Veterinary Bioscience: Cells to Systems

Practical class 3: Haematology

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Learning Outcomes

At the end of this practical class, you should be able to:

- Identify leukocytes in blood smears from domestic species and describe the differences between species
- Perform differential counts on blood smears
- Describe responses in blood leukocyte frequency between normal and infected animals

I. VENEPUNCTURE

Ia. Introduction

A blood sample can be analysed in various ways to provide essential information about the general health of an animal. In some circumstances, for example a blood count, whole blood is required; in others, the blood cells need to be separated so that plasma (the fluid fraction of whole blood) can be used. When plasma or serum is required, it is important to adopt procedures that will minimize damage to the red cells (haemolysis) – as released contents of the cells can affect other results. Blood is collected by inserting a hypodermic needle into a superficial vein (venepuncture), and collecting blood using either a syringe or a special vacuum blood collection tube ("Vacutainer"). If frequent blood sampling is required, then cannulation of a blood vessel would be considered.

Definitions:

Whole blood: Direct from the patient, uncoagulated. Contains cells, water, proteins and other solutes (e.g. glucose, salts etc)
Plasma: The liquid portion of blood obtained by spinning down uncoagulated blood. Contains water, proteins, salts, hormones… everything except the cells
Serum: The liquid portion of blood obtained by spinning down coagulated or clotted blood. The same as plasma except without proteins involved in clotting (clotting factors and fibrinogen) - these are 'tied up' in the clotting process.

Ib. Sites of Collection

In sheep, goats, cattle and horse the **external jugular vein** is the most commonly used. In dogs and cats the **cephalic vein** is the normal site of venepuncture (also the jugular vein), and the

cephalic vein can also be used in the sheep. Pigs have so much subcutaneous fat that suitable superficial veins are hard to find. The cranial vena cava or one of the ear veins is used instead.

Ic. Skin Disinfection

To reduce the risk of infection, the skin overlying the vein must be cleaned. The area is swabbed with 4% chlorhexidine scrub, alternating with 80% alcohol three times.

Id. Technique

i. An assistant restrains the sheep. There are two acceptable methods: (a) the assistant straddles the sheep and lifts its head and neck upwards and to one side by placing one hand under its lower jaw. Backing the animal against something solid, e.g. a wall, will help, or (b) the sheep is sat up on its haunches, its back between your legs, and its head and neck held up with one hand.

ii. The wool and skin are wet with 80% alcohol* and the wool overlying the jugular groove is clipped. *(The solution of 80% alcohol helps remove the grease from the wool and also facilitates clipping).

iii. The skin is swabbed with 4% chlorhexidine scrub, alternating with 80% alcohol three times.

iv. The thumb of your left hand (if right-handed) is placed into the jugular groove and pressed firmly to occlude blood flow through the jugular vein, thus raising the vessel so that it becomes distended and clearly visible.

v. Tapping the distended vein with the middle finger of your right hand can identify the site of the vein under the skin. This produces a distinct pulsation.

vi. If the grip of your thumb on the lower part of the jugular groove is released the fluctuation and swelling produced by engorgement of the released vein disappear.

vii. The vein is raised again by pressure with the thumb of the left hand in the jugular groove. Entry into the vein will be helped by backward and downward pressure with the thumb of the left hand. This stretches the skin over the vein at the same time as you apply pressure to the vessel to distend it.

Q (1) Why does the vein need to be occluded to be visible?

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viii. The syringe is held between thumb and the tips of your fingers. The bevel of the needle should face towards you as should the calibration marks of the syringe. The needle should be held at an angle of 30-45^{O} to the skin surface, and the needle directed along the axis of the vein, towards the head.

ix. The needle should then be pressed firmly, but gently, through the skin and into the vein. Sometimes a popping sensation or sudden give is felt when the needle enters the vein. When the needle is in the vein, the vein is kept distended with the thumb, while the blood is collected. Never withdraw blood excessively quickly.

Q (2) Would you expect a pulse to be visible normally?

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x. When the required volume of blood is collected the left thumb is removed, allowing the vein to collapse then the syringe and needle are withdrawn swiftly and evenly from the vein. Pressure is applied to the puncture site with a swab and hold for 30 – 60 seconds to halt the bleeding.

xi. The hypodermic needle is removed from the syringe before gently expelling the blood into a tube.

