CLINICAL LABORATORY DIAGNOSIS

Animal Medicine Department

Pathological variations in blood

- A. ANAEMIA
- B. POLYCYTHAEMIA

A. ANAEMIA

- Definition
- Classification

Definition

- Anaemia is a reduction below normal in the number of erythrocytes, haemoglobin or both in the circulating blood per unit volume of blood.
- In domestic animals, anaemia is seldom(rarely) a primary condition but most often is a secondary response following or associated with a disease condition.

Classification of anaemia

- Morphological classification
- Aetiological classification
- According to erythrocyte count

Morphological classification of Anaemia

- Abnormalities in Shape
- Abnormalities in Size.
- Abnormalities in Stain.

1. MORPHOLOGICAL CLASSIFICATION OF ANAEMIA

The RBCs of mammalian species are distinctly flat and disc shapes with little or no depression near the center.

The erythrocyte of dog and man is distinct biconcave disc; consequently, these cells have a distinct central pallor in stained smear

1. Abnormalities in Shape (Poikilocytosis)

- Changes in the Shape of RBCs include the following

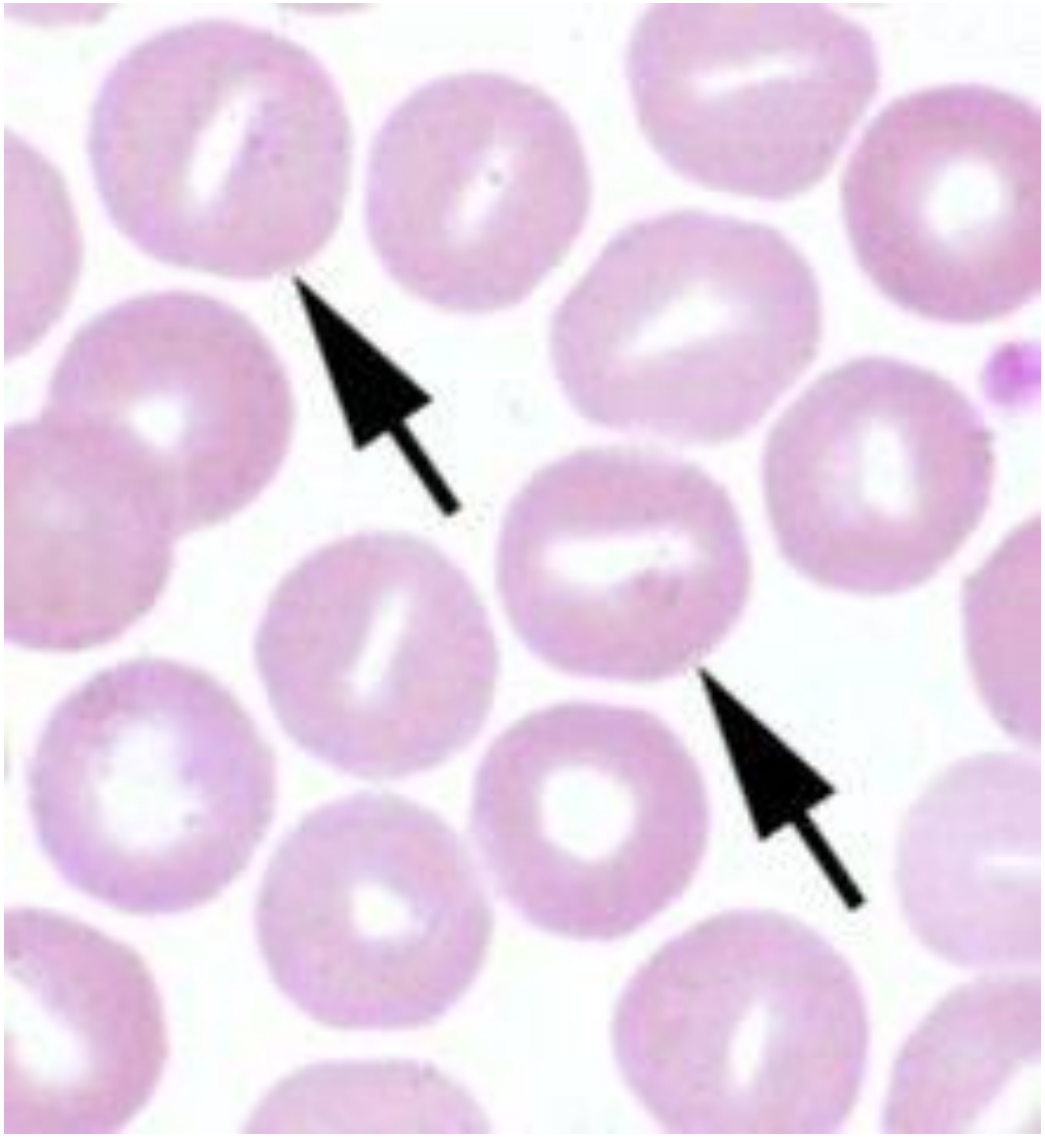
A. Leptocytes
B. Spherocytes
C. Ovalocytes
D. Target cells.
E. Eccentrocytes
F. Crenation
G. Nucleated cells

Leptocytes

- Thin erythrocyte
- Surface area increased , No change in cell volume.
- Cell membrane may fold
- More resistant to haemolysis in hypotonic saline solution.

Leptocytes

- Since such cells have a larger diameter, it is usually failed to participate in rouleaux formation
- Such large cells may become entrapped in the buffy coat and give a pinkish tinge to this normally white layer.



B. Target cell

- Described as shaped like a bull's eye **with a peripheral ring of haemoglobin separated by clear unstained zone from a dense central eye**.

Target cell

Most commonly seen in dog's blood indicate to:

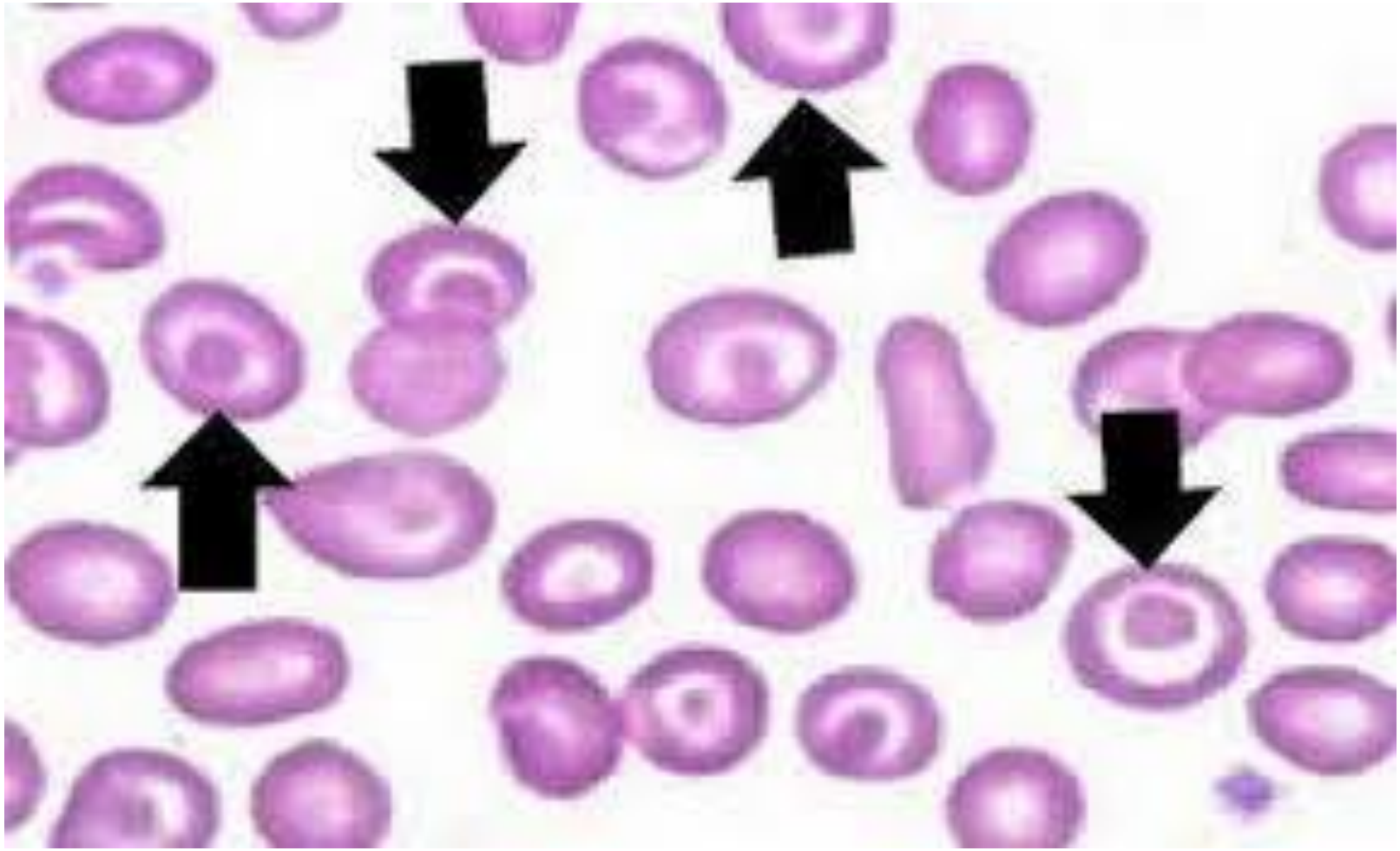
1- Artifact resulting from:

A- Fixation

B- Staining

2- Chronic disease.

