Detection of Some Blood Parasites in Domestic Pigeons and how can Preparing Their Permanent Smears

By

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Rock pigeon

**The rock pigeon is the world's oldest domesticated bird.

**It is mention that the domestication of pigeons more than 5,000 years ago, as do Egyptian Hieroglyphics .

** Researches suggests that domestication of pigeons occurred as early as 10,000 years ago.

Importance of Pigeons

**From the Past , pigeons have made contributions of considerable an importance to humanity, especially in war times(They called as a war pigeons) which have carried many vital messages (Messengers) that services in saving human lives.

**They are bred for meat which are known as utility pigeons, kept for the enjoyment or for sporting competitions (pigeon racing).

**Domestic pigeons are also commonly used in laboratory experiments in biology and medicine.

**Despite this, city pigeons today are seen as pests, mainly due to their droppings.

**Pigeons of the order Columbiformes are ubiquitous birds and can be found in virtually every towns and cities around the globe.

**Rock pigeons are native to Europe, North Africa, the Middle East, and South Asia, and they were probably domesticated at several times and places.

***Columba livia* is a species that descends from wild rock pigeons, which live in Mediterranean Europe.

**The domestic pigeon (*Columba livia domestica*) is a pigeon subspecies that was derived from the rock dove (also called the rock pigeon).

Domestic pigeon the scientific classification as:

Kingdom : Animalia

Phylum : Chordata

Class : Aves

Order : Columbiformes

Family : Columbidae

Genus : *Columba*

Species : *C. livia*

Subspecies : *C. l. domestica*

****The Trinomial name : *Columba livia domestica***

Synonyms: *Columba domestica*; *Columba livia rustica*

**Pigeons constitute a major source of infections and transmission of diseases.

**Humans are infected by inhaling fecal dust from cages or from sites that have been contaminated with dry feces, urine and other droppings.

**This usually occurs among breeders, veterinary doctors, industrial workers, and cleaning workers.

Humans are infected by contamination with dry feces, and other droppings.

**Parasites are endemic in Africa, Central and South America, certain Caribbean islands and parts of Asia, where vectors of all types transmit them.

**Over 65 species of parasites have been isolated from birds , but only a few species are the most pathogenic.

**Several health problems can affect bird, but parasite infections play a major role and they are a significant threat to the health and conservation of bird species.

** Haemosporidian parasites are blood parasites found globally in birds.

**More than 200 species of Haemosporidia have been described among the 4000 bird species investigated worldwide .

**These blood parasites have a non-specific, broad range of hosts and are transmitted by biting vectors (Blood sucking insects) that participate in the parasites' life cycle.

**Three genera of haemosporidian parasites: *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* and the most abundant genus *Haemoproteus*.

The classified of haemoprotozoan as:

**Order: Haemosporidia

Family: Haemoproteidae

Genus: *Haemoproteus*

Family: Plasmodiidae

Genus: *Plasmodium*

**Order: Achomatorida

Family: Leucocytozoidae

Genus*: Leucocytozoon*

**Most species of *Haemoproteus* and *Leucocytozoon* are relatively host-specific and restricted to closely related species.

**By contrast, species of *Plasmodium* have a much broader hosts range and often occur in several avian families.

**Infections with these parasites are common in many species of birds around the world.

**Infections with multiple genera and species of Haematosporidia are common .

**The prevalence of infection likely depend on environmental conditions such as temperature, humidity and rainfall that will in turn impact the numbers and distribution of the parasites insect vectors.

**Infection can occur during the breeding season or throughout the year.

**It is possible that migratory birds can serve as reservoirs and carriers of parasites.

** Migration plays an important role in the prevalence of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* parasites.

**Parasitic infections may persist for many years, but there is some thought that a moderate percentage of infected birds will cure themselves of infection.

** These parasites have been considered to be host-adapted and to cause little disease in the species that they infected.

**These parasites are single-celled, two-host parasites that cycle between birds and their insect vectors.

****_Leucocytozoon_ parasites are transmitted to birds by black flies, and mosquitoes transmit _Plasmodium_ parasites (responsible for malaria) and _Haemoproteus_ parasites.**

**Surveying haemosporidian parasites are essential to determine emerging or reemerging parasite infections, geographic distribution and expansion of the blood parasites, the bird species affected by the parasites, and disease outbreaks caused by different parasite species.

Common loons attack by black flies while sitting at their nest.

Dorsal view of *Pseudolynchia canariensis*

hippoboscid blood sucking fly

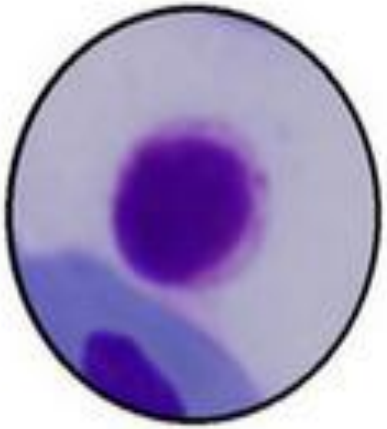
**Pigeons suffer from variety of metazoan and protozoan infections.

***Haemoproteus columbae*, transmitted by *Pseudolynchia canariensis*, a hippoboscid blood sucking fly affects domestic, as well as wild pigeons.

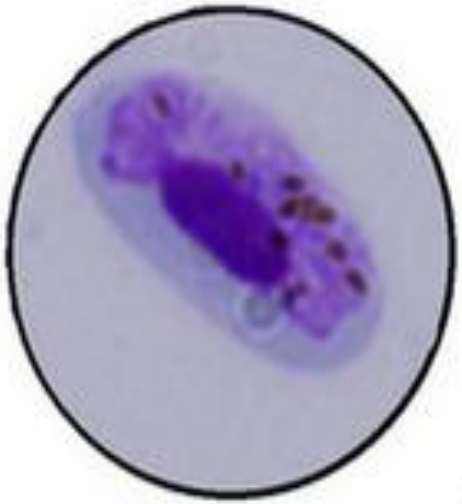
**The disease caused by *H. columbae* in pigeon is called as pigeon malaria or pseudomalaria and is lethal chiefly in young pigeons.

**The parasites widely occurs in pigeons of tropical and subtropical regions.

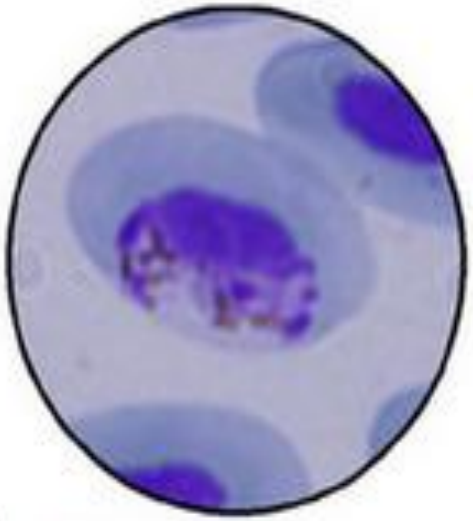
**The disease can be diagnosed by observation of halter or crescent shaped gamonts in the erythrocytes partially encircling the nucleus of the host cell.



Leucocytozoon sp.



Haemoproteus sp.



Plasmodium sp.

Life cycle

**The Haemosporidia have more complex life cycles that alternate between an arthropod and a vertebrate host. The trophozoite parasitises erythrocytes or other tissues in the vertebrate host.

**Microgametes and macrogametes are always found in the blood. The gametes are taken up by the insect vector during a blood meal. The microgametes migrate within the gut of the insect vector and fuse with the macrogametes.

**The fertilized macrogamete now becomes an ookinete, which penetrates the body of the vector.

**The ookinete then transforms into an oocyst and divides initially by meiosis and then by mitosis (haplontic life cycle) to give rise to the sporozoites .

**The sporozoites escape from the oocyst and migrate within the body of the vector to the salivary glands where they are injected into the new vertebrate host when the insect vector feeds again.