Q (3) Why should you remove the needle from the syringe when expelling the blood sample into the tube?

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Ie. Processing of Blood

If whole blood or plasma is required, then some form of anticoagulant must be added to the fresh blood to prevent the sample from forming an insoluble clot. There are four anticoagulants in common use: **oxalates, sodium citrate, a potassium or sodium salt of ethylenediaminetetraacetic acid (EDTA)** and **heparin**. The first three act by forming a complex with calcium, a necessary component of clotting. Heparin acts by preventing the action of thrombin on fibrinogen. The oxalates are toxic and should not be used in collecting blood for transfusion. In addition, oxalate precludes the use of blood for determination of non-protein nitrogen or urea nitrogen, due to the presence of the ammonium ion.

Sodium citrate is used for anticoagulation at the rate of one part of 3.8% aqueous solution to 9 parts of blood. A mixture of citrates, ("ACD"), is used in blood transfusions. It consists of 1.37g sodium citrate dihydrate, 0.44g anhydrous citric acid and 1.447g hydrous dextrose in distilled water, made to 100ml. ACD is used at a rate of one part to four parts of blood. If a citrate sample is used for routine haematology then the results need to be corrected for dilution of the blood sample.

Heparin, a natural anticoagulant occurring in various tissues, is found abundantly in the liver. Heparin does not alter erythrocyte volume but can interfere with staining of leukocytes. Ten international units (1 int. unit = 0.0077mg of Na salt) per ml of blood will prevent clotting.

EDTA is the preferred anti-coagulant for blood morphology studies, and the one you will use most commonly in practice. It is commonly used at 1.0 to 2.0mg/ml of blood. Excess EDTA will cause shrinkage of erythrocytes. The EDTA is a powder in the tube and thus there is no dilution effect on resultsleu.

Blood Film

Thin, well-stained blood films are essential for the examination and evaluation of erythrocytes and leukocytes. A good smear can be a very useful tool for a quick diagnosis – either in the laboratory or in a Veterinary practice.

Blood smears can be prepared on slides or coverslips. The slide technique, i.e. a drop of blood on a slide that is smeared across that slide with another glass slide, is suitable for routine work. A coverslip technique is sometimes used for birds and reptiles.

Task 1

Review the Clinical Skills Guide on Making a Blood smear and watch the video on Canvas LMS.

The distribution of leukocytes is not uniform since the large white cells accumulate at the edges of the blood film, or the ‘feathered edge’. This is not a problem, so long as you know where to look for them!

Task 2

Watch the video on how to examine a blood smear. This gives a clear procedure for identifying the regions of the smear, how to perform an initial survey, and how to do a differential cell count.