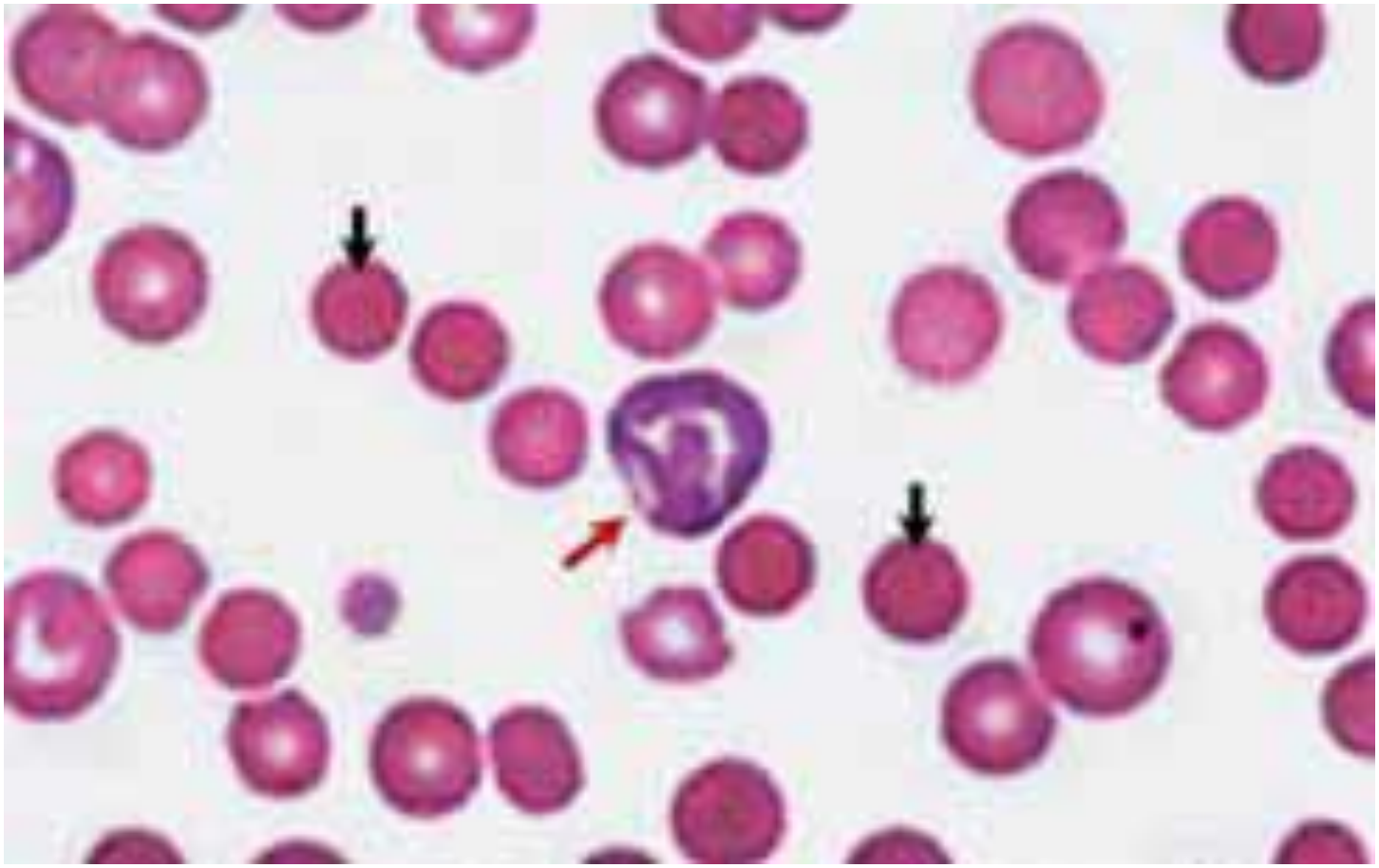
Target cell



C. Spherocytes

- C. Spherocytes
- Small
- Easily rupture and fragile
- Not common in domestic animals
- In man, spherocyte is a congenital disease.

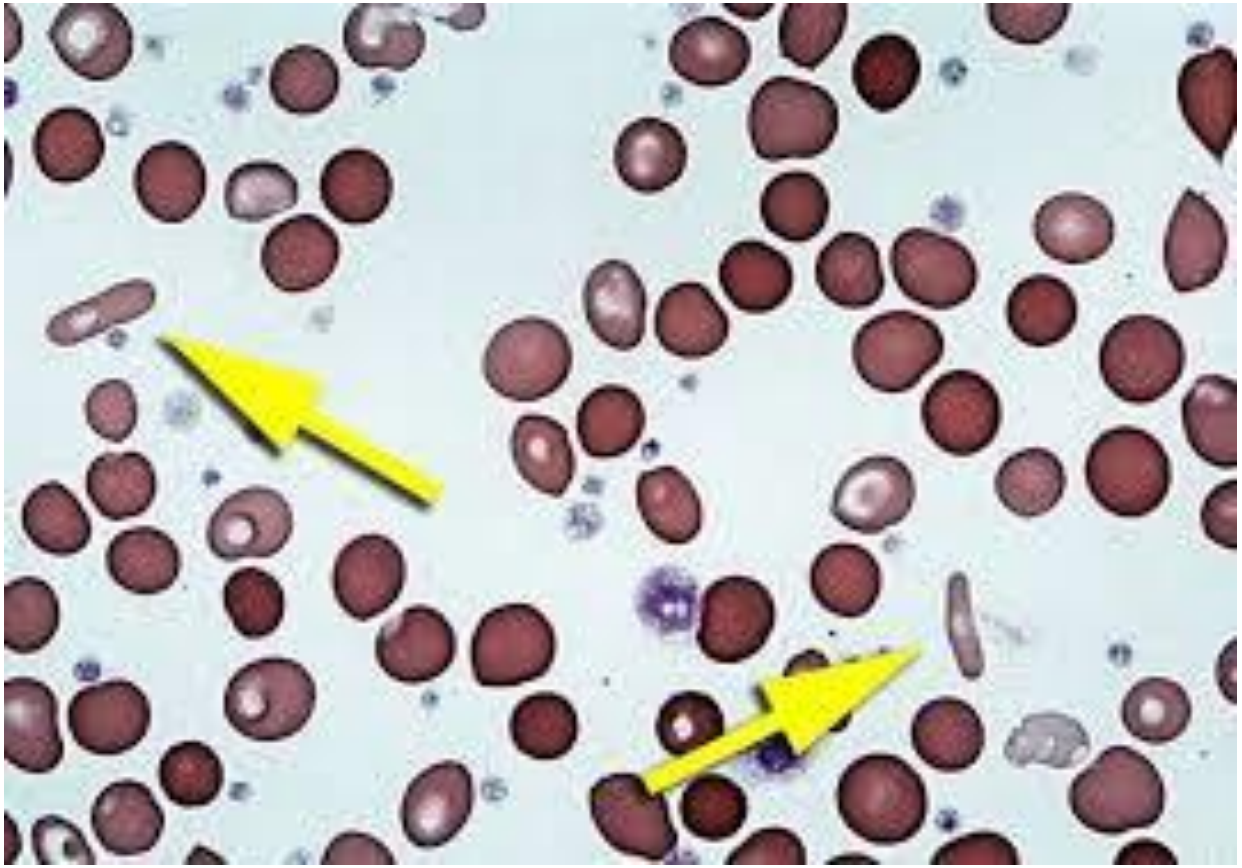
Spherocytes (*black arrows) and a polychromatophil (red arrow) in a dog (modified Wright's stain; 100).*



D. Ovalocytes

- D. Ovalocytes
- This indicates the existence of oval erythrocytes.
- Normal in camel & bird blood.
- Appear in:
- Advanced stage of anaemia
- Artifact during staining of smear

