





BLOOD EXAMINATION

By

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► **Blood:** is a fluid media:

- **Straw yellow in color.**
- **Slight alkaline PH (7.35 : 7.45 average 7.4) .**
- **Suspended with cellular components (RBCs, WBCs & blood platelets).**

Indications

1. All cases characterized by fever.
2. For diagnosis of blood parasites:

A. Intracellular:

- Babesia: B.caballi & B.equi, B.bigemina & B. bovis
- Theileria: T.annulata & T.parva.

B. Extracellular: Trypanosomiasis & filariasis.

Indications

3. **For diagnosis of some bacterial diseases as: pastereulosis, leptospirosis and anthrax.**
4. **CBC examination to estimate RBCs, WBCs & differential WBCs counts, PCV, Hb.**
5. **Serology and serum biochemical analysis**
6. **Before surgical operations (blood compatibility).**



Collected in different forms according to the type of examination:

- ▶ **Whole blood; with anticoagulant for hematological ex. Or viral isolation.**
- ▶ **Blood smear; for piroplasmosis, pasteurullosis.**
- ▶ **Blood swab, for bacteriological cultivation.**
- ▶ **Blood without anticoagulant (serum) for serological examination and serum biochemical analysis.**



Technique

- ▶ **Clipping and shaved the area over the vein**
- ▶ **Clean thoroughly with a detergent**
- ▶ **Dry and disinfection by ethyl alcohol 70%**
- ▶ **Collect blood sample by sharp wide pored needle according to size of animal**

Blood sample tools

Vacuum tube and syringe



1. Red cap (Plain)
2. Blue cap (Sodium Citrate anticoagulant)
3. Yellow cap (Clot Activator + Separator gel)
4. Green cap (Heparin anticoagulant)
5. Violet cap (EDTA anticoagulant)
6. Grey cap (Fluoride anticoagulant)

Common anticoagulants

1. EDTA:

- **Dose** : 1 mg /1 ml blood (1 gm / 1 litre blood)
- Used in determination of urea, creatinine, uric acid, ph and glucose.
- Not used in determination of some electrolytes such as Ca & Cl because they bind with them.
- **The best anticoagulant used for hematological examination.**

2. Heparin:

- Natural anticoagulant which prevent conversion of prothrombine to thrombine so fibrinogen not converted to fibrin (No Clot)
- **Dose** : 0.1 ml / 1 ml blood
- **Used in : blood transfusion.**

Common anticoagulants

3. Flouride :

- **Dose:** 2 drops as 40 % solution / 5 ml blood
- Can be used in sugar determination **Not** used in blood transfusion

4. Citrate:

- **Dose:** 2 – 4 mg / 1 ml blood
- Used mainly in blood transfusion

5. Oxalates :

- **Dose:** 1mg / 2ml blood
- **Contraindicated** to be used in blood transfusion why ?
 - a) Combine with calcium forming calcium oxalates.
 - b) Make shrinkage of RBCs.

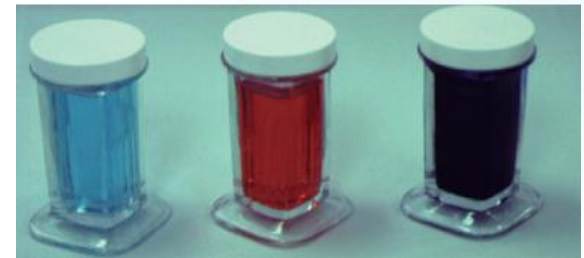
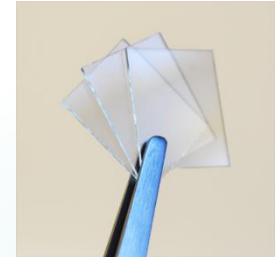
Site of blood sampling

- ❖ **Cattle : Jugular vein and milk vein.**
- ❖ **Camel and equine: Tibial vein & jugular vein
(caudal 2/3)**
- ❖ **Dog and cat**
 - **Fore limb in radial or cephalic vein.**
 - **Hind limb in saphenous and recurrent tarsal V.**



Tools

1. Coverslips
2. Microscope slide
3. Stains
4. Staining jars
5. Tap or deionized water
6. Methyl alcohol (Methanol)
7. Light microscope (Bright field)
8. Immersion oil (Cider oil)
9. Paper towel





Types of examination

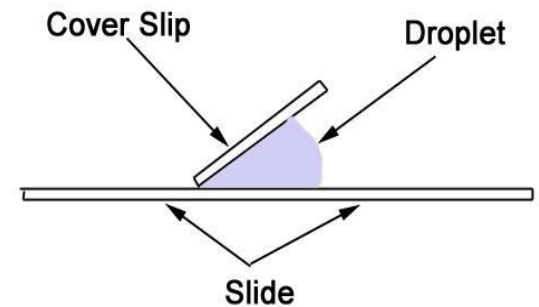
1. Microscopic examination of unstained blood films (wet blood film).

