

# From Aardvark to Zorilla: understanding the haematology of exotics

**PROFESSOR John Cooper once wrote, in an obituary for Dr Oliphant Jackson, how Dr Jackson exemplified that in order to understand an animal - and to provide optimum care for it - one needed to know, and fully comprehend, its natural history and biology.**

Haematology is an important facet of an animal's biology. With an increasing number of endangered species, and the growing number of exotic animals kept as pets, it is vital that as much knowledge as possible is obtained so that through careful management and veterinary care the welfare and health of these animals is assured.

Haematology can provide much information to aid the differential diagnosis in these animals.

The dictionary definitions of exotic are: (1) introduced from, or originating in, a foreign country; and (2) attractively or remarkably strange or unusual, bizarre. These definitions cover all the exotic species that could be seen by any veterinary surgeon either in general practice or specialist practice.

It has been calculated that there are approximately one million extant species with blood, of which there are about 42,000 species of mammal, bird, reptile, amphibian and fish.

It has been estimated (Hawkey, 1991) that reference ranges of the cellular components of blood are currently available for about 600 species of mammal, 350 species of bird and 80 species of reptile and around two per cent of amphibians and fish. The reference ranges currently available span a considerable number of years and were derived in many cases with methodologies that had not been standardised or validated.

Many are to be found in obscure journals and may prove difficult to track down.

The effects of handling, stress, immobilisation and anaesthesia are

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mostly missing from the data and even such obvious physiological variations such as sex, age and the clinical status of the animals are often not accounted for. It may be possible to predict ranges in an unstudied species by referring to the ranges of a closely related species.

Where possible, all haematology reports emanating from a laboratory should provide species-specific reference ranges.

Veterinary surgeons should be aware of these shortcomings when interpreting data, especially from laboratories that have not developed their own ranges or do not use reliable sources and validated methodologies.

Yet all is not doom and gloom because of an understanding of, and referral to, the basic haematological principles as described by Wintrobe in 1933, which show a relatively small variation in haemoglobin (Hb), packed cell volume (PCV) and mean cell haemoglobin concentration (MCHC) in mammals and birds (**Table 1**).

There is, however, a great deal of variation in red cell count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH) and white cell count (WBC). For example, goats have many small red cells but have an MCHC similar to an animal with few large cells, such as elephant.

An understanding of the basic

principles of Hb, PCV and MCHC can prove very useful in differential diagnosis or health monitoring, especially in assessing anaemia, polycythaemia and hypochromia. These conditions can be identified in any adult mammal or bird without consulting reference ranges or where reference ranges are unavailable.

These basic principles do not apply to reptiles, amphibians or fish.

There is considerable variation in the total white cell count and in the numbers of the various cell types that make up the white cell population among orders, classes, families and species. Again, referring to general

**TABLE 1. Variation in haemoglobin, packed cell volume and mean cell haemoglobin concentration**

	Mammals		Birds	
	mean	Standard deviation	mean	Standard deviation
Haemoglobin	14.8g/dl	±2.1	15.2g/dl	±1.7
Packed cell volume	14.8g/dl	±2.1	15.2g/dl	±1.7
Mean cell haemoglobin concentration	14.8g/dl	±2.1	15.2g/dl	±1.7

principles and noting the white cell morphology on a stained film, an assessment of the clinical status as presented by the haematology can be made.

### Physiological variations

- **Age.** Certain age-related differences are recognised in man, for example Hb, RBC, PCV and neutrophil numbers are lower - and lymphocytes and mono-cytes higher - in children than in adults. Similar age -

related differences have been found in other mammals and birds.

- **Sex.** In man, and in some domesticated species, adult males have higher Hb and PCV levels than females. There is a tendency for higher Hb and PCV levels in males of many exotic mammals and birds.

- **Stress.** Excitement, fear, the presence of humans and herd or group interaction can all result in the release of stress hormones (catecholamines and cortico-steroids) which, in turn, affect both the red and white cells.

It is the red cell numbers that are most notably affected by catecholamines.

Catecholamines induce spontaneous splenic contraction in animals with reactive spleens, for example canidae, felidae, camelidae, cervidae, bovidae and equidae. These animals have large, reactive spleens capable of sequestering up to 25 per cent of their total number of red cells.