APPROACH TO BLOOD SMEAR EXAMINATION

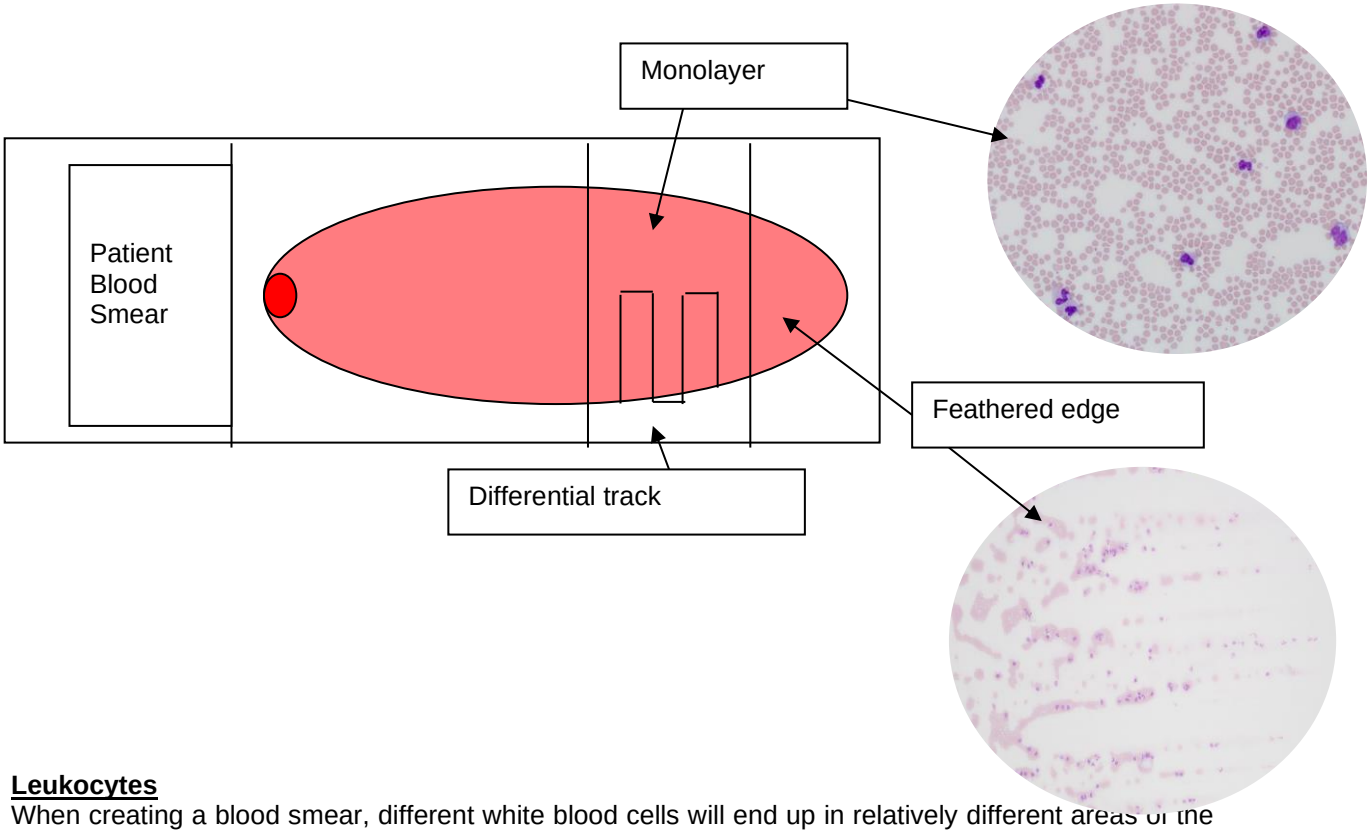
1. **Low power scan** (4-10x dry) - overall impression of the quality of the smear and stain

2. **Feathered edge scan** (4-10x dry) - large cells or organisms tend to be pushed towards the edge of the smear, however morphology of normal cells tend to be disrupted in this area. Check the feathered edge for the presence of:

- large, abnormal cells in circulation (e.g. mast cells, leukaemia cells)
- large blood parasites (e.g. microfilaria)
- platelet clumping (common in cats)
  Note: if platelet clumps are found it is likely the automated platelet count and/or estimated smear count will be inaccurate.

3. **Find the monolayer** – also called the "counting area" (40x dry, 100x oil)

- the monolayer is found between the streaky feathered edge zone and the thicker body of the smear (where cells are too close together and often overlap each other), typically one field of view from the feathered edge with the 10x lens
- cells in the monolayer are separated or barely touching, and morphology is therefore able to be evaluated more easily
- identification of abnormal morphology in areas outside the monolayer may be artefactual, e.g. red blood cells may appear to be spherocytes or echinocytes in the thicker body of the smear
- detailed examination of the **three cell lines** (RBC, WBC, platelets) occurs in the monolayer.



Leukocytes

When creating a blood smear, different white blood cells will end up in relatively different areas of the monolayer according to their size and weight. So when evaluating the smear ensure you are in the monolayer, and that you cover a representative area of the monolayer (typically in a "battlement" pattern as shown in the image above "differential track") to ensure your estimate will be as accurate as possible.

Evaluation of WBC's in the blood smear should include manual estimates of total white blood cell count and a differential, especially if you want to validate an automated analysis or you do not have access

to an automated analyser, and evaluation of cell morphology e.g. check for toxic change, left shift, atypical cells, parasites etc.

Estimated total white cell count (WCC) from the smear:

- Count number of leukocytes in 10 high power fields (40x)
- Calculate average number of leukocytes per high power field (i.e. divide total by 10)
- Multiply by 2 to get an estimated WCC in units 10^9/L to compare to reference intervals

The estimated WCC can be used to confirm the haematology analyser WCC result.