1. Microscopic examination of stained blood films (dry blood film).

1. Wet blood film

Indications:

1. Cytological examination to detect type of anemia acc.to shape and size of RBCs.
2. Diagnosis of extracellular blood parasites



Procedures:

1. Put a small drop of blood on the surface of very clean glass slide (Center).
2. Put cover slide on the drop.
3. Examine under microscope.

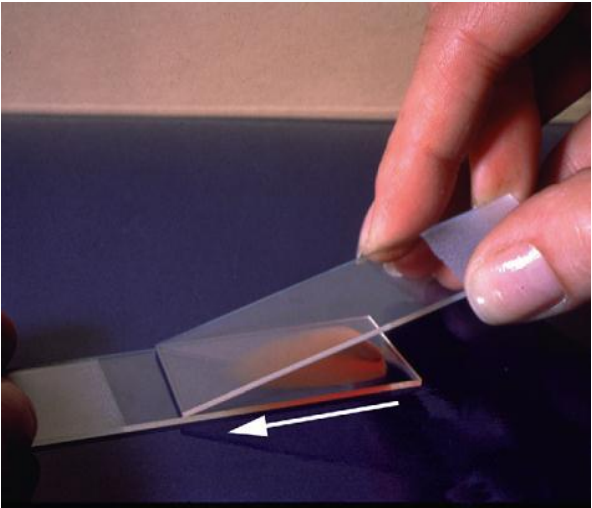
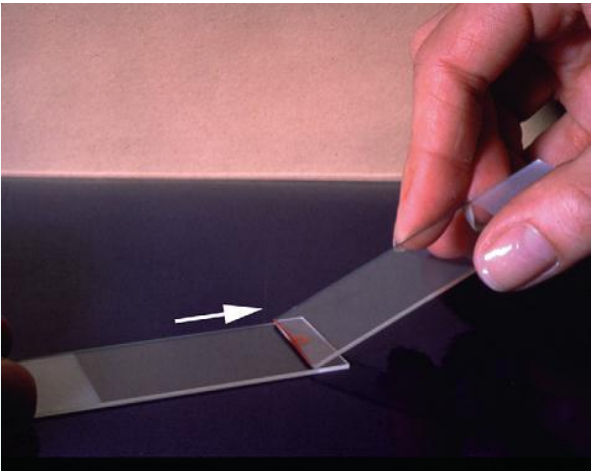
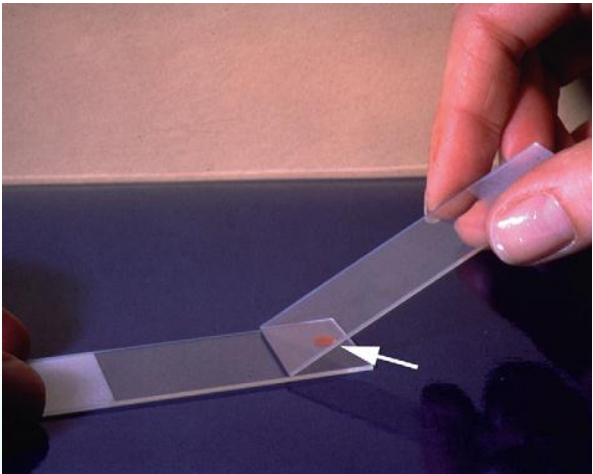
In case of microfilaria we can add one drop of acridine 1/5000 as a stain giving the microfilaria a yellow color.