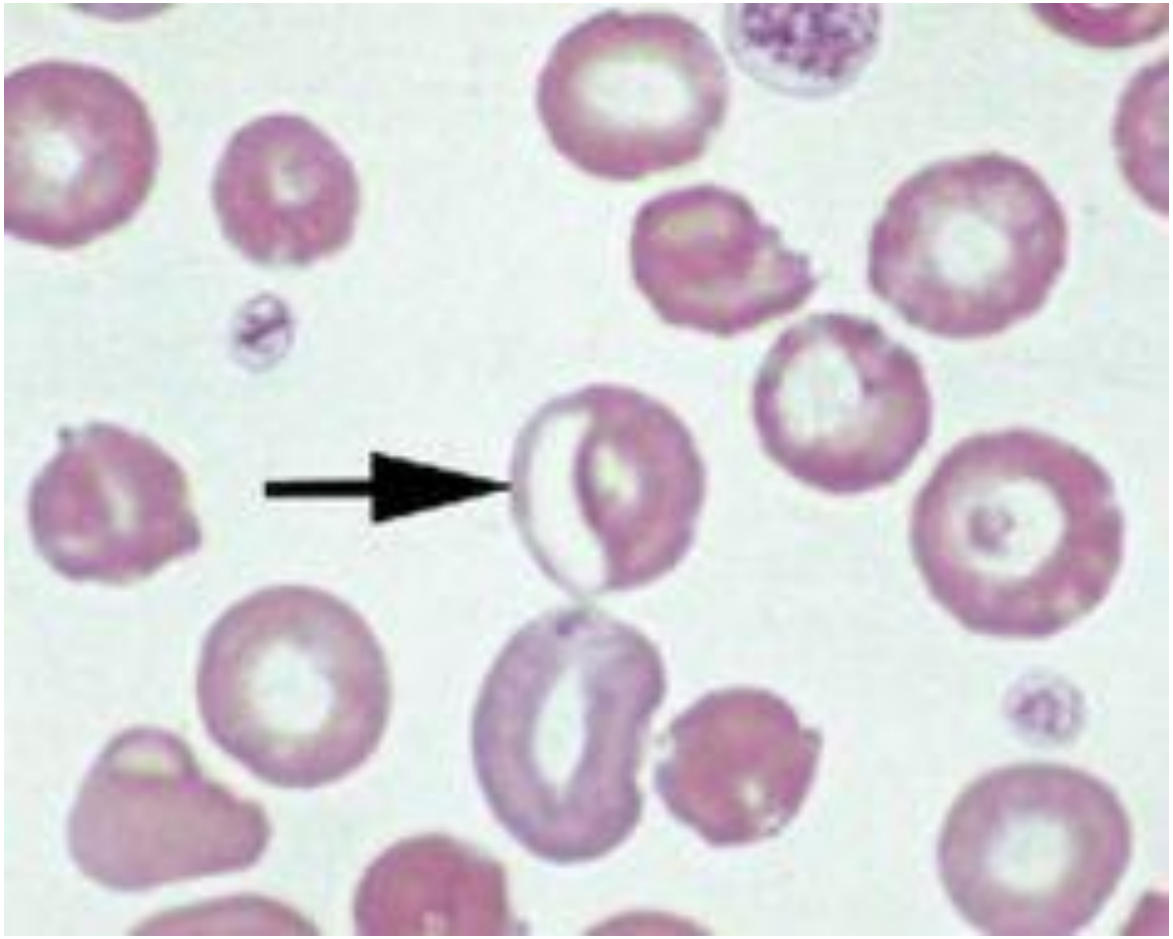
Ovalocytes



E. Eccentrocytes

- E. Eccentrocytes
- These are RBCs where the haemoglobin has coalesced together in one area.
- They develop in dogs poisoned with paracetamol or onions.

E. Eccentrocytes



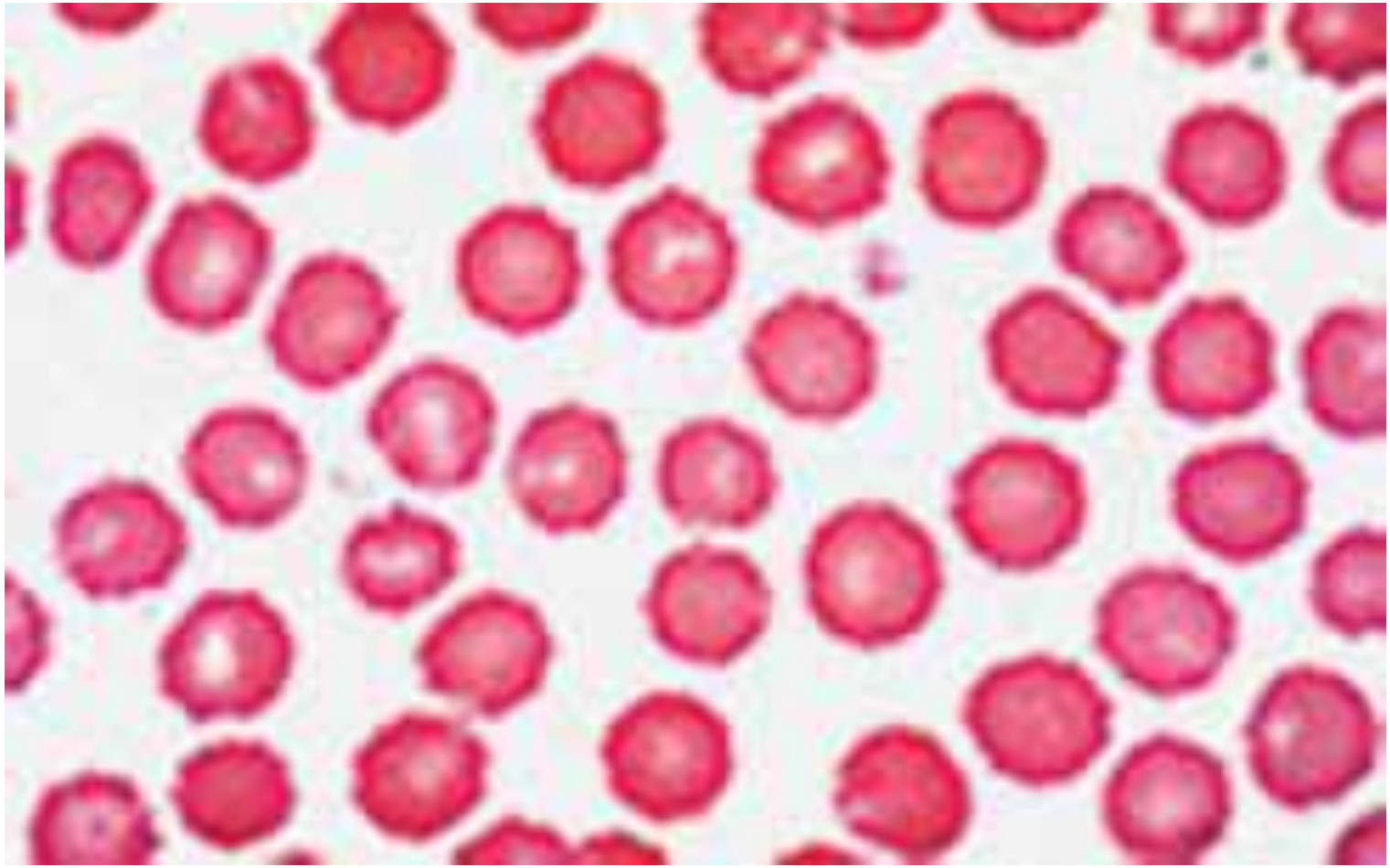
F. Crenation (acanthocytes)

- Not clinically significant
- Results from:
- Delayed drying
- Exposure to lytic agent,
- Presence of hypertonic solution
- When blood is allowed to stand

Crenation (acanthocytes)

- The RBCs may have blunt processes
- Crenated: cells appear `crinkle-edged'. Seen in old samples, also normally
- In smear from pig's blood, the processes have sharp points.
- in pig blood even when fresh. Extensive crenation typical of some acute haemolytic conditions.

