a cause of anaemia. Avians are reported to recover quickly from blood loss, with PCV reaching normal levels within three days to a week following a single episode of blood loss. The speed of recovery may depend on the quantity of blood lost. Blood smear changes that may be noted would include increased polychromasia and possibly earlier erythroid stages.

Chronic blood loss may result in iron deficiency, with the erythrocytes appearing hypochromic – with a possible reduction in the amount of polychromasia present.

- **Haemolytic anaemia**
The most common reports of haemolytic anaemia in avians are related to zinc or lead toxicosis, but occasional reports of haemolysis secondary to onion ingestion occur. Aflatoxins may also cause haemolytic anaemia. Immune mediated haemolytic anaemia is rare. When it is present, it is most likely to be secondary to an infectious agent, such as *Salmonella* or *Borrelia* septicaemia. Primary immune mediated haemolytic anaemia is very rarely reported in psittacines.

A variety of avian blood parasites may also be associated with haemolytic anaemia.

Birds may present with haemoglobinuria or biliverdinuria with haemolytic anaemia.

Mechanisms for the development of zinc and lead toxicosis are not fully understood. Presentation may be variable and the duration of exposure to the heavy metal prior to clinical signs developing is also unclear; in addition, the anaemia may be regenerative. In lead toxicosis, there may be increased numbers of early erythroid stages and hypochromasia of the erythrocytes. The basophilic stippling commonly reported in lead poisoning in mammals may not be a feature in many avians. The presence of basophilic stippling in a regenerative response may be a non-specific finding in the avian. The level of zinc plasma that may be associated with zinc toxicosis is not clearly defined. When measuring zinc in plasma, care must be taken to submit the sample in a tube that does not contain zinc. In volumes obtained from birds, this is less likely to be a concern, compared to samples from large animals that may be placed in a Vacutainer tube, where there is a rubber stopper that contains zinc.

Heinz bodies may be less commonly identified on blood film examination in avians, but may be noted in cases of onion toxicity, or toxicity relating to exposure to oil or petroleum-related products.

**Decreased production**

A mild non-regenerative anaemia may be noted as a response to inflammation or other chronic disease process. This will resolve when the underlying disease does. Hypochromasia has been reported alongside anaemia due to inflammation, which is different to mammals, where non-regenerative anaemia is expected to be normocytic and normochromic.

Myelopthesis, or bone marrow disease, may also result in anaemia. If this is due to abnormal maturation in the erythroid line, moderate to high numbers of atypical erythrocytes may be noted in the peripheral blood smears. As with other species, if non-regenerative anaemia is persistent without the identification of an underlying cause, then bone marrow examination may be helpful.

**Blood parasites**

Parasites may be commonly noted in avian blood films. Parasites most commonly associated with anaemia include *Haemoproteus*, *Plasmodium*, *Leukocytozoon* (all Apicomplexan protozoal organisms) and *Aegyptianella* (a rickettsial, Anaplasmataceae organism). However, these parasites
may also be presented without any apparent anaemia. Parasite numbers may also be increased, due to unrelated illness. *Haemoproteus* (Figure 4) has been reported in increasing numbers in raptors presenting with injuries, with parasitaemia decreasing as the injuries resolve. *Haemoproteus* macrogametocytes appear as tubular structures in the erythrocytes, or occasionally noted as round, extracellular structures. The gametocytes often have iron pigment associated with them. Microgametocytes appear as small, spindle-shaped structures.

*Plasmodium* (malaria) gametocytes, schizonts and trophozoites may be noted in erythrocytes, thrombocytes and leukocytes.

*Leukocytozoon* (Figure 5) macrogametocytes may be seen – they largely distend the host cell so that identification of the cell occupied is difficult. However, *Leukocytozoon* may have a varied appearance on a peripheral blood smear.

• Iron

Iron deficiency anaemia is due to a lack of iron in diets, and may also be caused by chronic blood loss. The blood smear would be expected to have hypochromic erythrocytes, possibly with a reduced regenerative response.

Haemachromatosis (iron storage disease) has also been reported alongside anaemia, although this is rare. In this case, the anaemia was strongly regenerative. The erythrocyte parameters and examination of a blood smear may be helpful to determine the underlying cause of anaemia in a bird.

Although not discussed here, assessment of leukocytes and thrombocytes may also be required to form a full haematologic picture. The combination of findings may provide a more complete assessment of the individual patient.

Assessment of the clinical presentation, urinalysis and biochemistry, and review of the patient’s environment, may also be useful in the work up of an anaemic bird.

**References**


*Figure 1 (left). A mature avian erythrocyte (left) and a hypochromic polychromatophilic erythrocyte.*
Figure 2 (right). This image displays an avian polychromatophil in the centre of the field.
Figure 3 (left). Erythroplastid on the right and mature avian erythrocytes on the left.
Figure 4. An avian erythrocyte containing Haemoproteus species. Note the iron associated with the parasite.
Figure 5. Two Leukocytozoon parasites in avian blood.