Life cycle

salivary glands of vector

↓

sporozoite Infective stage bites a new host

↓

Endothelial cells of blood vessels & lung, liver and spleen

↓

asexual reproduction schizonts

↓

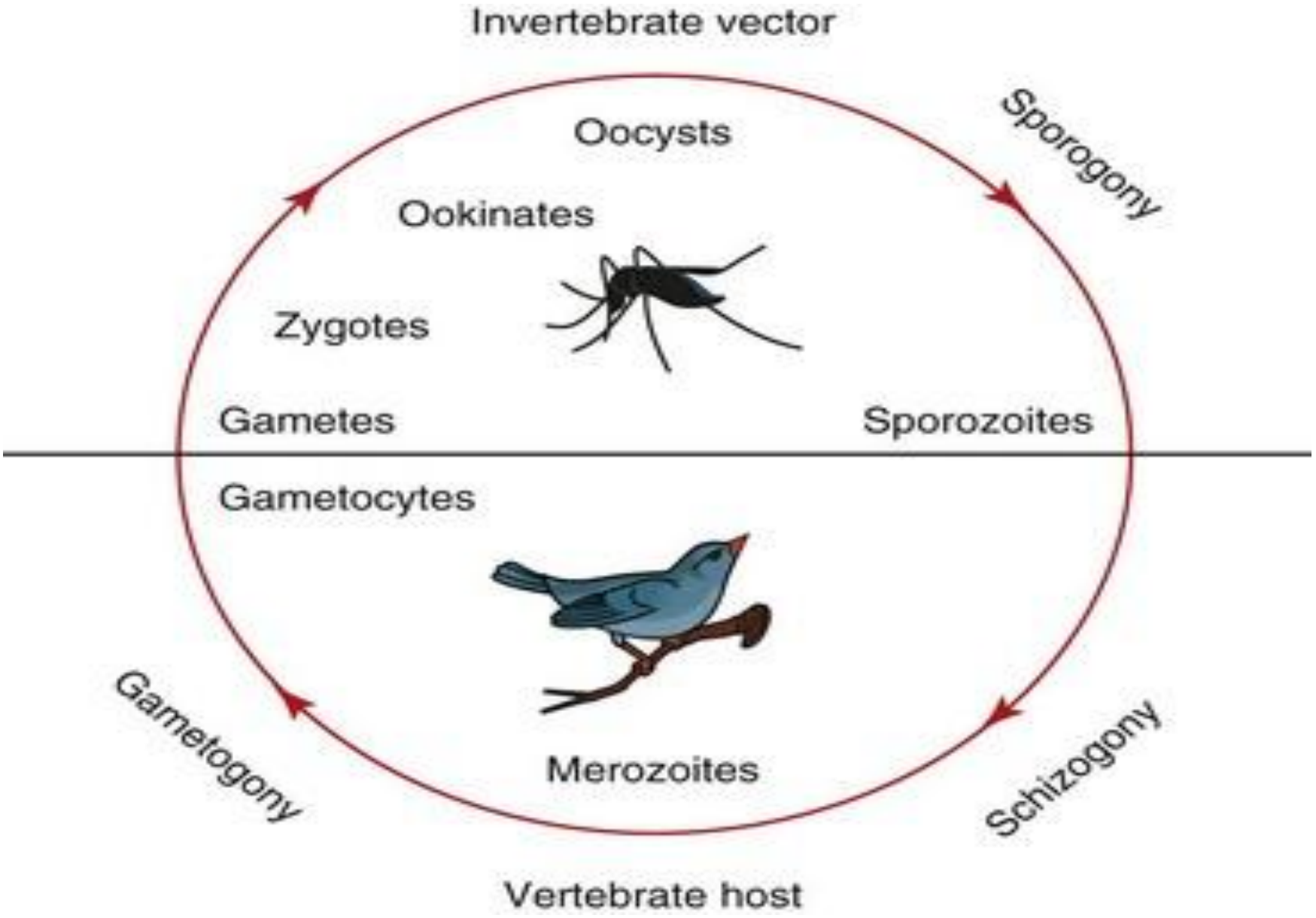
Numerous merozoites penetration erythrocytes