2. Dry blood film

Indications:

1. Diagnosis of intracellular blood parasites such Babesia, theileria and anaplasma.



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1. Put a small drop of blood near one end of the slide.
 2. By using the second slide (spreader) make back word movement toward the blood drop (acute angle) until touching it (wait 2 sec.).
 3. Make forward spreading of the blood film evenly.
 4. Dry by moving the slide in air for 3-5 minutes,
 5. Identify the slide (write the name of patient, date & number).
- OR**

6. Staining of blood film & examine under microscope



Staining of blood film

1. Giemsa stain

Film flood with methyl alcohol for **3-5 minutes**
(fixation)



Air dry, then put Giemsa stain 8-10 % for **45-60 minutes**



Wash with water, dry with filter paper, put **cedar oil**
and examined using oil immersion lens.

2. Leishman stain

Cover the film with stock Leishman stain (15 drop),
leaves for **2-3 minutes (fixative period)**



Dilute with equal quantity of distilled water and
leave for **5-10 minute**



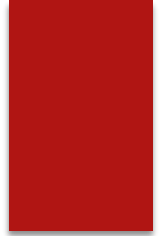
Decant the stain, wash with distilled water, dry with
filter paper, **put cedar oil** and examined

| Items | Thin Film | Thick Film |
|--------------------|-------------------------|-------------------------|
| Size of drop | Small | Large |
| Angle of spreading | Narrow | Wide |
| Speed of spreading | Rapid | Slow |
| indication | Intracellular parasites | Extracellular parasites |

Characters of good blood film

1. Covers $\frac{3}{4}$ of the slide
2. Thick at drop end and thin at opposite end
3. Occupy central portion of slide
4. Microscopically: even distribution of cells and not overlapping

Common faults of blood film



1. Too thick:

- Too large blood film, Too large angle of spreading & Too slow spread speed.

2. Too Thin:

- Too small drop, Too small angle of spreading & Too fast spread speed.

3. Streaks throughout the film :

- Irregular or notched edges of spreader.
- Presence of dried blood at edges of spreader.

Common faults of blood film

4. Presence of spots on blood film :

- Presence of greasy material on slide that absorb blood

5. Alternative thick and thin bands on slide :

- Jerky movement (hesitation) of the hand during spreading.

6. Too narrow and thick blood film :

- Raise edges of spreader from surface of slide during spreading.
- When spreading occurs before blood had run along the edges of the spreader.

Characters of good blood film

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4. Microscopically: even distribution of cells and not overlapping.

1 – Babesia

- ❖ *B. bigemina* (large 2:4 microns – pair pear shape, Located at periphery).
- ❖ *B. bovis* (small 1:2 microns – pair pear shape, Located at center)