Differential white cell count from the smear:

- Following the battlement pattern count 100 consecutive white blood cells using high power (40x or 100x) and categorise them into whether they are neutrophils, lymphocytes, monocytes, eosinophils or basophils
- Note any unusual or atypical cells or morphology, such as bands, metamyelocytes, mast cells, reactive lymphocytes, toxic change, and atypical leukocytes.
- The percentage value must then be used to create an **absolute value**, as the significance of the proportion will depend on the total white cell count:

e.g. if the estimated WCC = 10.4 x 10^9/L and differential shows neutrophils at 70% then the actual (or absolute) neutrophil proportion is 0.70 x 10.4 = 7.28 x 10^9/L

Erythrocytes

You will learn more about evaluating erythrocytes in Veterinary Bioscience: Respiratory System in semester two. When evaluating erythrocytes you should evaluate density, size, shape, colour, formation and check for presence of inclusions. Erythrocyte size and shape varies amongst species.

Platelets

Examination of platelets should include evaluation of the number of platelets present and checking the smear for clumps, particularly in the feathered edge. Morphology of platelets is often variable particularly in cats (including large platelets) and they are prone to clumping which leads to falsely low analyser platelet counts and smear estimates. You will learn more about evaluating platelets in Veterinary Bioscience: Respiratory System in semester two.

Identification of leukocytes in domestic species

There are 5 main types of leukocytes that can be identified in common domestic species using Wright's Giemsa or Diff Quik stain:

- Neutrophils – generally have a segmented or multi-lobed nucleus and neutral staining cytoplasm (hence the name neutrophil). These are the most common leukocytes in the blood of most domestic species other than ruminants. Mature neutrophils have multiple lobes or segments, while immature neutrophils (band neutrophils) have simpler, less-segmented nuclei.
- Lymphocytes – these are round cells with a nucleus that takes up most of the cell and that often has a slight indentation. The cytoplasm stains pale blue. These are the next most common blood leukocyte type in domestic species, and the most common type in ruminant blood.
- Monocytes – larger cells with a nucleus that can range from oval to varying degrees of indentation from single kidney bean shape to multiple indentations and lobular shapes. The cytoplasm is blue-grey and can contain vacuoles. Monocytes usually are less than 10% of the blood leukocytes.
- Eosinophils – these have a segmented or multi-lobed nucleus, that is less defined than a neutrophil nucleus. Eosinophils are rare in normal blood of most species. The cytoplasm

stains pale blue but is generally obscured by granules that stain reddish to orange (eosin-loving). The shape and colour of the granules differ between species.
    - Dog granules are round and variable in number
    - Cat granules are rod shaped and fill the cytoplasm
    - Horse granules are large, round or oblong and fill the cytoplasm, often hiding the nucleus
    - Ruminant granules are small, round and fill the cytoplasm
- Basophils – similar in size to neutrophils but very rare in the blood of most species. They have a segmented nucleus and a light purple cytoplasm (basic staining). The granules may or may not be visible, depending on the stain used and the species.
    - Dog granules have low numbers of small purple granules
    - Cat granules are small, oval and lavender
    - Horse and ruminant granules are small, numerous and purple and may obscure the nucleus

Task 3

Examine smears from common domestic species and draw diagrams of the main cell types you can find. You can take screen shots as well but drawing the cells will help you to remember the main features. Describe the general appearance of each cell type and note both the similarities and differences between species. This will provide you with your own resource of images that you can build on as you progress through the course.

The images are reached in the Slice database by the following hyperlinks:
Normal sheep http://www.best.edu.au/s/5zq4sg5d
Normal cow http://www.best.edu.au/s/ra9v5tar
Normal dog http://www.best.edu.au/s/d8ducpzu
Healthy cat http://www.best.edu.au/s/ekj7kjgz (has a mild eosinophilia)
Normal horse http://www.best.edu.au/s/nwgjlzga

Resources to help identify leukocytes:

1) Canvas LMS prac folder contains images of each leukocyte type for these species "**Comparative leukocyte morphology**"
2) The College of Veterinary Medicine at Cornell University has an excellent online resource of images for identifying and contrasting leukocyte morphology, and is available at: https://eclinpath.com/atlas/hematology/different-species/
3) The MSD Veterinary Manual also has a library of images and is available at: https://www.msdvetmanual.com/circulatory-system/hematopoietic-system-introduction/white-blood-cells-in-animals