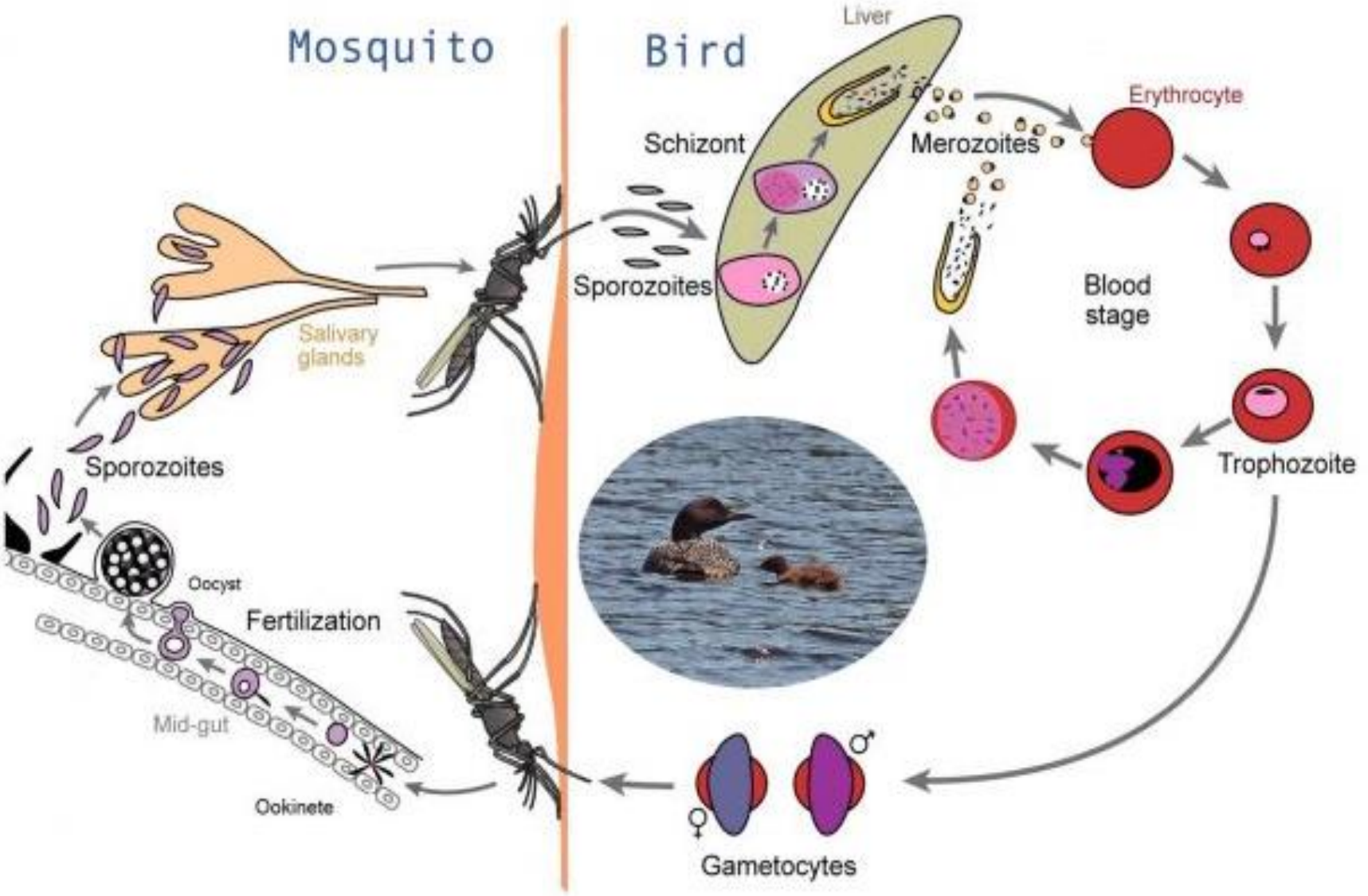
↓

Mature either macrogametocytes or microgametocytes

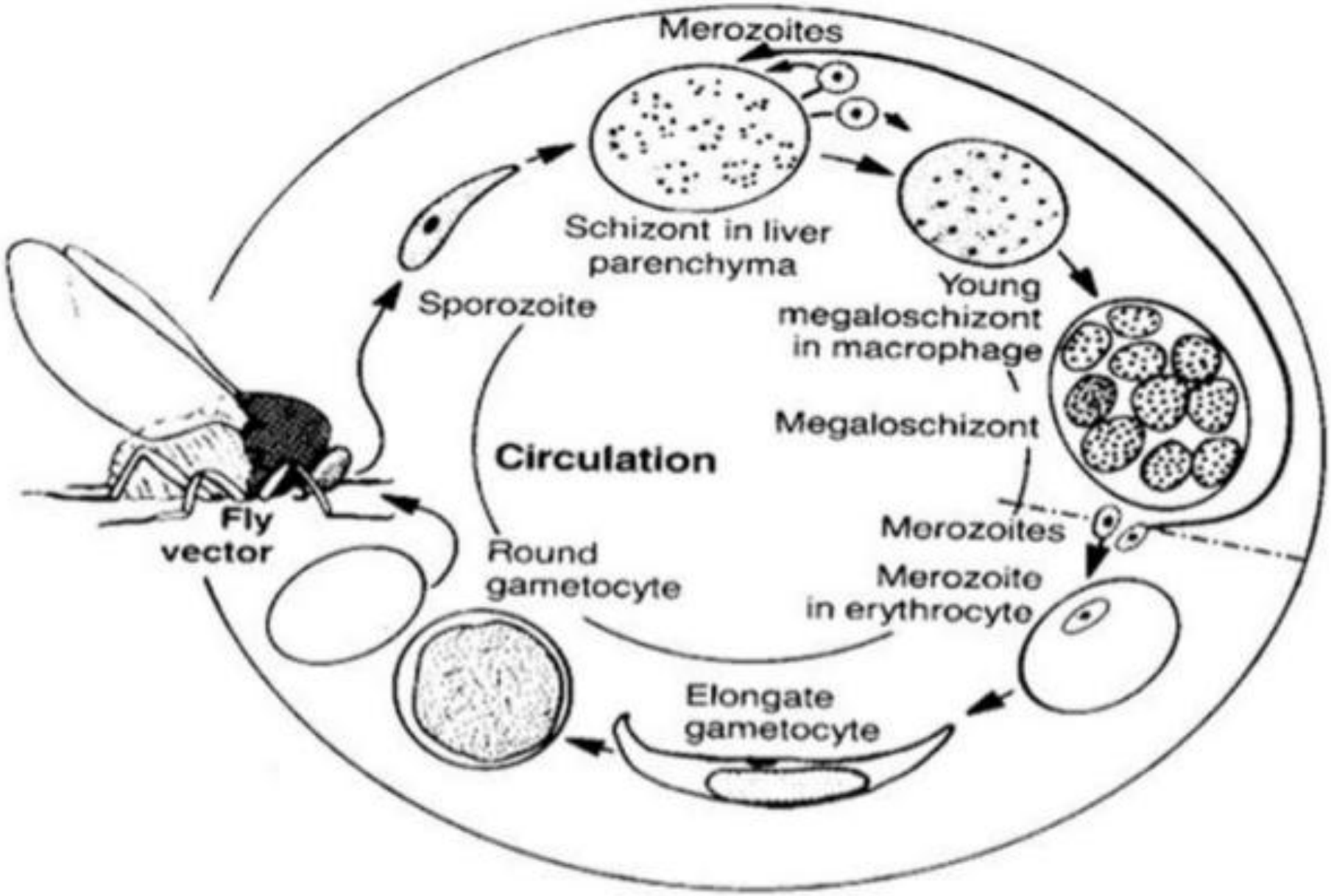
↓

Another blood-sucking insect


Invertebrate vector
Oocysts
Sporogony
Ookinates
Zygotes
Gametes
Sporozoites
Gametocytes
Gametogony
Merozoites