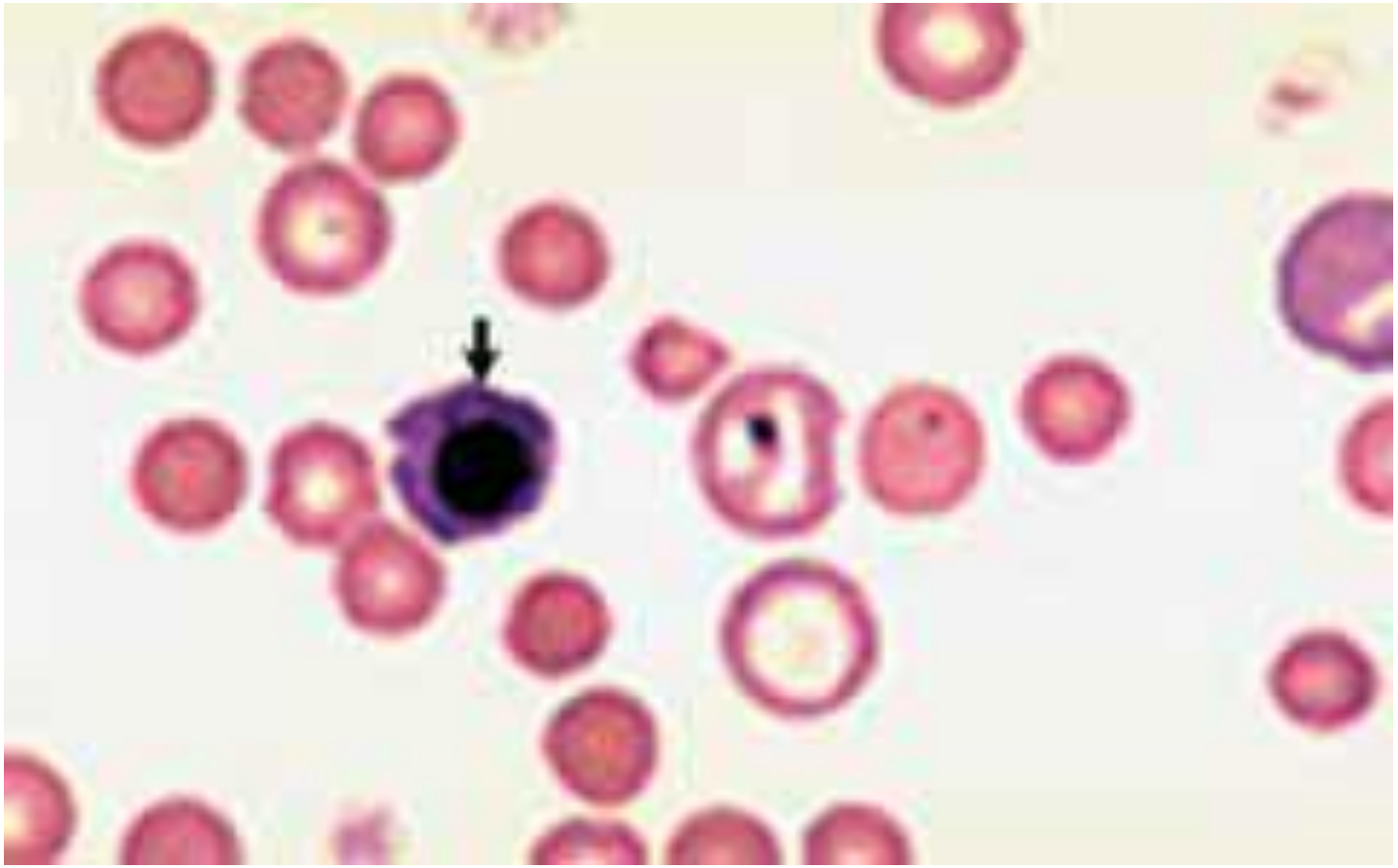
Crenation (modified Wright's stain; 100).



G. Nucleated

- G. Nucleated
- Normally in small number in young dog, cat and in young pigs as at the 1st 3 months of life. Appear in case of severe anaemia and leukemia
- Appearance of nucleated RBCs for long time means defect in Erythropoiesis

A nucleated RBC (*arrow) in a dog (modified Wright's* stain; 100).



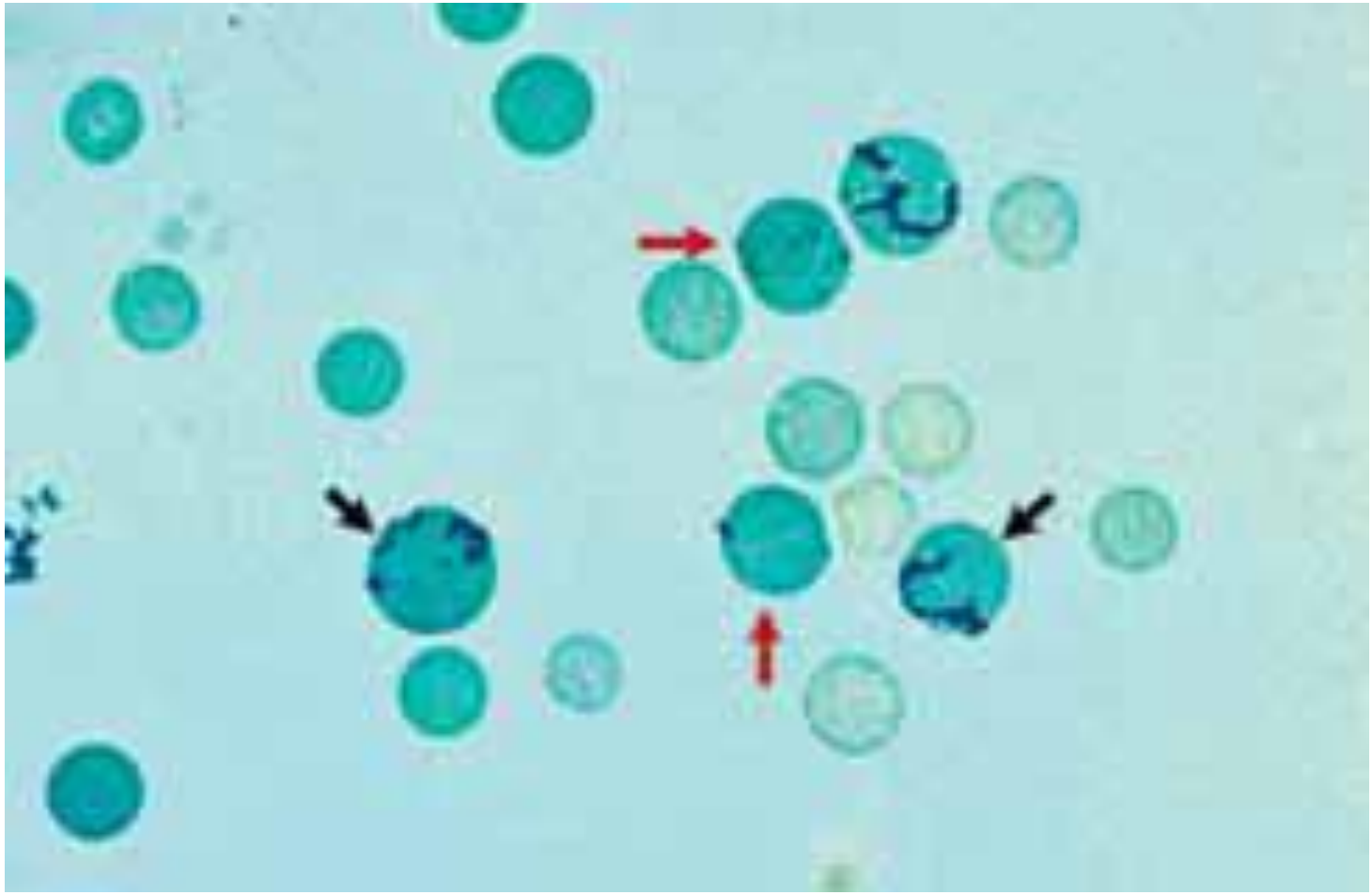
Inclusion bodies of erythrocytes

- 1. Reticulocytes
- 2. Basophilic stippling
- A. Punctate basophilic granules.
- B. Diffuse basophilic granules
- 3. Howell Jolly body
- 4. Heinz bodies
- 5. Distemper inclusion bodies
- 6. Protozoan parasites

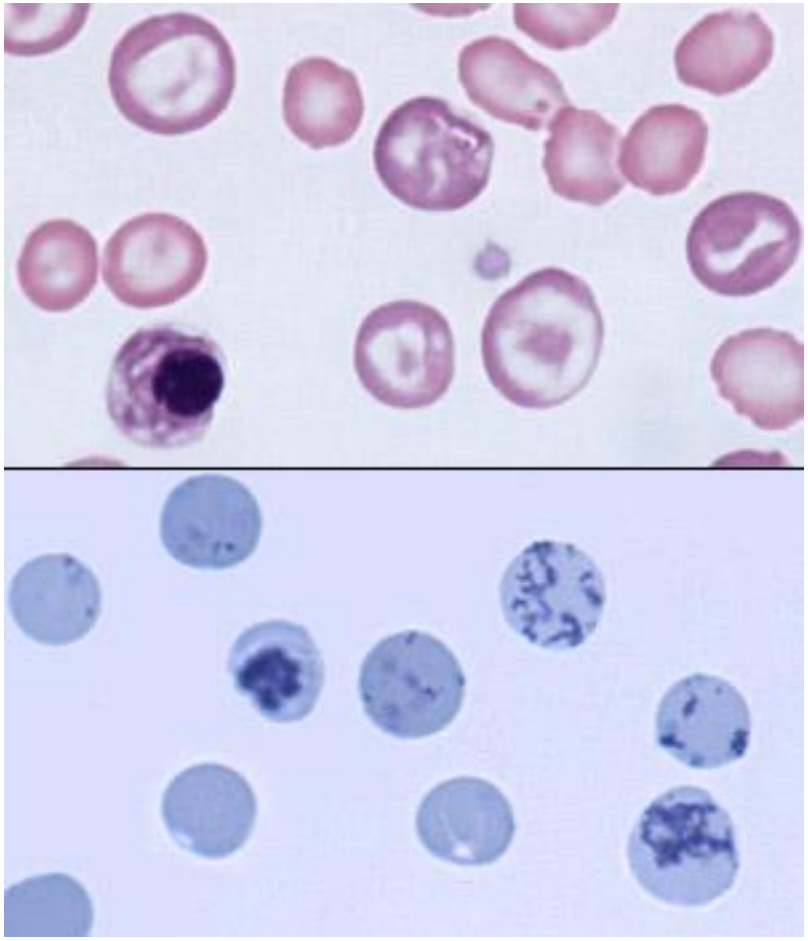
1. Reticulocytes

- 1. Reticulocytes
- Reticulocyte is a non-nucleated cell of the erythrocytic series which when stained with brilliant cresyl blue, present one or more granules or diffuse network of fibrils.