Task 4

Perform differential leukocyte counts on the five digitised blood smears:

1) Choose a magnification that allows you to both scan across the image quickly and identify cells. You may have to zoom in if the cell is hard to identify.
2) Begin the count by moving back and forth across the smear in a battlement pattern that avoids covering the same territory (and that keeps you in the optimal viewing area as much as possible). This can be done by moving sideways across the slide in one direction, then down to the next field and moving back across the slide in the other direction. Identify each leukocyte that is encountered until 100 cells have been counted and sorted by type, giving you a percentage of each cell type or a relative differential leukocyte count.

3) Calculate the absolute count for each cell type (% x Total WBC count). e.g. If % lymphocytes is 20% and the animals total WBC count is 10 x 10^9/L the absolute count for lymphocytes is 20% x10 = 2x10^9/L

Record your results in the tables below.

Sheep

Total white cells counted: _____

Total WBC count 5.1 x10^9/L			
Cell Type	Number counted	% of total	Absolute differential count x10^9/L
Lymphocytes			
Neutrophils			
Monocytes			
Eosinophils			
Basophils			

Cow

Total white cells counted: _____

Total WBC count 7.7 x10^9/L			
Cell Type	Number counted	% of total	Absolute differential count x10^9/L
Lymphocytes			
Neutrophils			
Monocytes			
Eosinophils			
Basophils			

Dog

Total white cells counted: _____

Total WBC count 15.3 x10^9/L			
Cell Type	Number counted	% of total	Absolute differential count x10^9/L
Lymphocytes			
Neutrophils			
Monocytes			
Eosinophils			
Basophils			

Cat

Total white cells counted: _____

Total WBC count 13.8 x10^9/L			
Cell Type	Number counted	% of total	Absolute differential count x10^9/L
Lymphocytes			
Neutrophils			
Monocytes			
Eosinophils			
Basophils			

What process/es could cause an eosinophilia in this cat?

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Horse

Total white cells counted: _____

Total WBC count 7.9 x10^9/L			
Cell Type	Number counted	% of total	Absolute differential count x10^9/L
Lymphocytes			
Neutrophils			
Monocytes			
Eosinophils			
Basophils			