Schizogony
Vertebrate host

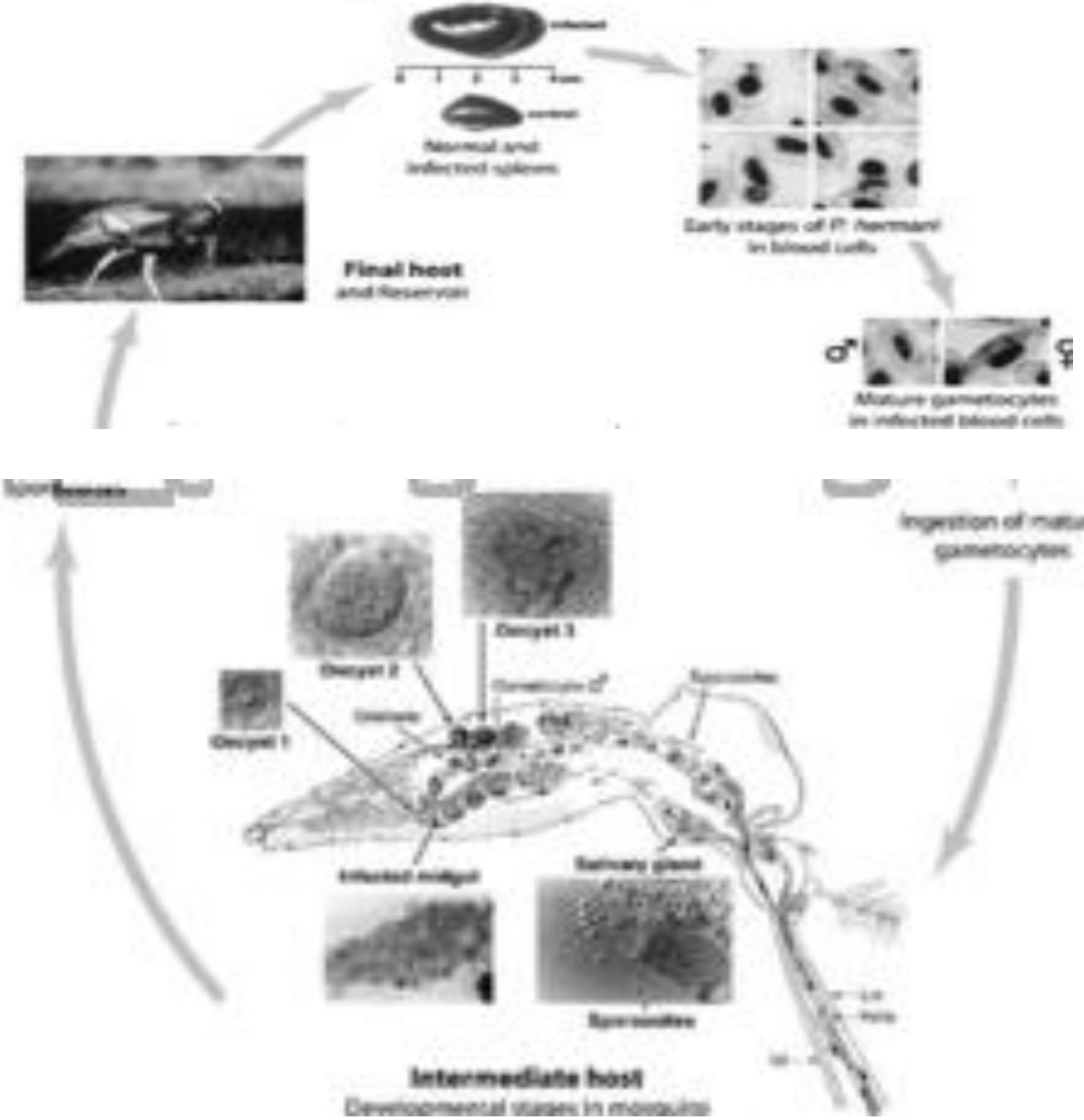


General life cycle of Haemosporidian parasites.



Leucocytozoon

Life cycle of avian (bird) malaria



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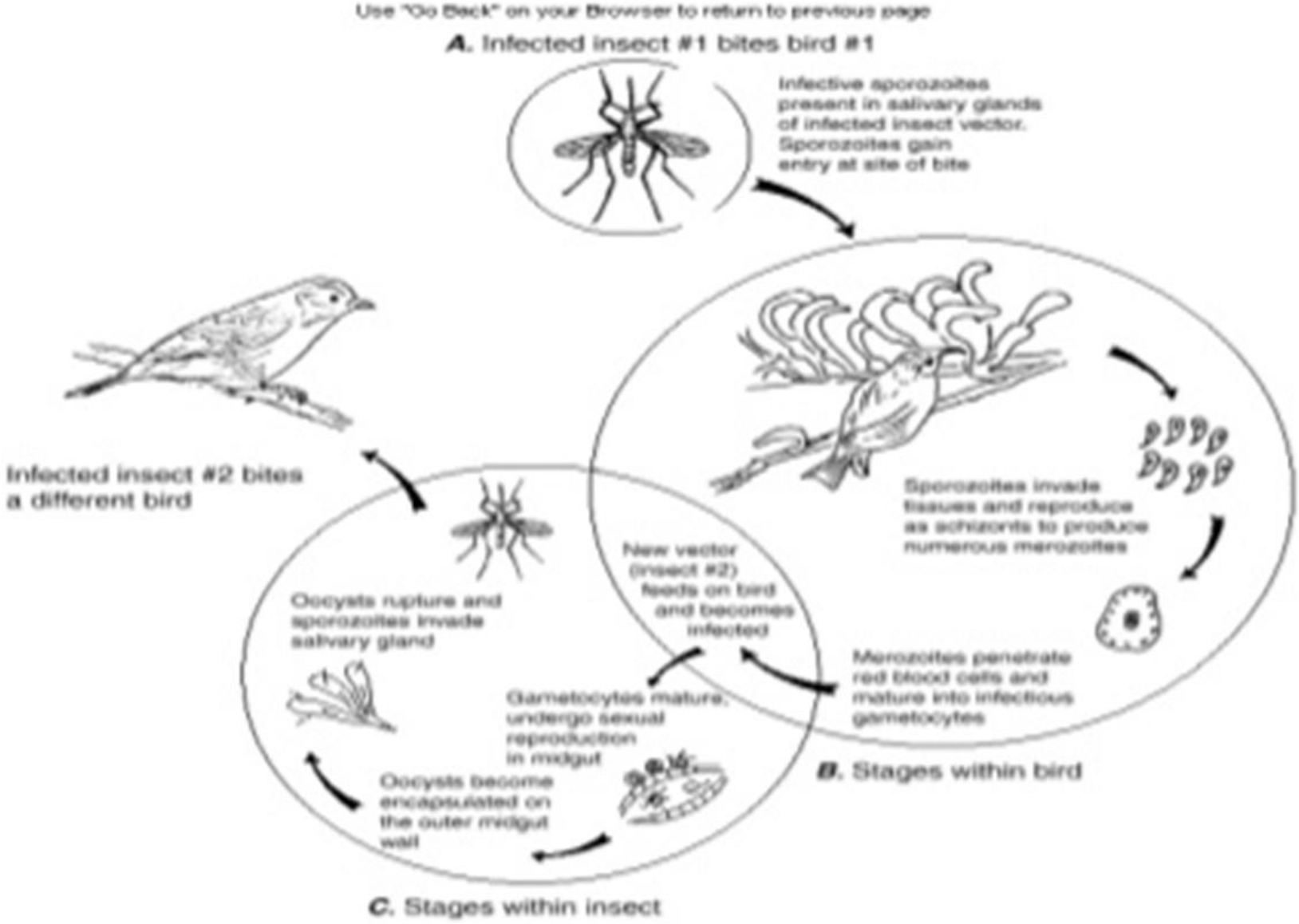