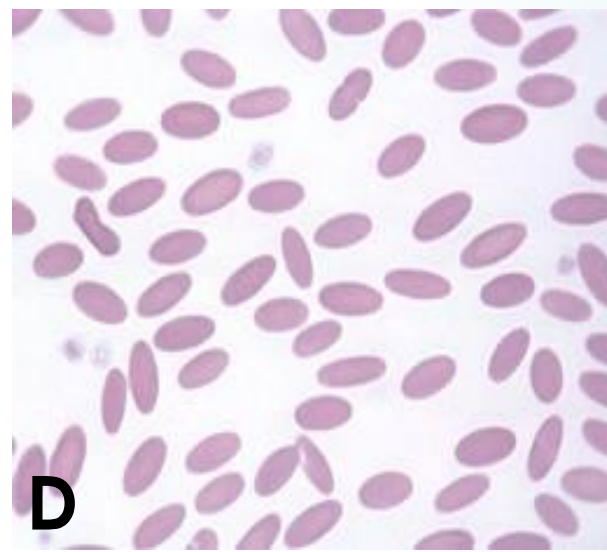
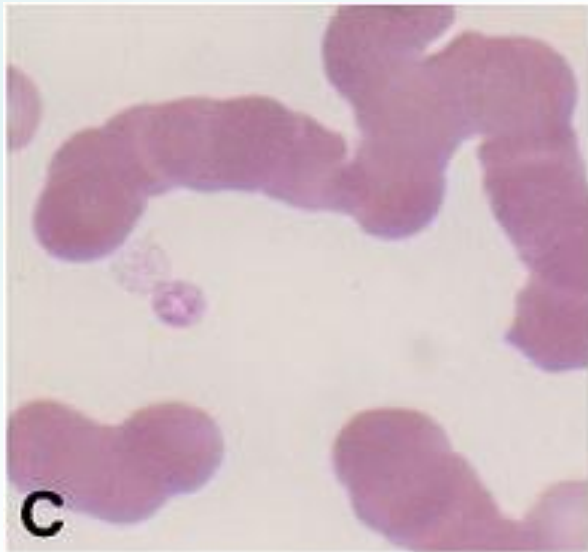
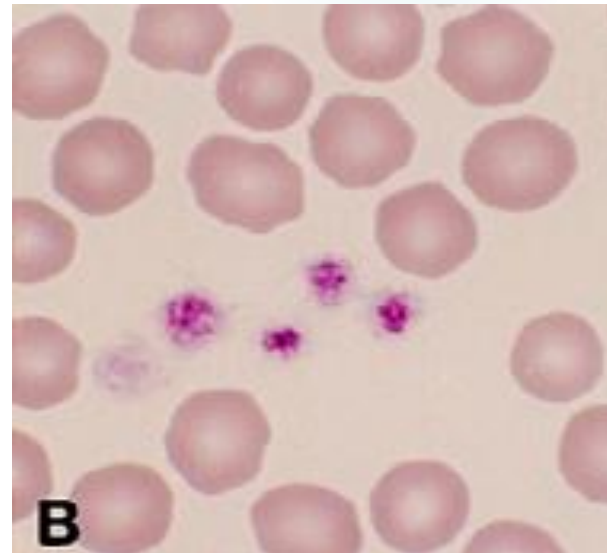
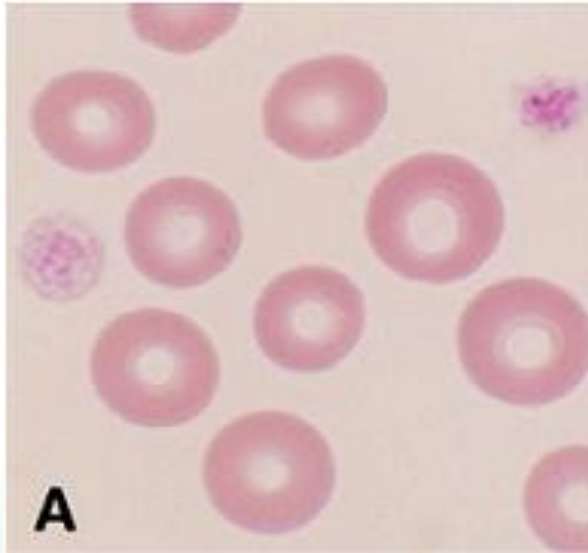
2 - Theileria

- ❖ *Th. annulata* (ring shaped – at center)
- ❖ *Th. parva* (comma shaped – at center)