Aggregate (*black arrows) and punctuate (red arrows)* reticulocytes in a cat. Note that aggregate reticulocytes contain greater than or equal to five basophilic specks (new methylene blue stain; 100).



Canine Reteculocytes



The reticulocytes differ from erythrocytes in:

- a Larger in size
- The size of the cell in the erythrocytic maturation series decreases as the cell mature. Thus, the reticulocyte is larger than the erythrocyte.
- b. More resistant to crenation
- c. Has a lower specific gravity
- d. More resistant to hypotonic saline solution.
- e Reticulocytes don't participate in Rouleaux formation
- f. It is more resistant to specific hemolytic serum.

1. Reticulocytes

- Reticulocytes are not found in health in the blood of the horse, cattle, sheep and goat, this means that the reticulocytes ripen in the bone marrow of these animals.

1. Reticulocytes

- Dogs and cats may have an average of 0.5- 1 % reticulated cells in peripheral blood. On the other hand, rabbit guinea pig, rat and mouse are good subject for the demonstration of reticulocytes, this because blood of these animals contain between 2-4 %reticulocytes.

1. Reticulocytes

- Reticulocytosis:
- Occur in cases of:
- Acute hemorrhage.
- Chronic hemorrhage or
- Hemolytic anaemia,

2. Basophilic stippling

- 2. Basophilic stippling A condition of the erythrocyte in which blue staining basophilic granules are scattered over the cell

2. Basophilic stippling

- There are two types of basophilic stippling:

- A. Punctate basophilic granules.
- B. Diffuse basophilic granules

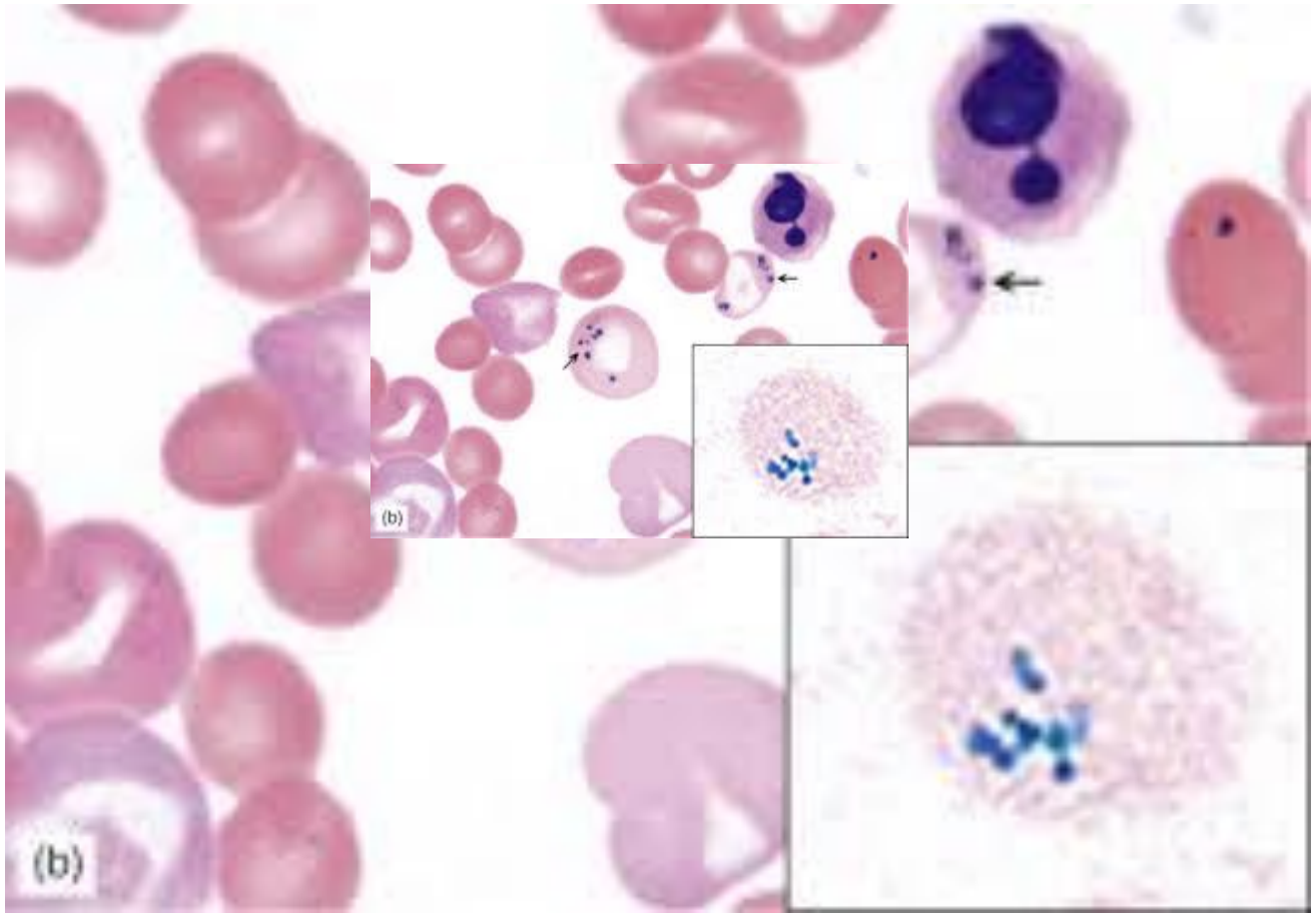
A- Punctuate basophilic granules.

- Means the appearance of punctuate aggregation of basophilic staining material in the form of large number of fine or coarse granules in the erythrocytes.
- The number of the granules present in an erythrocyte vanes in inverse ratio to the size of the granules

Punctuate basophilic granules

- They stain deep blue with Wright's stain.
- Stippling is generally attributed to degenerative changes in the cytoplasm involving RNA in the young cells
- Occurs in anaemia of bovine and ovine species.

Punctuate basophilic granules



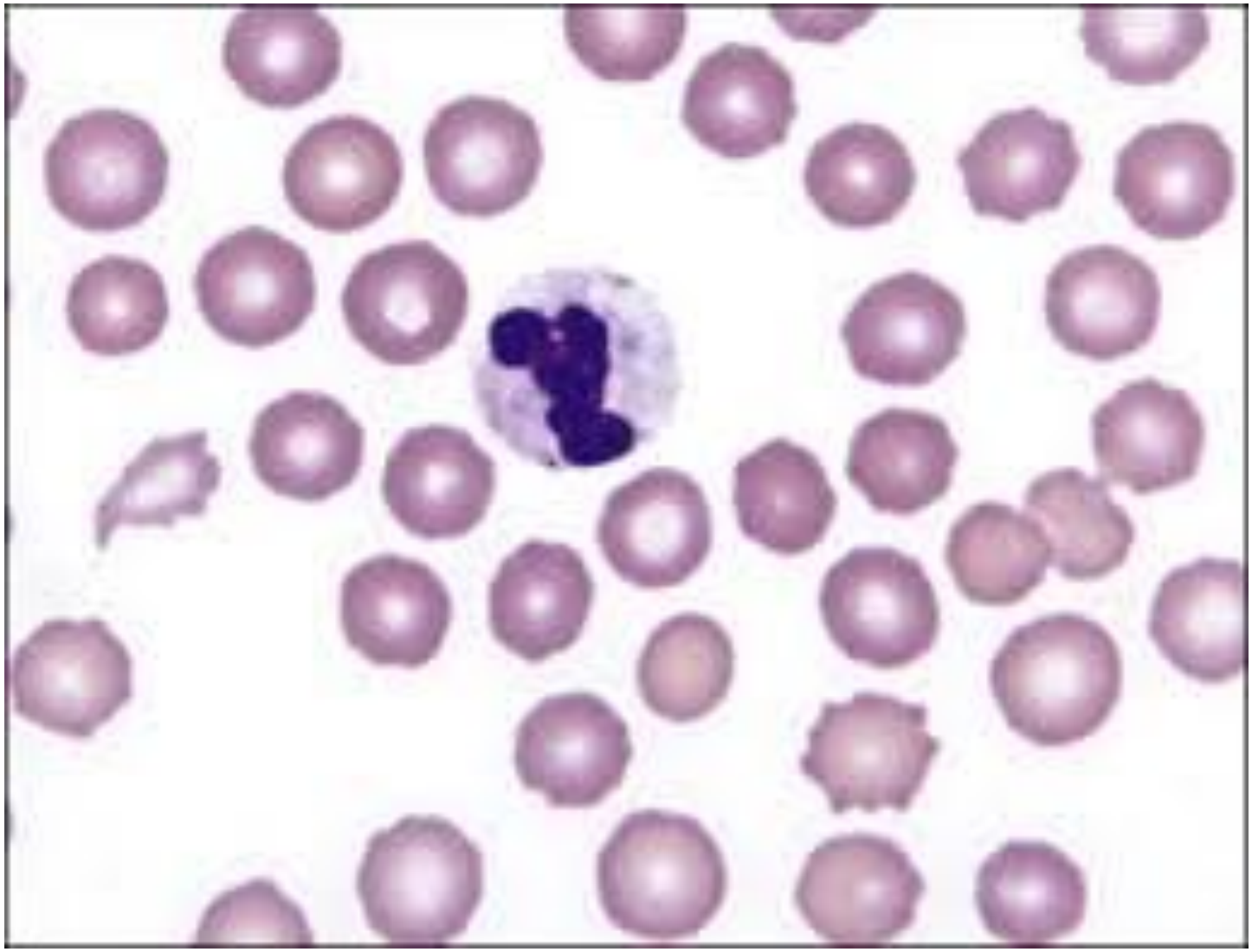
B. Diffuse basophilia

- B. Diffuse basophilia
- Characterized by an overall bluish color to the nomally red stained erythrocytes, it is a remnant of RBCs nucleus mostly RNA
- In addition to the overall distribution of this basophilic substance, the cell may in fixed preparations have a patchy appearance of bluish basophilic area.