plex general life cycle of hemosporidian parasites begins with (A), an infected insect biting a susceptible bird. Separate s and developmental stages occur in (B), the bird host, and (C), the insect vectors.

Clinical signs and lesions in the infected birds

The clinical disease and lesions are associated with fever, depression, anorexia, loss of body weight , dyspnea, hepatomegaly, splenomegaly, ocular haemorrhage, haemolytic anaemia, haemoglobinuria , leukocytosis, lymphocytosis, hypoalbuminaemia, nephritis, fatty liver, edema of the lungs, hydropericardium and occlusion of capillaries of the brain.

**Infections with *Haemoproteus* spp. are generally asymptomatic owing to low pathogenicity of the parasite.

**Clinical examination of the pigeon revealed dullness, depression, dyspnea, torticollis and frequent diarrhea.

Clinical signs/symptoms

***The majority of birds affected with Leucocytozoonosis exhibit no clinical signs.**

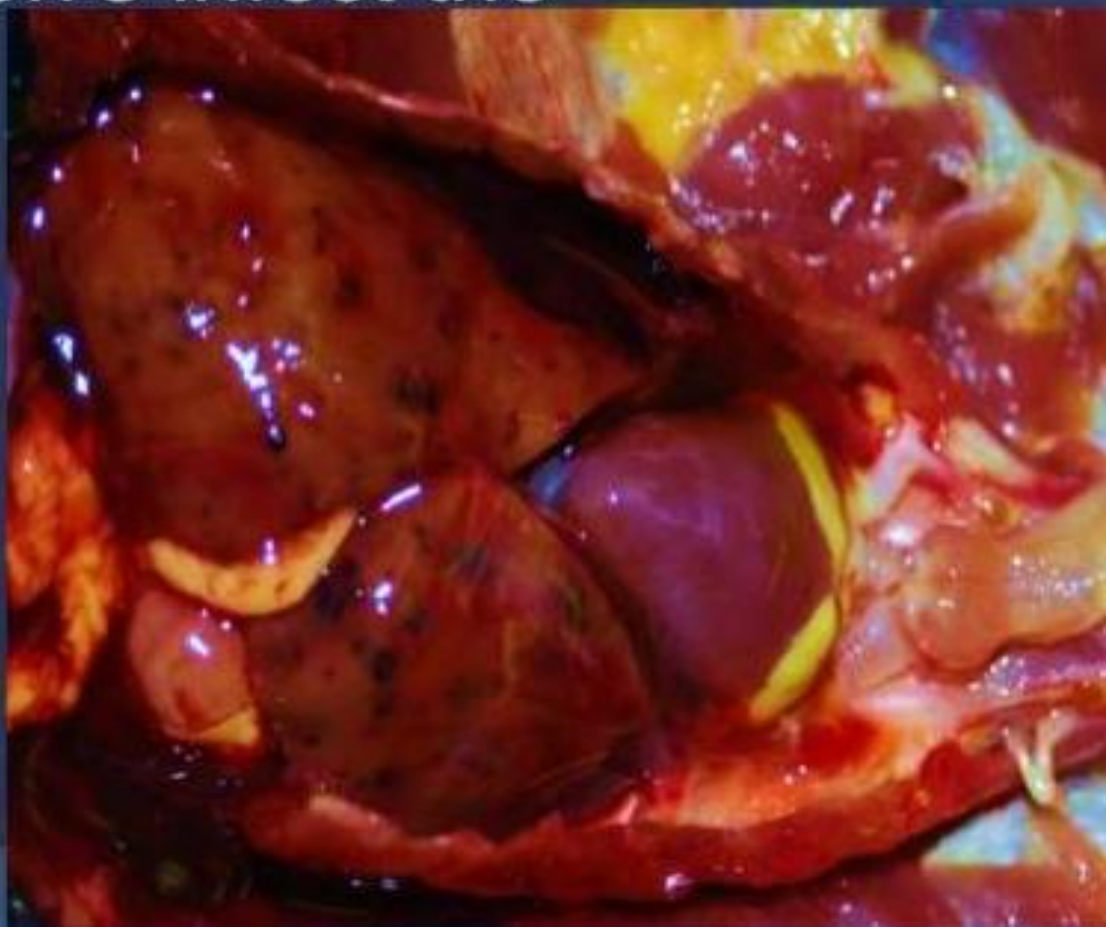
***Visibly affected shows mild to severe signs of weakness, anemia, emaciation , difficult breathing with leukocytosis in blood.**

***Young birds manifest in appetence, weakness, and some times death within 24 hrs.**

Area of Infection

Leucocytozoon's infect the

- Heart
- Liver
- Lungs
- Spleen
- Brain



**The primary target organs of the Haematosporidian parasite can be one or more of the lungs, liver, spleen or brain and spinal cord.

**Damage to the capillaries in the brain and other cells can result in neurologic signs including mentation changes, problems with balance and blindness.

**Disease in the eye may result in intraocular hemorrhage and blindness.

** Damage to the capillaries in the lungs can cause difficulty breathing.

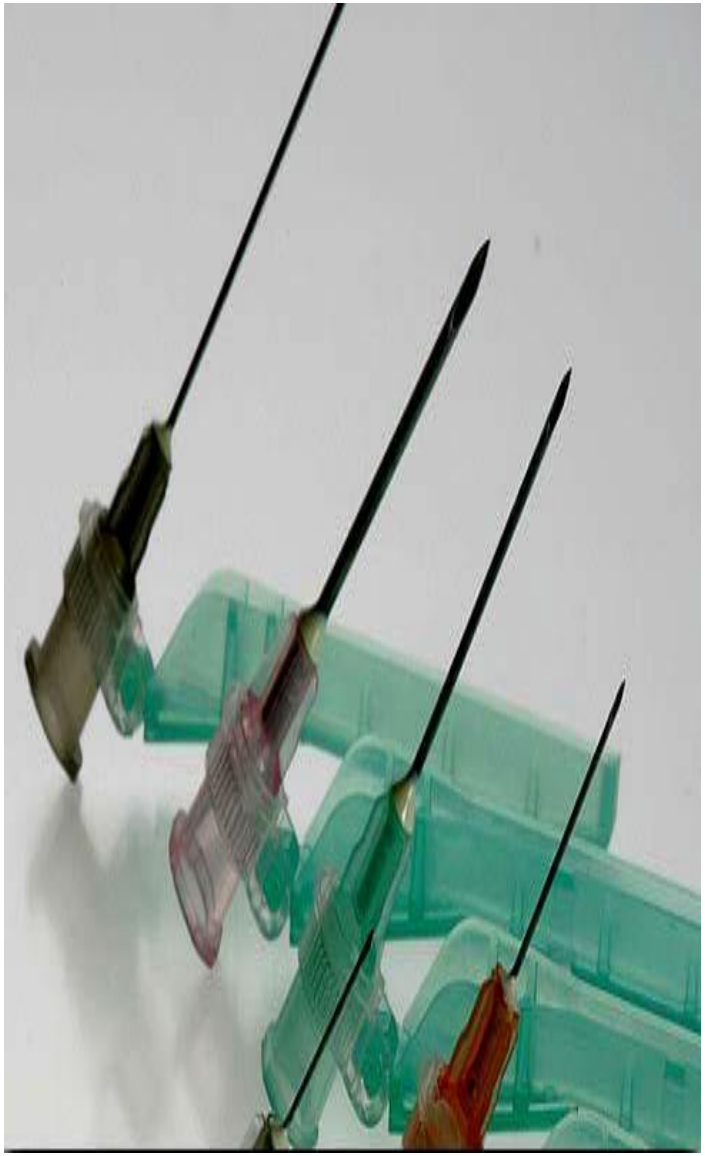
**Birds may be lethargic , anemic, and pale.