3 – Anaplasma

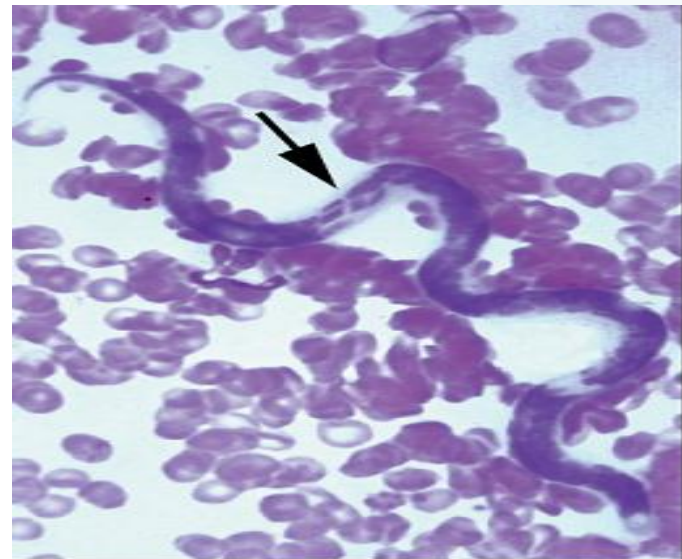
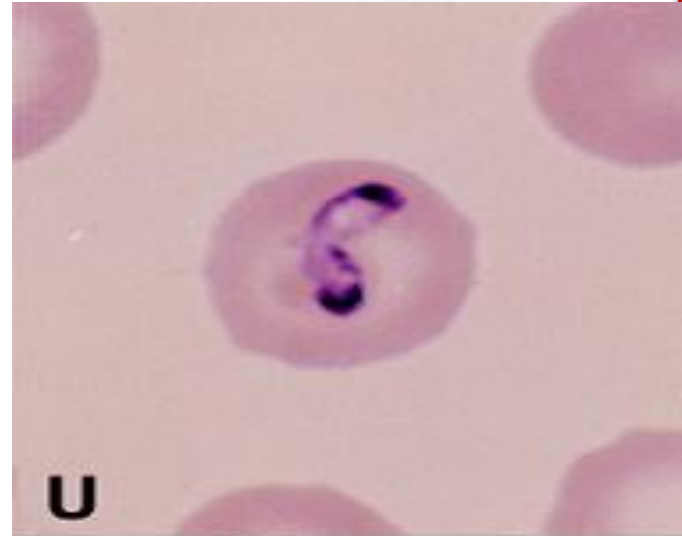
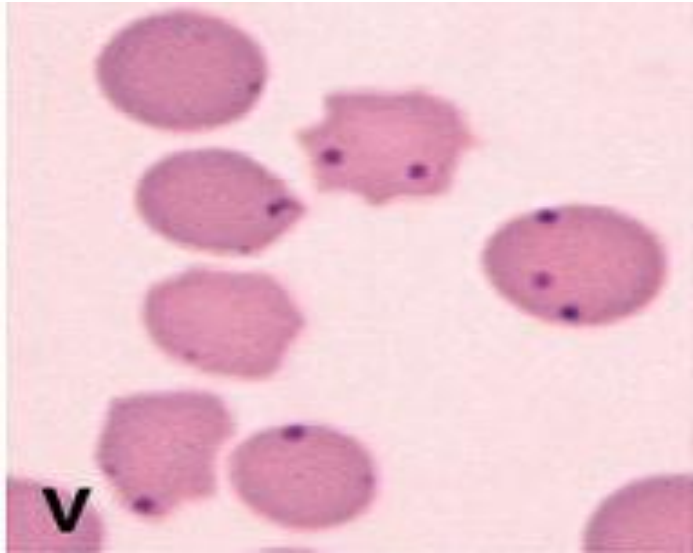
- ❖ (*A. centrale* – *A. marginale*) bluish purple dots.