This contraction releases red cells into the circulation resulting in high RBC, Hb and PCV levels. Splenic relaxation takes approximately 45 minutes after the stress stimulus. Many exotic species need to be anaesthetised prior to examination and blood sampling and the use of adrenolytic immobilising drugs suppresses the action of catecholamines and the red cells are re-sequestered. This does not occur in primates or birds.

Care must be observed when assessing the RBC, Hb and PCV levels in animals that have been anaesthetised with adrenolytic agents. It could be argued that the anaesthetised state is more "haematologically normal" than the stressed state.

The effect of stress on white cells is less pronounced, is slower and usually lasts much longer than that of the red cells. White cell changes are mainly effected by corticosteroids.

Stress may produce neutro-philic, lymphopaenia and eosinopaenia and in dogs a mono-cytosis. Eosinopaenia is considered as diagnostic of stress in dolphin but the effects on white cells of the majority of exotic species

is unknown, but it is possible that mammals react the same as for man and domestic mammalian species.

### Problems

Of the 42,000 or more species with "conventional" blood, the average veterinary surgeon or veterinary diagnostic laboratory will see only an extremely small percentage, usually a few of the more common exotic species such as grey parrots, common iguana, Greek tortoise and perhaps an occasional snake.

Even these common species need special care with haematology and suitable reference ranges. It is not sufficient to have ranges for "tortoise" as even the usual species such as Greek or Hermann's have differing ranges.

Veterinary surgeons should be mindful that not all laboratories have the necessary skills, experience and appropriate instrumentation and methodologies to produce meaningful data.

### Materials and methods

All the technical requirements that apply to domestic species apply equally well to exotics, but with some additional requirements. The site of venepuncture is sometimes an important factor, for example when bleeding tortoises from the tail vein

majority of exotic species is EDTA, but for several species EDTA causes rapid and progressive red cell lysis, for example crowned crane, wood duck and tortoises: for these species heparin is the anticoagulant of choice. However, it should be borne in mind that heparin may cause white cell aggregation making the total white cell count inaccurate, if not impossible.

The author advises taking another sample into EDTA solely for the total white cell count.

### Red cell counts

Automated, or semi-automated, cell analysers were developed for human blood but several are suitable for many exotic mammals.

However, there are some species that the majority of analysers are unable to produce accurate results for, such as animals with MCV < 25fl, for example goats, and those that have red cell counts above  $9.0 \times 10^{12/l}$  as most cell analysers are not linear with counts above 9.0, for these further dilution is required.

The most suitable instruments for counting these species, and for non-mammalian species, are those that employ the impedance method developed by Coulter Electronics and that have manually adjustable aperture currents and threshold

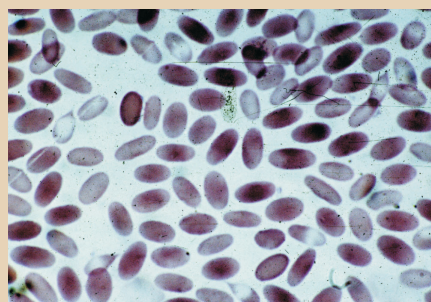


Figure 1. Bactrian camel oral red cells

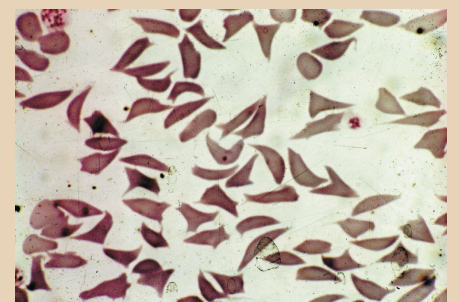


Figure 2. Genet showing sickling

haemodilution with lymph very often occurs resulting in erroneous results. It is known that the blood of some species, for example penguin, clots very quickly and it is recommended that the venepuncture needle is flushed through with heparin solution to reduce the risk.

The choice of anticoagulant for the

settings - or have "hunting" thresholds - built into the soft-ware. These instruments are now either no longer manufactured and/or are difficult to obtain in the UK.

Many workers in avian haematology still prefer to count red cells manually using suitable microscopy (Samour, personal communication).

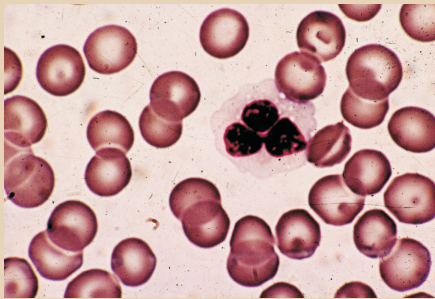


Figure 3. Indian elephant showing trilobed monocyte.