**Red blood cell destruction results in the release of biliverdin in the urine staining the urates lime green or sulfur yellow in some cases.

**Liver enlargement is common.


Thanks for Listening

Practical Applications



بعض أنواع المحاقن والإبر المستعملة في جمع الدم من الطيور

Tube type:	Glass	Glass	Glass + gel[1]	Plastic + gel[2]	Glass	Glass
Anticoagulant:	Heparin	None	Heparin	None	EDTA	Citrate
Yields:[3]	Plasma[4]	Serum	Plasma[4]	Serum	Whole blood[5]	Plasma
Uses:	Chemistry	Chemistry Electrophoresis (ELP)	Chemistry	Chemistry ELP	Hematology Cytology Flow cytometry Clonality (PARR) Coombs test	Coagulation

VACUTAINER TUBES

Color	Anticoagulant	Uses
	No anticoagulant	Serological examination in biochemistry
	**Sodium Fluoride**	Glucose estimation
	**EDTA**	Hematological examination like complete hemogram, ESR
	**3.2% sodium citrate**	Coagulation studies like PT, APTT
	**Heparin**	Bone marrow studies
	**Citrate**	Blood culture
	**(K2)EDTA**	Blood Bank tests. Blood typing. ABO grouping etc

Blood Samples

**Two extremely important components of blood testing in birds and exotics include the complete blood count (CBC) and biochemistry analysis.

**Anatomic differences necessitate the modification of venipuncture techniques between bird species and selection of appropriate blood collection sites. Moreover, a venipuncture site is chosen after consideration of a number of important factors such as:

**The volume of blood required dictates whether a relatively small or large blood vessel is sampled.

**Health status, from healthy to critical, influences how is restrained and thereby affects the vein that is selected.

**History of recent venipuncture in a particular vessel may preclude the clinician from sampling the same vessel again.

**Risk of hematoma formation varies between blood collection sites and should be considered.

**Finally, the clinician's experience also plays a role in the site selected.

**Several common and uncommon blood collection sites will be describe .

**A general rule of thumb for phlebotomy in avian and exotic bird is that 1 ml of blood can be safely removed per 100 grams of body weight.

**This equates to removal of 1% of bird's body weight, or 10% of its total blood volume.

*For example, a parakeet weighing 30 grams can have 0.3 ml of blood taken; a parrot weighing 300 grams can be taken 3 ml of blood .

**In most birds, jugular venipuncture is the most accessible and reliable technique for obtaining adequate blood samples.

**This approach has the following two advantages:

1) It allows one to tent up the blood vessel, allowing expansion of the jugular vein and engorgement of the vessel.

2) There is less risk of puncturing the medial wall of the jugular, as the distance the needle must be inserted is comparatively less when the bevel is oriented down.

**Alternate venipuncture sites include :

**The ulnar vein (hematoma formation is likely, so prolonged pressure is needed in this location) and the medial metatarsal vein (Prominent in pigeons and waterfowl).

**Toenail clips are generally discouraged as sample contamination can lead to altered biochemistry or DNA probe results and micro-clotting or hemolysis may occur.

Table 1. Needle and syringe guide

Body Weight (grams)	Needle gauge	Syringe size (ml)
<60 e.g. canary	28–30	Insulin 0.3–0.5
60–200 e.g. cockatiel	25–27	1.0
> 200 e.g. cockatoo	25 or larger	3.0 or larger

Blood Handling

**Following collection of the sample, the blood must be properly stored or preserved to prevent changes and enable diagnostic accuracy.

**Multiple sizes of blood containers are available; for small blood samples under 1 ml, 0.5 ml-capacity containers may be used.

**The proper anti-coagulant to be used; it is usually dependent on the diagnostic tests that will be performed.

Blood Smear Preparation

**A stained blood smear is an essential part of the hematologic examination.

**Blood films are made using blood with or without anticoagulant and using a variety of techniques.

**Standard push-slide methods are often used; alternatively, blood films can be made by using a slide and coverslip.

**Blood-Cell Stains include :

Wright, Wright-Giemsa, and Wright-Leishman, staining methods.

**Quick stains or modified Wright stains are the most commonly used stains in veterinary practice settings.

**The blood samples were collected using an insulin syringe inserted through a brachial vein catheter.

**Each sample provided two blood smears, fixed with methanol and stained with Giemsa dyes .

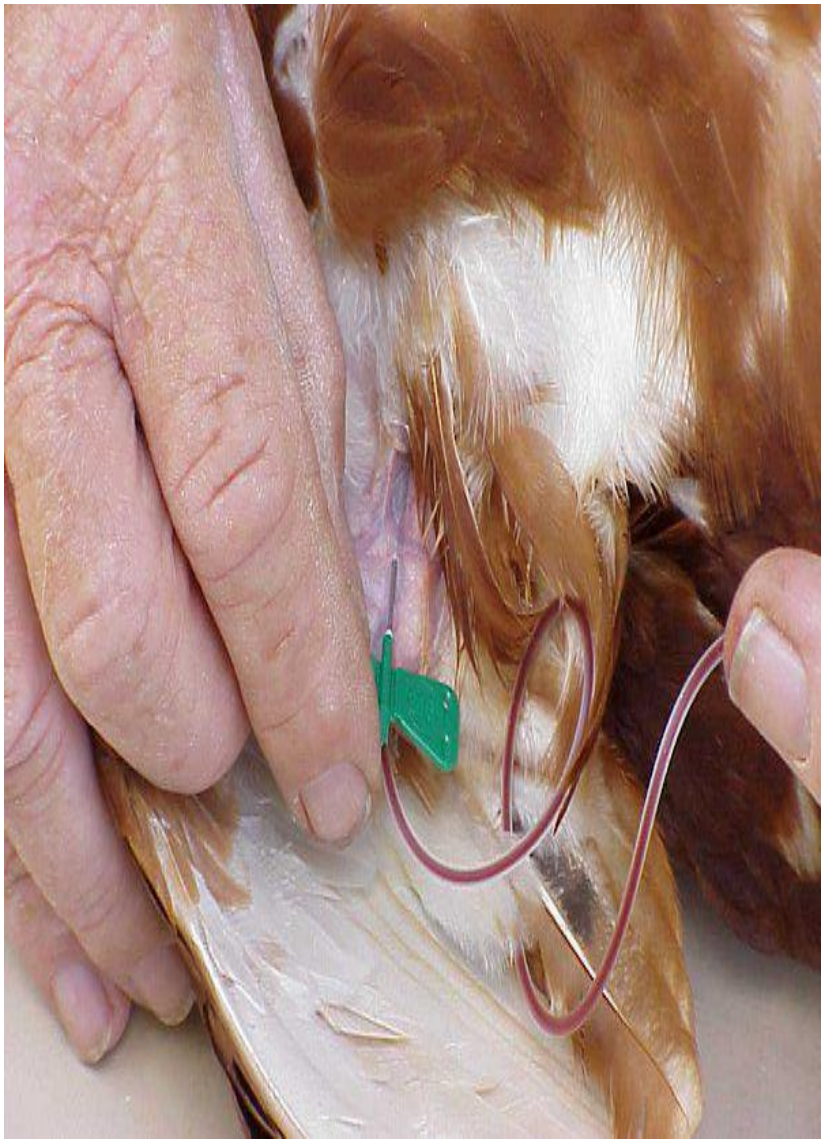
**The slides were analyzed under light microscopy using an oil immersion objective.