RBCs morphology of different species

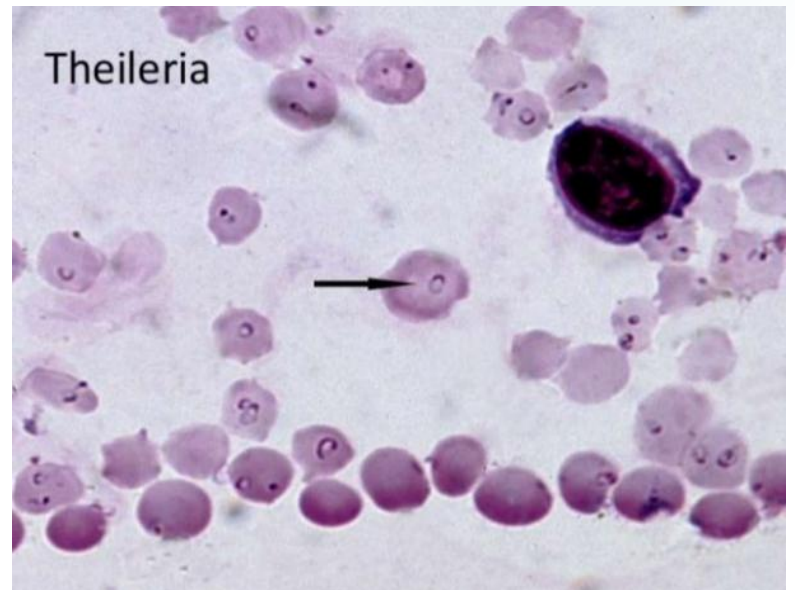
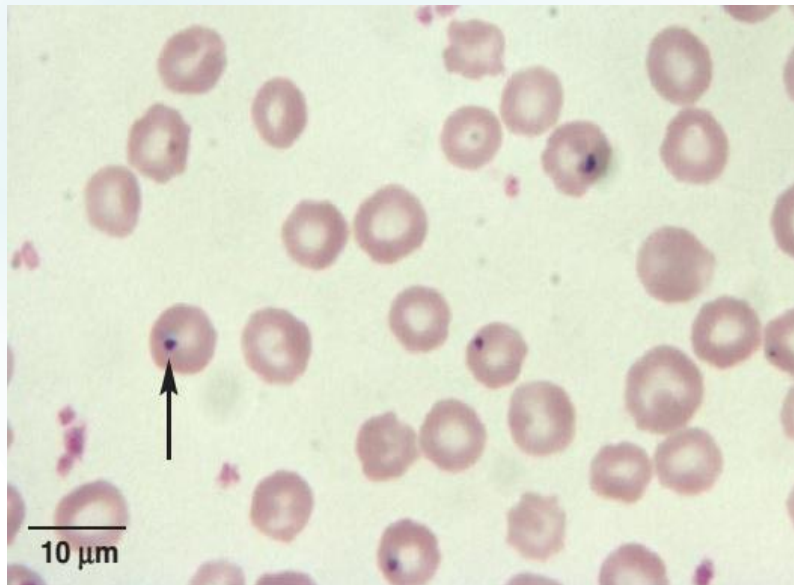
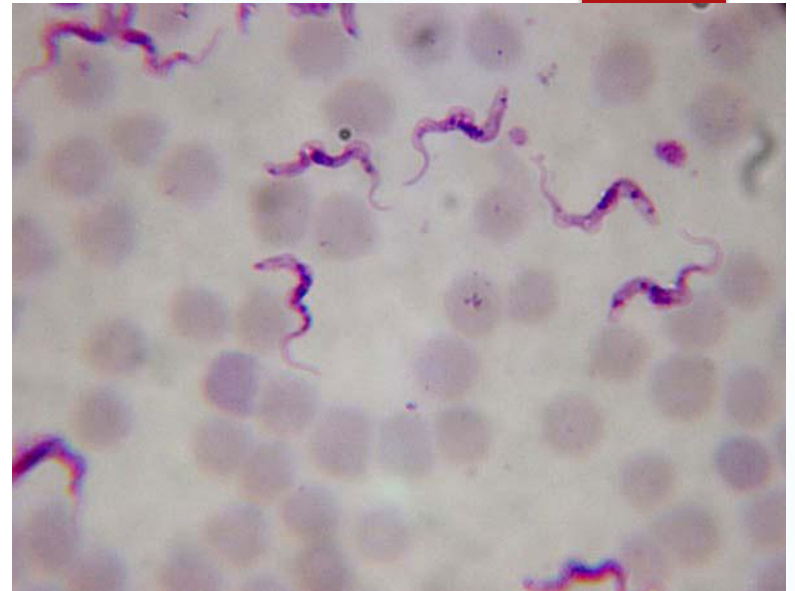
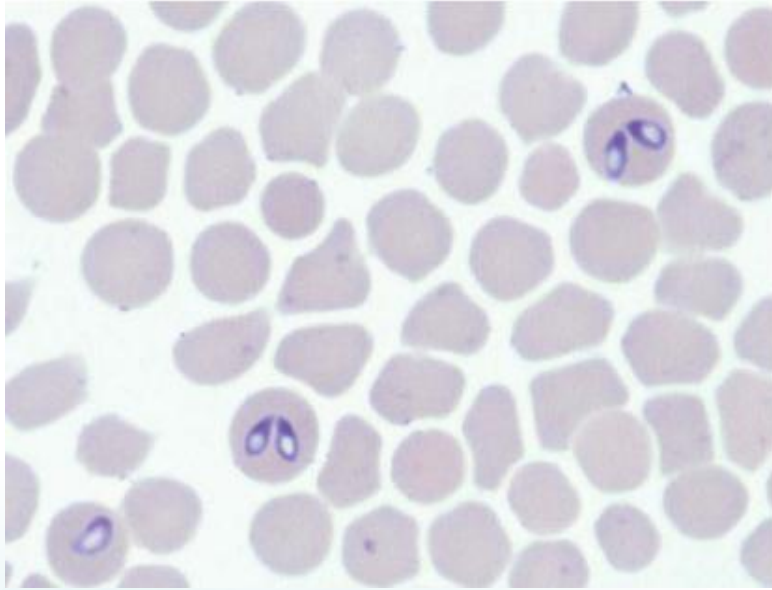


- A- Dog**
- B- Cat**
- C- Horse**
- D- Camel**

Canine blood parasites



Equine blood parasites



Camel trypanosoma