Task 5

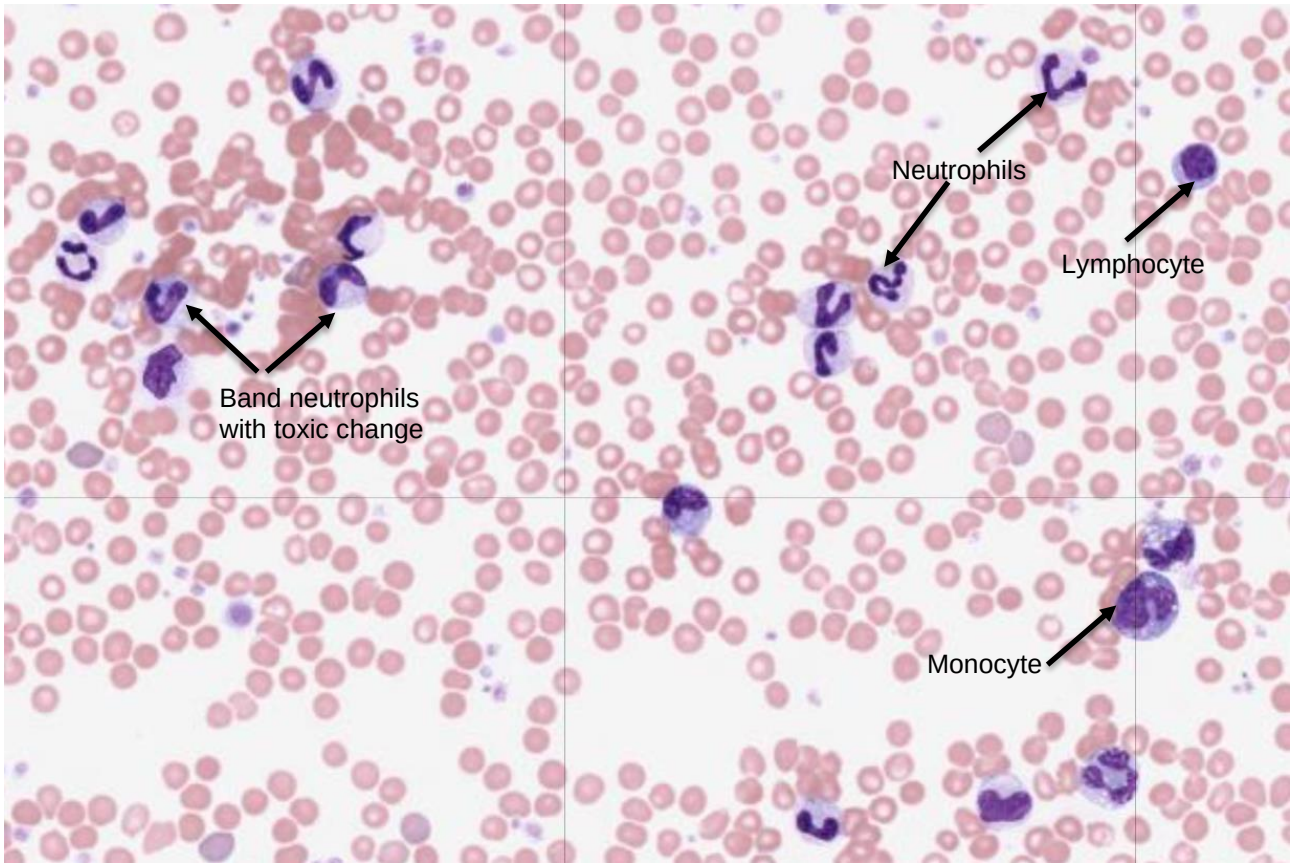
Case Study

You are presented with a 5-year old female Japanese Chin dog with a history of 3 days of lethargy, increased thirst and occasional vomiting. She has a fever and a tense caudal abdomen.

You run haematology on your clinic analyser and prepare a blood smear. The analyser has provided you with a differential count, however there are instrument flags suggesting it is inaccurate due to the presence of suspected band neutrophils (immature neutrophils with a U-shaped nucleus) and toxic change (blue tinting of the cytoplasm due to increased RNA associated with immaturity).

Evaluate the haematology results below and the scanned blood smear and perform a manual differential count then calculate the absolute counts using the total WBC count from the analyser. Find some band and metamyelocyte neutrophils in the smear (examples shown below). Note that the monocyte is the larger and bluer than the neutrophils and bands.

http://www.best.edu.au/s/pmsvrjvd



Haematology		**Manual differential %**	**Manual absolute count**	**Analyser Results**	**Reference Values**
Red cell count	x 10^{12}/L			**4.5**	5.5 – 8.5
Haemoglobin	g/L			**100**	120 – 180
Haematocrit	L/L			**0.28**	0.37 – 0.55
Platelets	x 10^{9}/L			500	200 – 500
White cell count	x 10^{9}/L			**135.0**	6.0 – 17.0
Metamyelocytes	x 10^{9}/L				0
Bands	x 10^{9}/L				0 – 0.3
Neutrophils	x 10^{9}/L			**108.4#**	3.0 – 11.5
Lymphocytes	x 10^{9}/L			**8.1#**	1.0 – 4.8
Monocytes	x 10^{9}/L			**17.5#**	0.2 – 1.4
Eosinophils	x 10^{9}/L			0	0.1 – 1.3
Basophils	x 10^{9}/L			0	< 0.1
Total protein (ref.)	g/L			**102**	60 - 80
# Potential differential error due to suspect bands/toxic change					

What process do the blood smear findings and haematology results suggest is going on in this bitch? Justify your answer

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Task 6 Haematology Questions:

1. Why would a veterinarian wish to measure the total numbers and types of cells in blood?

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2. What might cause a decline in the numbers of erythrocytes?

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3. What might cause an increase in the percentage of neutrophils?

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4. What might cause an increase in the percentage of eosinophils?

Reference

Weiss, D.J and Wardrop, K.J (eds) *Schalm's Veterinary Hematology*, 6th Edition, 2010