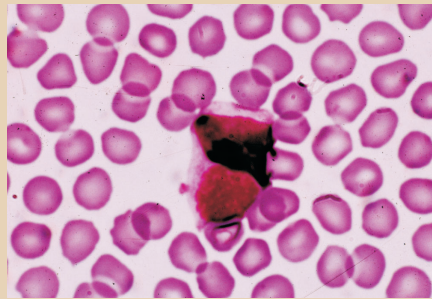


Figure 4. Guinea pig showing Kurloff body in lymphocyte

### Haemoglobin estimation

Any cell analyser capable of measuring human haemoglobin will be suitable for exotic mammals. These instruments use a modified Drabkin's solution which haemolyses the red cells and converts the released haemoglobin to cyanmet-haemoglobin.

This method is unsuitable for non-mammalian species as a turbid solution results which interferes with the light path in the instrument, producing grossly elevated results. The turbidity is caused by the release of the red cell nuclei which are unlysed.

Several workers suggest a manual method using Drabkin's fluid with centrifugation to spin down the nuclei and to read the clear supernatant in a spectrophotometer or haemoglobinometer. Avian red nuclei contain haemoglobin (Campbell, 1996): this would be lost to the measurement if the preparation is spun. The nuclear haemoglobin represents one to two per cent. The conversion of haemoglobin to oxyhaemoglobin was the method of choice prior to the development of the azidemet-hemoglobin method (Vanzetti, 1966).

### White cell counts

At present there are no automated cell analysers capable of reliably counting non-mammalian white cells and thrombocytes due to the presence of the red cell nuclei. Some workers are investigating the possibility of using flow cytometry. White cell counts of these species

are best performed manually with a counting chamber and micro-scope with a suitable diluent solution (Natt and Herrick, 1923) or fluid (Rees and Ecker, 1952), or by phase contrast microscopy.

Whatever method is used, difficulties will be encountered as no method is perfect. The white cells of most mammalian species can be counted using automated or semi-automated cell analysers. Thrombocyte numbers are estimated from a stained blood smear with reference to the total white cell count.

### Red cell morphology

• **Mammalian species.** The red cell morphology of most mammalian species is very similar, i.e. bi-concave discs which usually vary only in size, degree of anisocytosis and polychromasia.

However, a few species show very different characteristics: for instance, all the camelidae species have oval, anucleate flat cells (Figure 1), while others, such as the cervidae and some felidae, show an in vitro sickling tendency (Figure 2).

Heinz bodies are often seen in rhinoceros and in some New World primates.

• **Non-mammalian species.**

All animals in these classes have nucleated, oval red cells which are larger than mammalian cells. Their immature red cells are

round, which become more oval as they mature, and are more polychromatic: up to five per cent polychromatic cells in birds is considered as a normal degree of erythropoiesis. Some species are more, or less, ovoid than others. Many reptile species show intra-erythrocytic inclusions variously identified as pirhemocytin, iridovirus inclusions or degenerate organelles.

### White cell morphology

• **Mammalian species.** The white cells of the majority of mammals is very similar, but there are some variations. Elephant monocytes are large with bi- or tri-lobed nuclei (Figure 3).

The neutrophils of camels often appear left shifted (the Pelger-Huet phenomenon) as a normal finding. Guinea pig lymphocytes often show a large basophilic inclusion known as a Kurloff body (Figure 4).

Seals and dolphins often have Dohle bodies in their neutrophils. The neutrophil granules vary greatly in their amount and in their staining characteristics, varying from agranular to heavily granulated, and from pale pink to deep purple, and the nuclei vary in the degree of lobulation. The numbers, size and shape of granules and the intensity of staining is also variable in eosinophils and basophils.