Diffuse basophilia

- The later is known as polychromatophilia
- Such cells occur in anaemia and represent an immature form of erythrocyte.
- Diffuse basophilic stippling is of common occurrence in the cow and sheep under conditions of intense erythrogenesis e.g. acute anaplasmosis and Haemonchosis,

Diffuse basophilia



3. Howell Jolly body

- 3. Howell Jolly body
- This is a nuclear remnant of 1-2 microns in diameter, after the nucleus has been extruded.
- In Wright's stained smear, Howell jolly bodies appear as single and at times double spherical bluish bodies within red blood cells.
- They are also found in small numbers in the cat and horse.

3. Howell Jolly body

- Spleen remove the Howell jolly bodies by its pitting function without destruction of the erythrocyte, following splenectomy the number of Howell jolly bodies in the circulation may increased
- Howell jolly bodies are most commonly observed in anaemia in remission

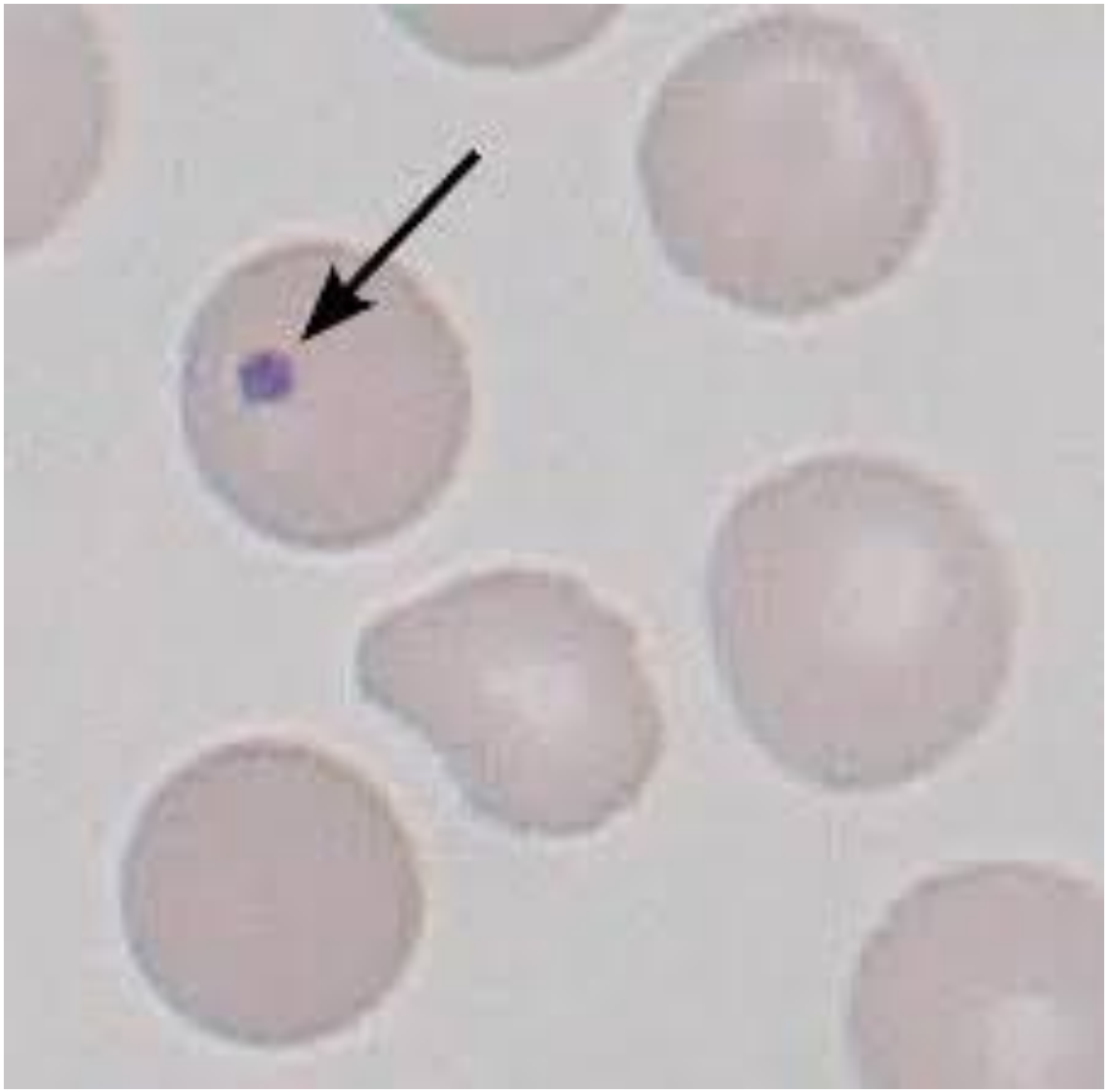
3. Howell Jolly body

- Howell jolly bodies are most commonly observes no differences in size between erythrocytes with and those without Howell jolly bodies

3. Howell Jolly body

- It must be differentiated from Anaplasma marginale in bovine by:
- Howell jolly bodies may appear anywhere within the RBCs and not confined to the periphery as anaplasma.
- Anaplasma are usually uniform in size, however, Howell jolly bodies may vary considerably in size and location within the cell.
- Howell jolly bodies seen occasionally in horse blood where it located eccentrically.

Howell Jolly body



4. Heinz bodies

- Heinz bodies are small, round to irregularly shaped retractile inclusion bodies that may occur as a single or may be multiple within a single cell.
- They formed within the RBCs of man and animals exposed to toxic drugs and chemicals and often resulting in hemolytic anaemia.