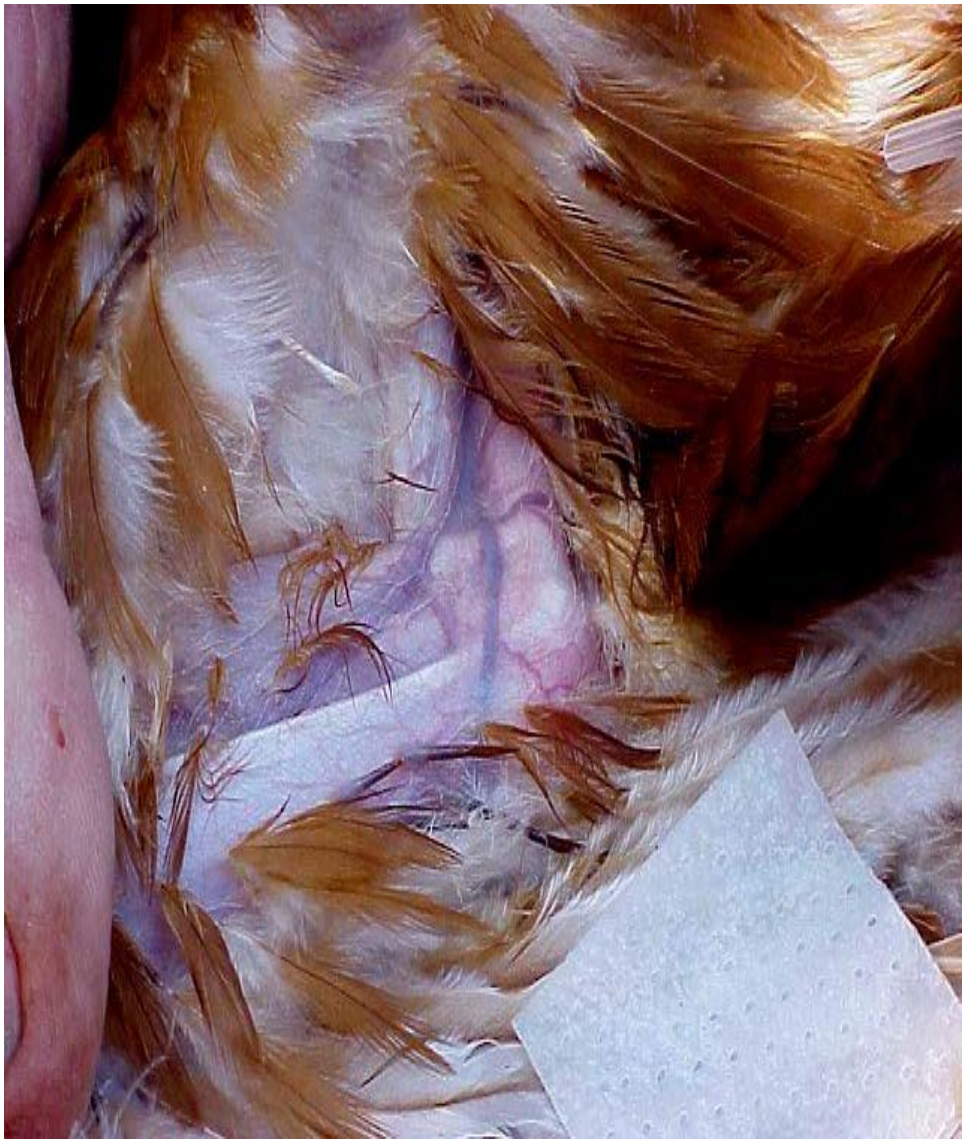
Jugular venipuncture

Blood Collection from Jugular Vein

Venipuncture of ulna vein

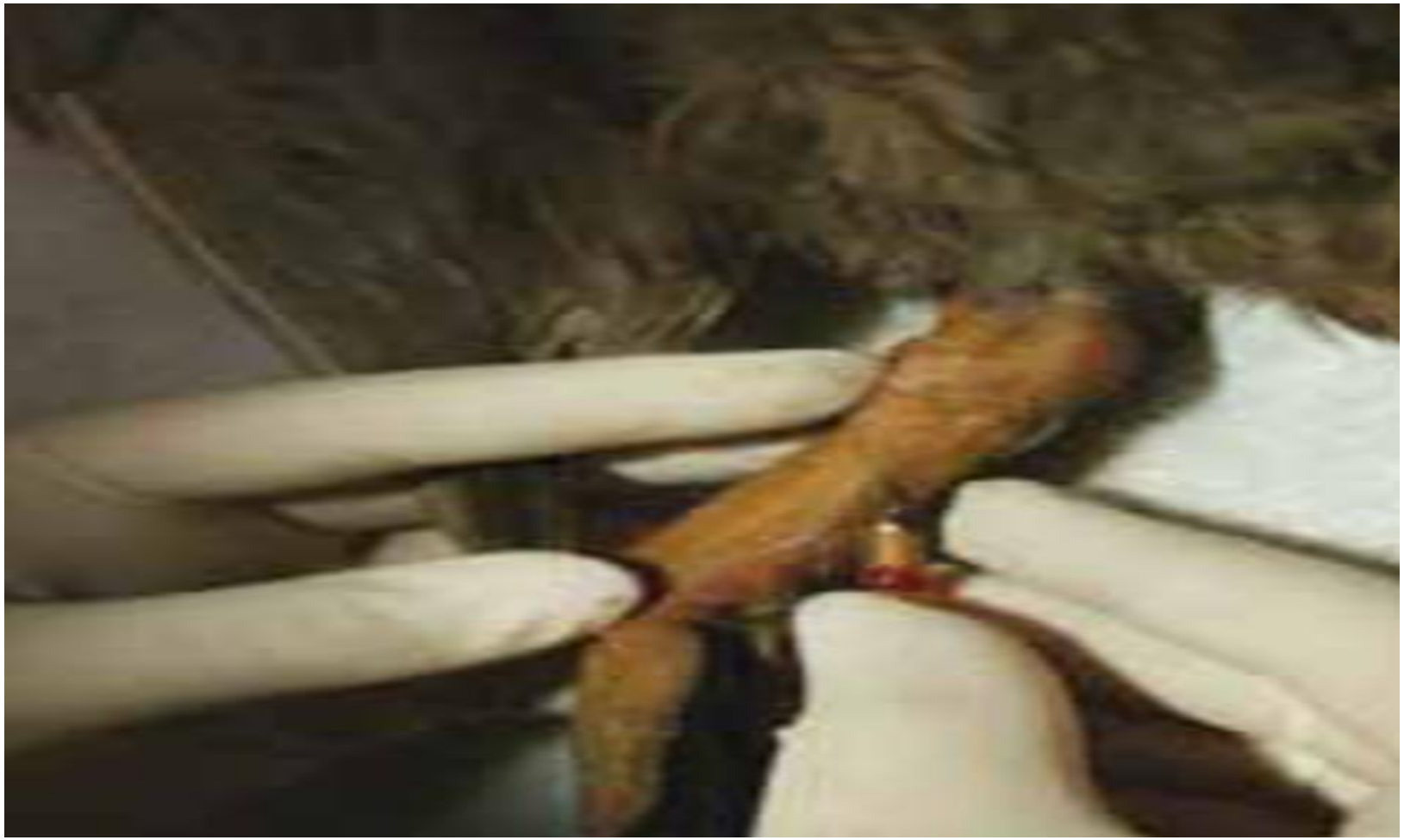
Ulna vein





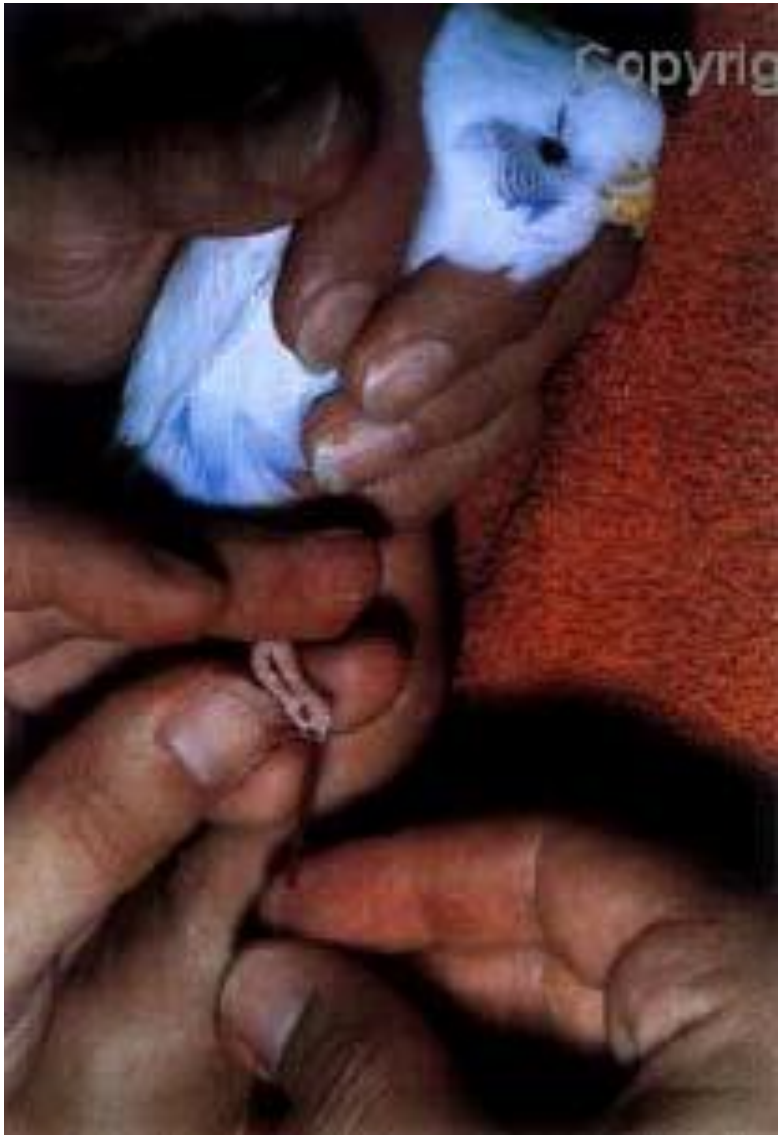
Blood Sample Collection from Ulna Vein

Blood Sample Collection from Ulna Vein



Venipuncture of medial metatarsal vein

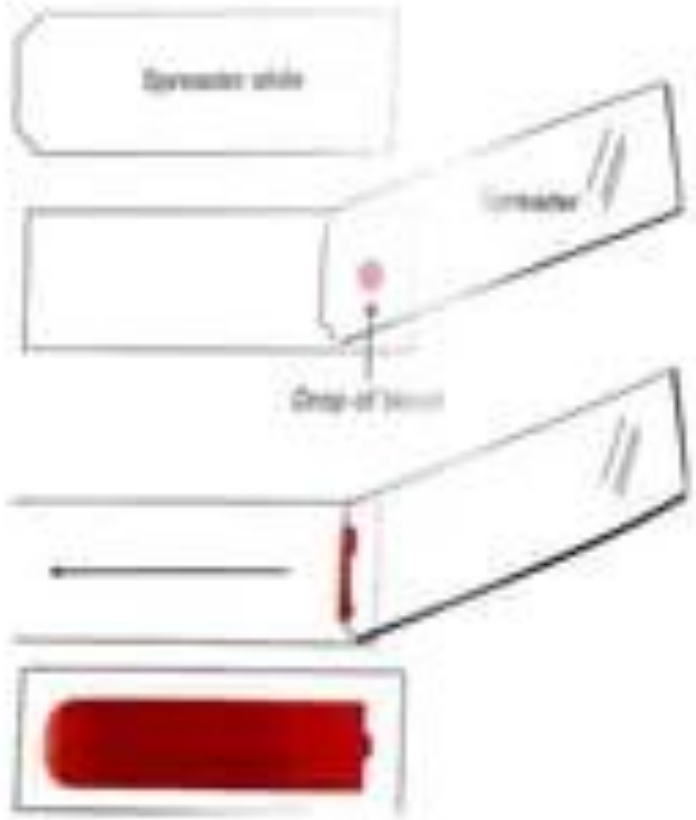
Blood collection from metatarsus vein



Blood collection by Toe clip

Preparation of thin smears

SMEAR PREPARATION



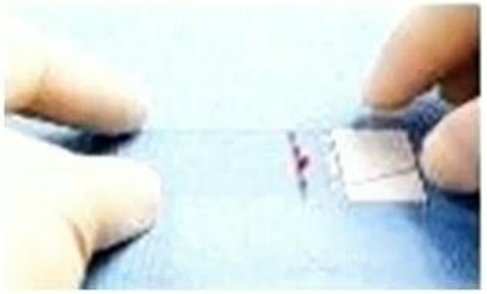
1. Place a drop of blood, about 2-3 mm in diameter approximately 1 cm from one end of slide.
2. Place the slide on a flat surface, and hold the other end between your left thumb and forefinger.
3. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.
4. Hold the spreader slide at a 30°- 45 angle, and draw it back against the drop of blood
6. Allow the blood to spread almost to the edges of the slide.
7. Push the spread forward with one light, smooth moderate speed. A thin film of blood in the shape of tongue.
8. Label one edge with patient name, lab id and date.

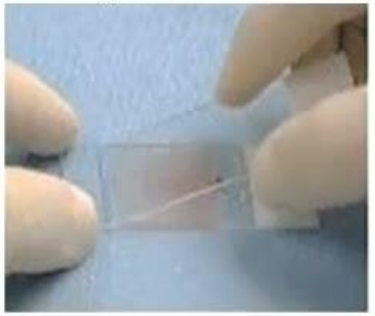
9. The slides should be rapidly air dried by waving the slides or using an electrical fan.

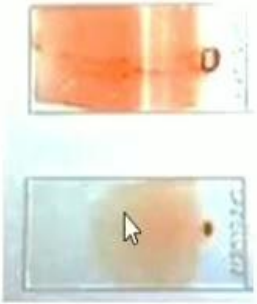
Preparation of thin blood film: Slide Method

Procedure :

1. Place a drop of blood in the centre of a clean glass slide 1 -2 cm from one end.
2. Place another slide (spreader) with smooth edge at an angle of 30 – 45 near the drop of blood.
3. Move the spreader backward, so that it touches the blood drop. Blood will spread along the edge of spreader by capillary action.
4. Push the spreader forward along the length of slide rapidly.
5. A thin peripheral blood film is thus prepared.
6. Then allow the smear to dry in the air before staining.
7. When dry, Id/Name/Number may be written on the slide with a marker/pencil.