• **Non-mammalian species.**

There is considerable variation in the granulocytes of these classes of animals, especially in the heterophils. The granules of these cells are generally fusiform or rod-shaped and stain in a range of colours from muddy brown to bright orange in birds (Figure 5), but many reptiles

TABLE 2. Cell types seen in animals

Mammals	Birds	Reptiles
Erythrocytes	Erythrocytes	Erythrocytes
Neutrophils	Heterophils	Heterophils
Lymphocytes	Lymphocytes	Lymphocytes
Monocytes	Monocytes	Monocytes
		Azurophils
Eosinophils	Eosinophils	Eosinophils
Basophils	Basophils	Basophils
Platelets	Thrombocytes	Thrombocytes

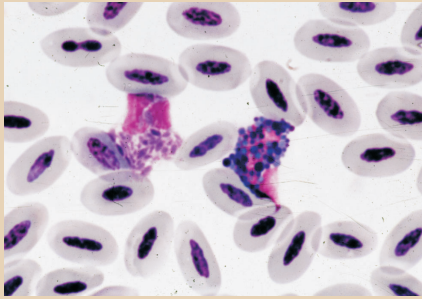


Figure 5. Lesser sulphur crested cockatoo showing heterophil (H) and eosinophil (E).

have oval or round reddish granules (Figure 6).

The eosinophils of birds normally have small, round, brighter eosinophilic granules although a small number of species have rod-shaped eosinophilic granules, but the eosinophil granules of parrot, macaw and cockatoo spp are round, blue (Figure 5) and occasionally blue and purple.

The eosinophil granules of reptiles vary in shape but are usually roundish and paler staining. Iguana, and very rarely snakes, also have blue staining eosinophil granules. Reptiles are unique in that they have a cell called an azurophil, although some workers report them as atypical monocytes, reactive monocytes or azurophilic monocytes (Figure 7). They are

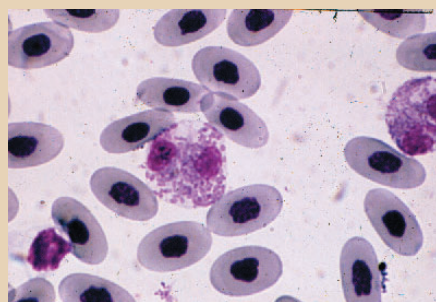


Figure 6. Common iguana showing heterophil.

rarely found in tortoises, but are common in most snakes.

Fish granulocytes are often classified as Type I, II, III, IV and there appears to be considerable morphological variation between cartilaginous and bony fish, and possibly between fresh water, sea water, warm water and cold water fish.

### Platelets and thrombocytes

• **Mammalian species.** Although platelet numbers vary between species their morphology is fairly similar but, of course, there are exceptions for example elephant have relatively large numbers of very small platelets, and echidna often have two distinct populations of platelets. Granularity may vary from species to species and the platelets of some, such as felidae, may become activated and aggregate rapidly.

Activated platelets, non-activated platelets and aggregates may all be seen together on stained smears of any mammalian blood. Large increases in numbers in a given species will often indicate a disease process or other abnormality occurring.

• **Non-mammalian species.**

There are many varieties of thrombocytes in these classes of animals but they have one feature in common: they are all nucleated. They are easily mistaken for small lymphocytes, especially if they are activated when there is often increased cytoplasmic basophilia, or when they have had prolonged exposure to anticoagulant causing them to swell. Thrombocytes may also increase in number as a result of a disease or abnormality occurring.

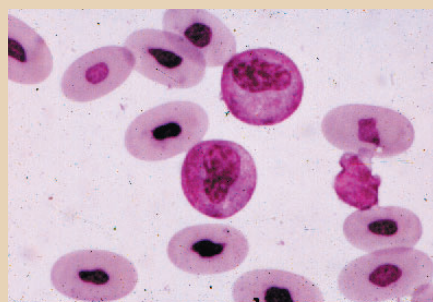


Figure 7. Reticulated python showing azurophils (x2).

### Fibrinogen

A few species of animal do not always show a leucocytosis in infection but will often show a large increase in fibrinogen, for example wallabies. The range across the animal kingdom is approximately 1.0-5.0 g/l.

Fibrinogen is a very useful test in all species as a possible indicator of infection/inflammation.

### Conclusion

Haematology is already a useful tool in the diagnosis of disease in domesticated animals and is becoming a very useful diagnostic tool for many exotic species.

However, much still needs to be done, more reliable reference ranges of unstudied species need to be established, and existing ranges need to be increased.

Improved and standardised methods for cell analysis need to be developed so that more reliable data are generated.

With an increasing amount of information available on the Internet, and with interested and important organisations such as the British Veterinary Zoological Society and the Association of Comparative Haematology in the UK and several American and European organisations, the future of exotic animal haematology as an important diagnostic aid is assured.

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