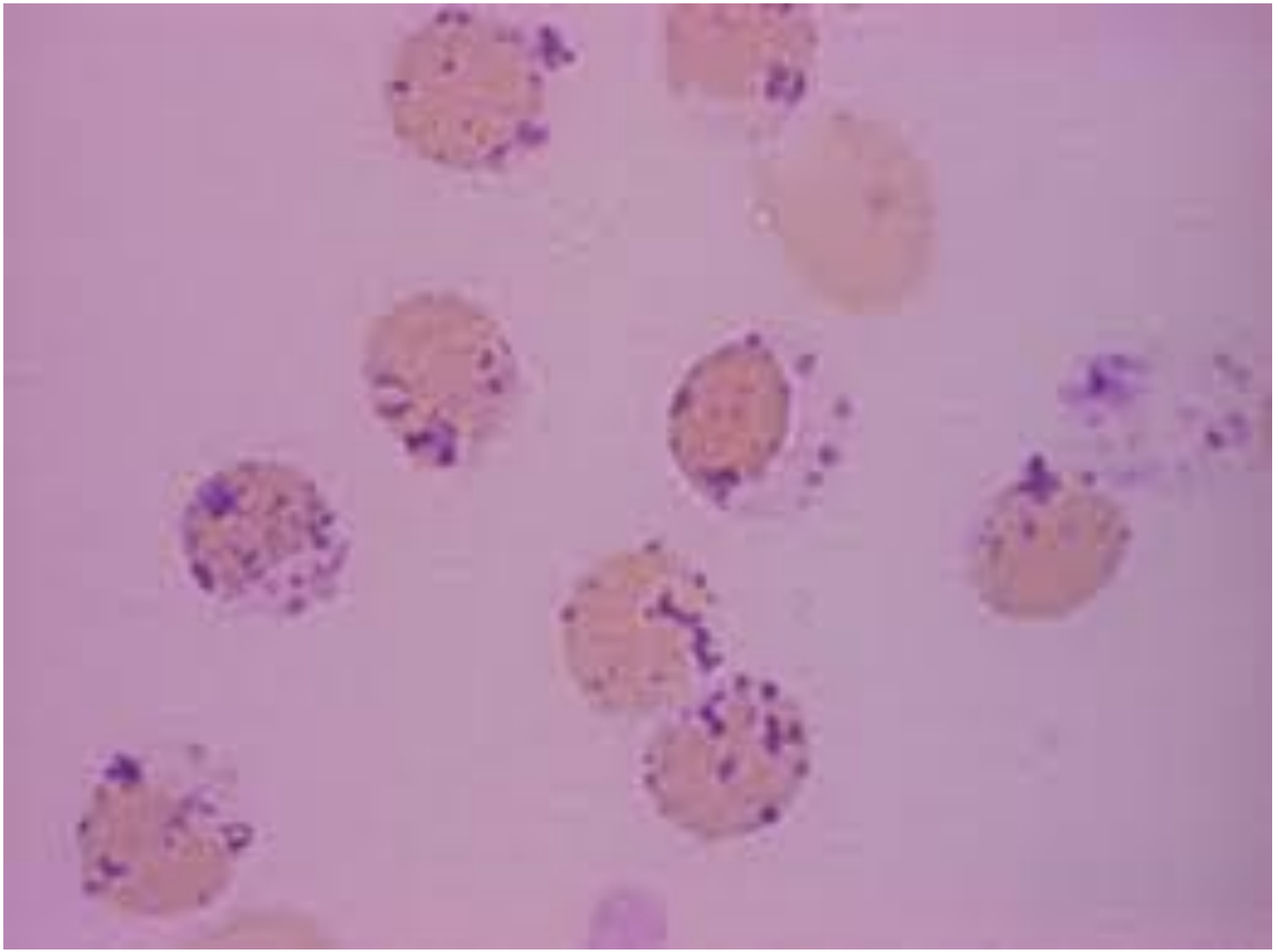
4. Heinz bodies

- Heinz bodies may be one or more within the red cell wall and they move around (Brownian motion) or remain fixed in one position, ordinarily they are eccentrically placed located near the margin,

4. Heinz bodies

- True Heinz inclusions are not visible in Wright's stained preparation but may be seen in reticulocyte smears as well as in unfixed and unstained smears.
- They disappear after fixation with either methyl or ethyl alcohol

Heinz bodies



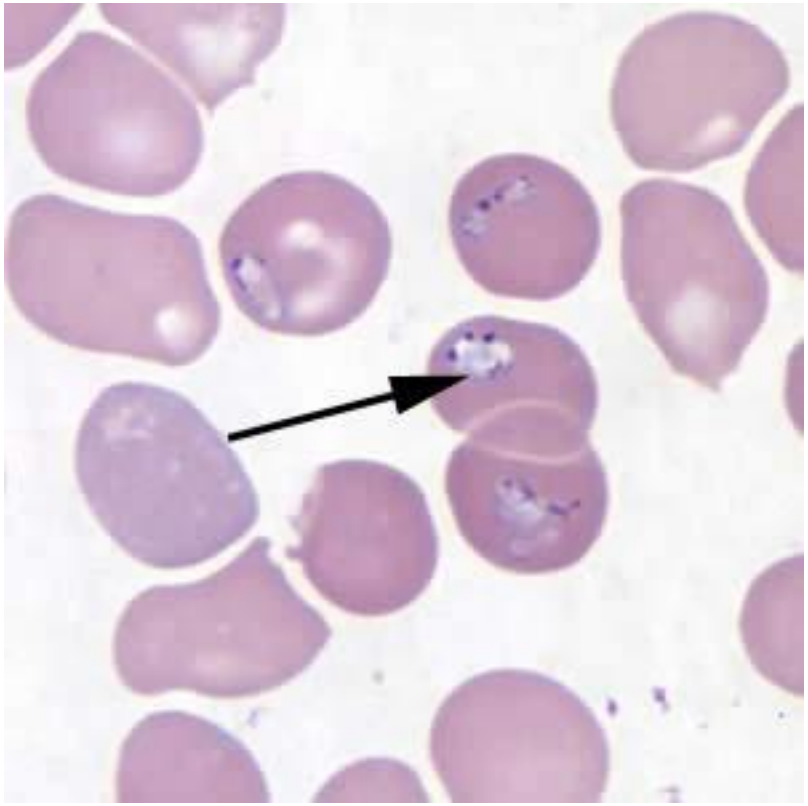
5. Distemper inclusion bodies

- In Wright's or leishman stained blood film, the inclusion bodies take a pale blue stain and it is larger than Howell jolly bodies
- When the blue inclusion bodies occur in a target cell the inclusion was found in the central bull's eye.
- Such as the bar extending across the middle of the cell With new methylene blue, the inclusion appears pink to light red in color.

6. Protozoan parasites

- 6. Protozoan parasites These include intracellular blood parasites such as Babasia Spp. Theileria Spp, and Anaplasma Spp.

Babesia



3. Abnormalities in size of the RBCs (Anisocytosis)

- 3. Abnormalities in size of the RBCs (Anisocytosis)
- • Means change in the size of the RBCs.
- • The erythrocyte of the dog is the largest and that of the goat (3-4u) and sheep (4-5u) is the smallest
- These include normocytic anaemia, microcytic anaemia, and macrocytic anaemia.

Macrocytic anaemia

- a) Macrocytic normochromic anaemia
- b) Macrocytic hypochromic anaemia

Macrocytic anaemia

- a) Macrocytic normochromic anaemia
- 1. Cobalt deficiency (Vitamin B12 deficiency).
- 2. Congenital porphyrinuria.

b) Macrocytic hypochromic anaemia

b) Macrocytic hypochromic anaemia
Transitory condition occurring during active phase of erythrocyte regeneration following acute blood loss or erythrocyte destruction.

Macrocytic hypochromic anaemia

a. Spontaneous haemorrhage from hypoprothrombinemia caused by sweat clover poisoning

b. Bacillary haemoglobinuria.

C. Leptospirosis

d. Parturient haemoglobinuria,

e. Anaplasmosis.

f. Piroplasmosis.

Microcytic hypochromic anaemia

Microcytic hypochromic anaemia

1. Deficiency of iron
- 2. Defect in utilization of iron store in the body

Microcytic hypochromic anaemia

- 1. Deficiency of iron
- a. Chronic blood loss from injury to vascular bed, which does not heal.
- b. Heavy infection with blood sucking parasites (Haemonchus, ticks)
- c-Dietary iron deficiency.
- 2. Defect in utilization of iron store in the body
- a. Copper deficiency.
- b. Molybdenum poisoning.

Normocytic normochromic anaemia

- Normocytic normochromic anaemia
- 1. Stomach worm infection (excluding Haemonchus, which causes chronic blood loss).
- 2. Leukaemia or other marrow displacement.
- 3. Hypoplastic anaemia
- a. Bracken fern poisoning.
- b. Radiation injury

4. Abnormalities in stain:

- 1. Hypochromic anaemia: This implies RBCs with reduced haemoglobin content (hypochromic).
- 2. Hyperchromic anaemia: Normal RBCs with dense coloration of the RBCs wall.
- 3. Normochromic anaemia: Value of haemoglobin in individual RBCs increase or decrease with increase or decrease in size of the RBCs,

II. AETIOLOGICAL CLASSIFICATION OF ANAEMIA

- 1. Haemorrhagic anaemia
- a) Acute blood loss anaemia
- b- chronic hemorrhagic anemia
- 2- Haemolytic anaemia

1. Haemorrhagic anaemia

- 1. Haemorrhagic anaemia
- a) Acute blood loss anaemia
- Def.
- Causes
- Laboratory findings
- b- Chronic hemorrhagic anemia

a) Acute blood loss anaemia

- a) Acute blood loss anaemia
- The anaemia of acute blood loss occurs when large proportion generally 25 % of the circulating blood volume is lost in a period of several minutes to several hours.

a) Acute blood loss anaemia

- Causes
- 1. Trauma, cutting of major blood vessel or surgery.
- 2 Haemorrhage from highly vascular malignant neoplasm

a) Acute blood loss anaemia

3. Congenital or acquired defect of coagulation mechanisms such that occur with in poisoning with:

- Sweet clover
- Warfarin
- Bracken fern.
- Trichloroethylene extracted soybean oil meal.

a) Acute blood loss anaemia

Laboratory findings

Sings of regeneration

- Reticulocytosis.
- Anisocytosis.
- Decrease Myeloid/ Erythroid ratio (M/E ratio).

2 Increase MCV.

3. Decrease MCH.

4. Leukocytosis (especially neutrophils, in early stage of bone marrow regeneration).

b- Chronic hemorrhagic anemia

- Anaemia associated with chronic haemorrhage is usually microcytic hypochromic.
- Firstly, the body compensates the loss in RBCs, but with time consumption of iron store occurs, so that the anaemia is iron deficiency anaemia (↓MCV, ↓MCHC and Poikilocytosis).
-

b- chronic hemorrhagic anemia

- Causes
- Internal parasites such as **stomach worm**, hookworms, coccidia, nodular worm and liver flukes, which produce anaemia by combination of blood loss and poor nutrition
- 2 External parasites such as ticks, blood sucking lice and certain types of flea,
- 3. Hemorrhagic gastritis and enteritis

b- chronic hemorrhagic anemia

- 4. Gastric ulcers.
- 5. Chronic haemorrhage in the genitourinary tract.
- 6. Haemorrhage into body cavities from neoplasm.
- Chronic blood loss anaemia is almost usually microcytic hypochromic and result physiologically from lack of iron for formation of new haemoglobin

2- Haemolytic anaemia

- 2- Haemolytic anaemia
- This type of anaemia is associated with excessive destruction of erythrocytes.

2- Haemolytic anaemia

- Causes

1- Blood parasites causing anaemia

- -This includes the genera piroplasmosis, haemobartonella and erythrozoon
- -All these conditions are characteristically diagnosed by demonstration of typical inclusions or parasites on or in RBCS.

Haemolytic anaemia

- Parasitized erythrocytes are most easily detected during acute stage of disease.
- Parasitized erythrocytes tend to be larger and of lower specific gravity than none parasitized ones.
- Parasitized erythrocytes present in large number in capillary than in venous blood.

2- Haemolytic anaemia

- 2. Anaemia and bacterial infection.
- There are two common bacterial infection in which anaemia occurs
- Which are leptospirosis and Clostridium haemolyticum infection

Haemolytic anaemia

- The haemolytic activity of Leptospira has been shown to be the result of formation of a haemolysin probably a lecithinase that splits the lecithin of erythrocyte membrane resulting in lysis of erythrocytes.
- Within the genus clostridia two member of group, Clostridium perfringens type A and Clostridium haemolyticum both produce a potent haemolysin.

2- Haemolytic anaemia

- 3. Viral infection
- Equine infectious anaemia characterized by chronic illness. animal infected with the virus become carrier.
- Clinically the disease characterized by an intermittent fever.
- jaundice, oedema and petechial haemorrhages in the mucosa.

2- Haemolytic anaemia

- 4. Chemical agents
  - Copper
  - lead, phenothiazine.
  - Saponin, acetanilide, nitrofurantoin.
  - Neoarsphenamine, phenacetin and some sulfonamide.

2- Haemolytic anaemia

- Copper
- Sheep are the most susceptible animal to excessive intake of copper. This element accumulates in the liver and under certain conditions of stress may be released into the blood stream resulting in rapid haemolysis.
- Bone marrow is characteristically hyperplastic as a result of response to acute blood destruction

2- Haemolytic anaemia

- Phenothiazine
- • Used as anthelmintic especially in horse.
- • This drug accelerates the lytic action of naturally occurring lysolecithin present in horse blood
- Heinz bodies reported

2- Haemolytic anaemia

- 4. Poisonous plants
- Caster bean, oak shoots, Broom and frosted turnips.

2- Haemolytic anaemia

- 5. Some Metabolic diseases
- Post-parturient haemoglobinuria.
- Cold haemoglobinuria of calves and occasionally of older cattle, which occurs following ingestion of cold water. characterized by intravascular haemolysis and haemoglobinuria, and associated with cardiac insufficiency and pulmonary oedema.

2- Haemolytic anaemia :

- 7. Immune mediated diseases: Immune mediated anaemia is divided into:
- a- Autoimmune haemolytic anaemia: Where the body forms antibodies against its own RBCS.

2- Haemolytic anaemia

- b- Isoimmune haemolytic anaemia: The antibodies agnist RBCs come from another individual which either:
- 1. Incompatible blood transfusion
- 2. Neonatal isoerythrolysis: Maternal antibodies against the antigen of the offspring's RBCs are transferred in the colostrum, from a mother previously Formation of antibodies in mother against fetus.

3- Nutritional deficiency anaemia

- 3- Nutritional deficiency anaemia Anaemia due to nutritional deficiency seldom occur as a single entity in domestic animals and more commonly associated with disease condition that result in anorexia debilitation or absorption of nutrients.
- Nutritional deficiency anaemia occurs either due to deficiency of mineral, vitamins or proteins.

3- Nutritional deficiency anaemia

- A. Mineral deficiency anaemia
- The principal minerals include iron, cobalt and copper.
- 1. Iron deficiency anaemia
- Dietary iron deficiency may be observed in animals grazing on pasture deficient in iron.
- Also in calves suckling milk and which are not provided with additional supplements.
- Severe chronic haemorrhage may deplete the store of iron in the body
- Lack of intestinal absorption of iron due to intestinal disturbances

3- Nutritional deficiency anaemia

- Molybdenum poisoning interferes with copper metabolism, which in turn may interfere with the utilization of iron
- The fundamental defect in these deficiencies is reduced haemoglobin synthesis, but the red cell count is not markedly lowered as haemoglobin concentration.
- Characterized by microcytic hypochromic anaemia.

3- Nutritional deficiency anaemia

2- Copper deficiency .

- In copper deficient areas.
- Molybdenum in excessive quantities interferes with copper
- utilization affecting the availability of iron and its utilization in
- haemoglobin synthesis
- Cattle pastured on soil with high molybdenum content scour profusely, the pigmentation of hairs decrease and animals become debilitated

3- Nutritional deficiency anaemia

- 3. Cobalt deficiency
- • Accompanied by a decrease in synthesis of Vit.B12 in sheep.
- The morphology of the blood cells suggest aplastic anaemia and as the disease progress a marked macrocytic anaemia with an accompanying poikilocytosis and polycythaemia develop.

3- Nutritional deficiency anaemia

- B. Vitamin deficiency
- Restriction of nicotinic acid in the diet may decrease folic acid synthesis.
- Vit B and folic acid are called RBCs maturation factors
- In presence of low protein and niacin intake, black tongue may develop

3- Nutritional deficiency anaemia

- • Nicotinic acid deficiency results in severe macrocytic anaemia with leucopenia develop
- • Vitamin B12 deficiency rarely to occur in animals.
- Also pyridoxine deficiency rarely occurs in animals, it has been reported that B6 is required by the dog for utilization of iron in haemoglobin synthesis

3- Nutritional deficiency anaemia

- C. Protein deficiency
- • Dietary deficiency of proteins may interferes with haemoglobin production and result in the development of anaemia.
- Lysine deficiency in swine produces normocytic normochromic anaemia.

4- Anaemia due to bone marrow depression

- 4- Anaemia due to bone marrow depression
- A. Hypoplastic anaemia.
- B. Aplastic anaemia.
- A. Hypoplastic anaemia
- The hemopoietic tissues fail to produce the required amount of erythrocytes to maintain the normal erythrocyte count in the circulating blood.

4- Anaemia due to bone marrow depression

- B. Aplastic anaemia
- Failure of the bone marrow to produce erythrocytes.
- Causes
- 1. Radiation (X Ray)
- 2 Certain chemicals as sulphonamides, chloramphenicol, lead, copper, arsenic and mercury.
- 3-Exhaustion of the bone marrow may follow chronic blood loss

4- Anaemia due to bone marrow depression

- 4-Chemical agents as:.
- Bracken fern poisoning.
- Arsenical compounds.
- Certain antibiotics (chloramphenicol)

B. POLYCYTHAEMIA

- Definition
- Polycythaemia means increase the number erythrocytes above the upper limit specific for each animal species per unit volume of blood.
- Classification
- 1. Relative (apparent) polycythaemia.
- II. Absolute polycythaemia,
- 1. Relative (apparent) polycythaemia.
- It is an apparent or relative increase in the number of erythrocytes due to loss of water from the blood,

- Aetiology
- 1. Haemoconcentration as in cases of diarrhea, diuresis, excessive vomiting or shock
- 2. Excitement, which results in release of epinephrine that stimulate contraction of the spleen.

B. POLYCYTHAEMIA

- Laboratory findings
- 1. Increase total RBCs count, haemoglobin concentration and PCV %
- 2. Decrease plasma volume.
- 3. Increase plasma protein level.
- 4. Normal platelets and leucocytes counts.
- 5. Normal erythropoietin level.
-

B. POLYCYTHAEMIA

- II. Absolute (true) polycythaemia
- It is a real excess of total erythrocyte count and it is always associated with increase blood formation,
- It may be:
- 1. Primary polycythaemia.
- 2. Secondary polycythaemia.

B. POLYCYTHAEMIA

- 1. Primary polycythaemia
- Definition
- A disease condition characterized by well-marked increase in the number of red corpuscles.

B. POLYCYTHAEMIA

- Causes
- a. Excessive erythroplastic activity of the bone marrow (hyperplasia of hemopoietic tissues of the bone marrow).
- b. Unknown causes
- 2. Secondary polycythaemia (Erythrocytosis)
- Erythrocytosis should be regarded as a conservative vital reaction i. e. an effort on the part of the organism to compensate for some difficulty in the oxygenation of blood and tissues of the body.

B. POLYCYTHAEMIA

- True polycythaemia appears following hypoxic stimulation of the bone marrow under the following conditions:
- Exposure to high altitude.
- Any disease that interferes with the oxygenation of the erythrocytes as in obstructive lesion in air passage ways
- Circulatory insufficiency that permits stagnation of blood accompanying hypoxia..

B. POLYCYTHAEMIA

- Laboratory findings
- 1. Increase total RBCs count, haemoglobin concentration an PCV %
- 2. Normal plasma volume.
- 3. Normal plasma protein level.
- 4. Increase platelets and leucocytes counts in conditions accompanied with infections.


Thank you