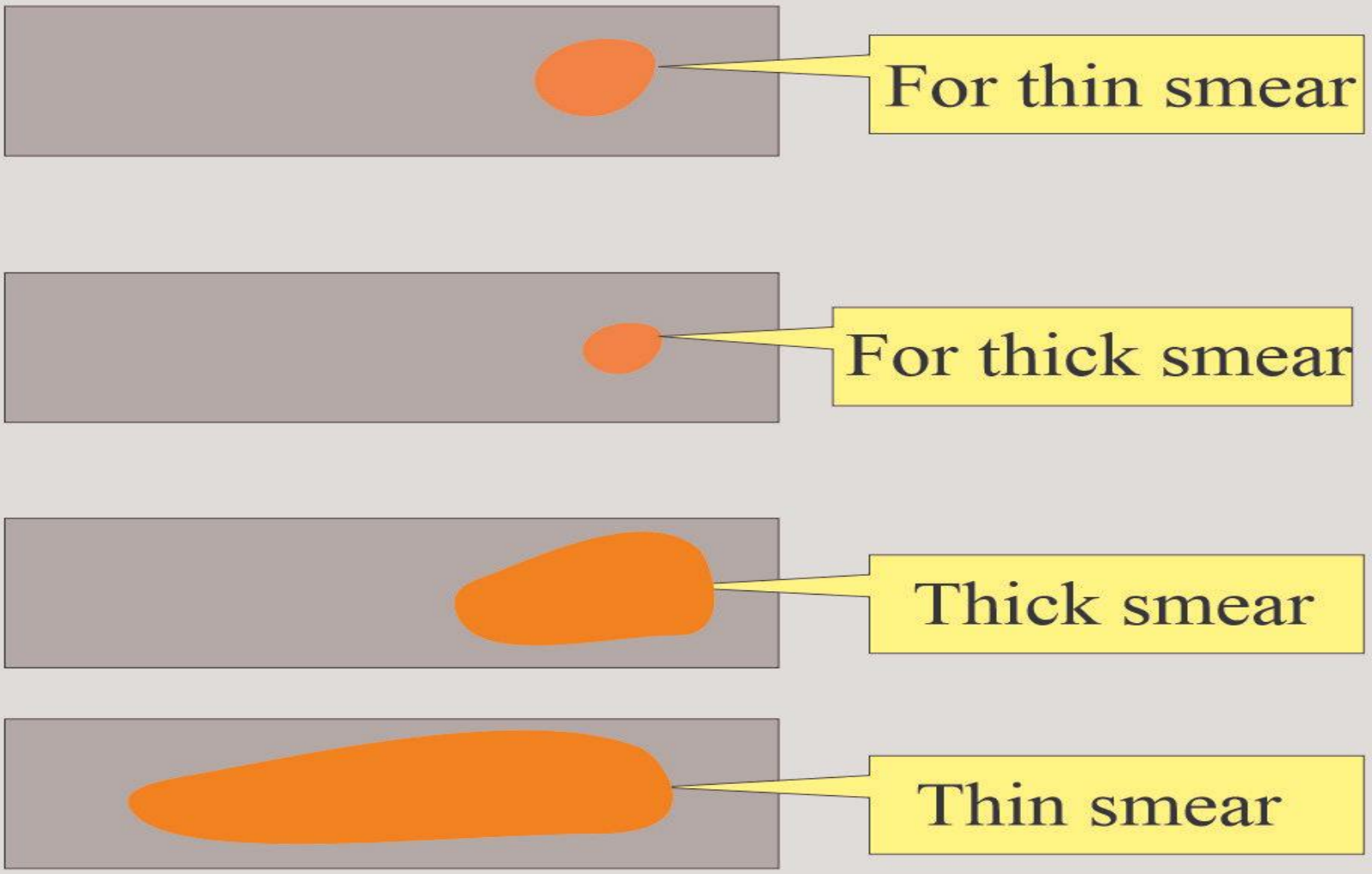


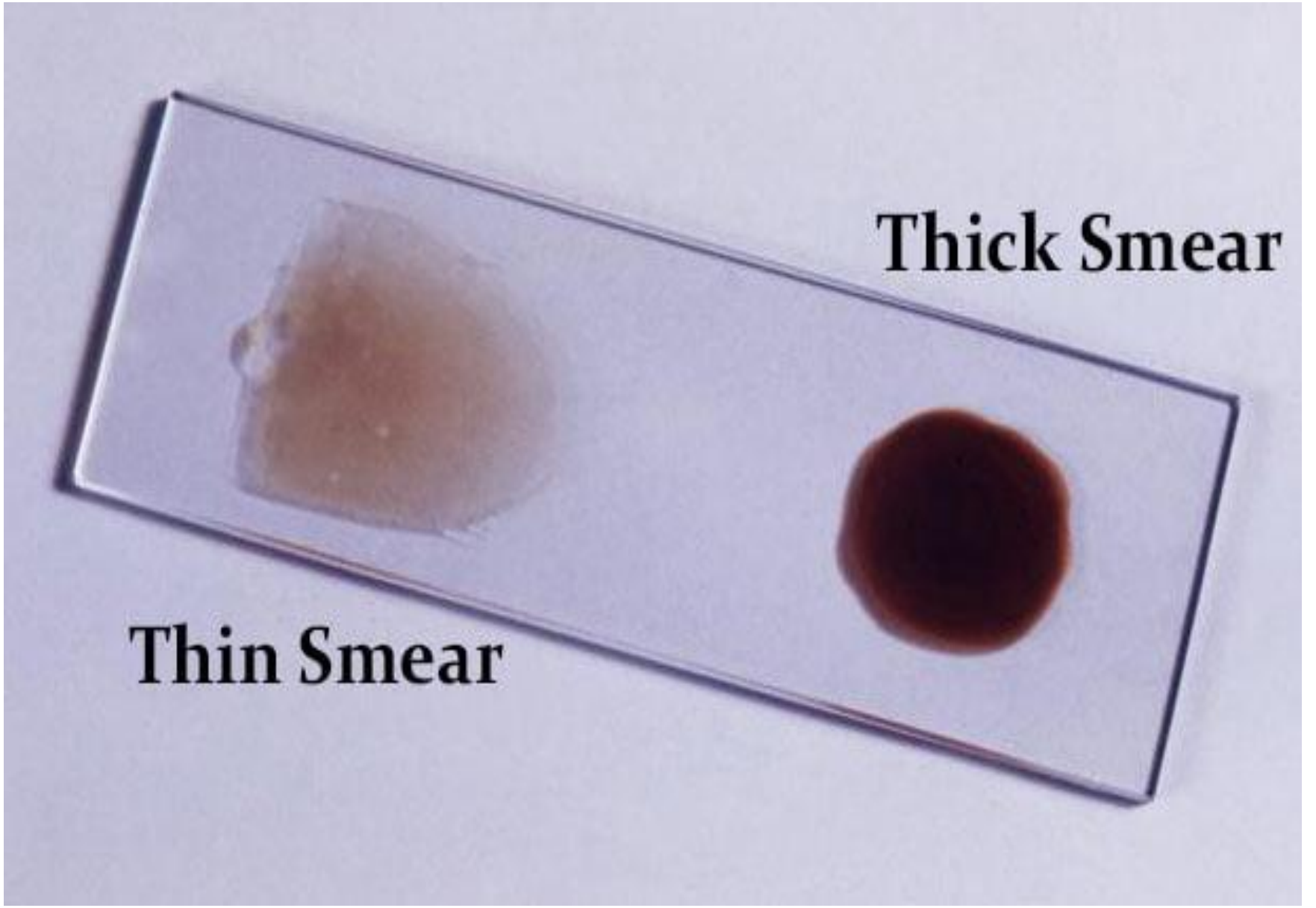


a. Bad Blood Film

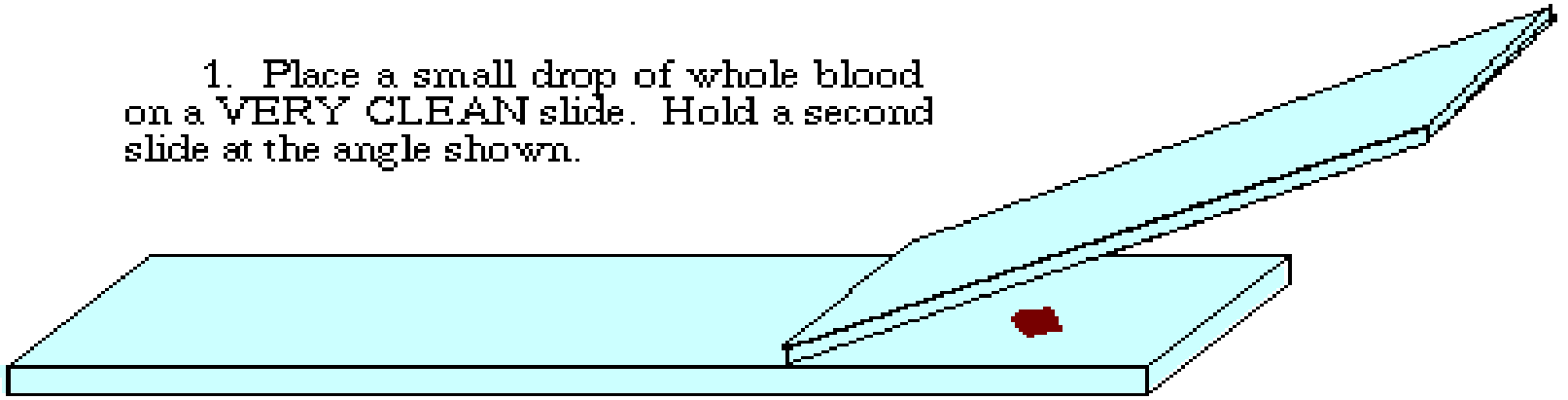
b. Good Blood Film

How to make thin and thick smear

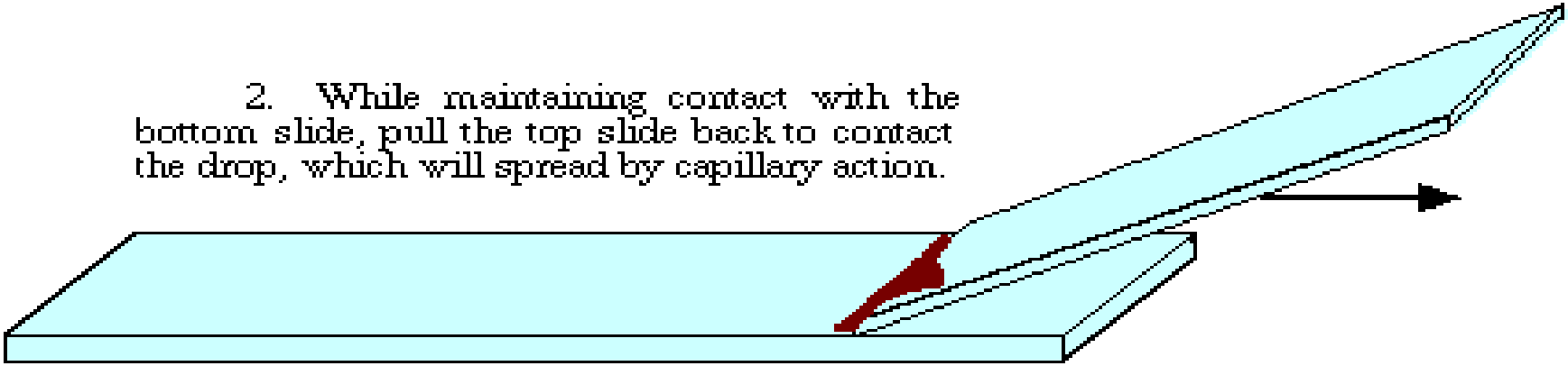



Thick Smear
Thin Smear

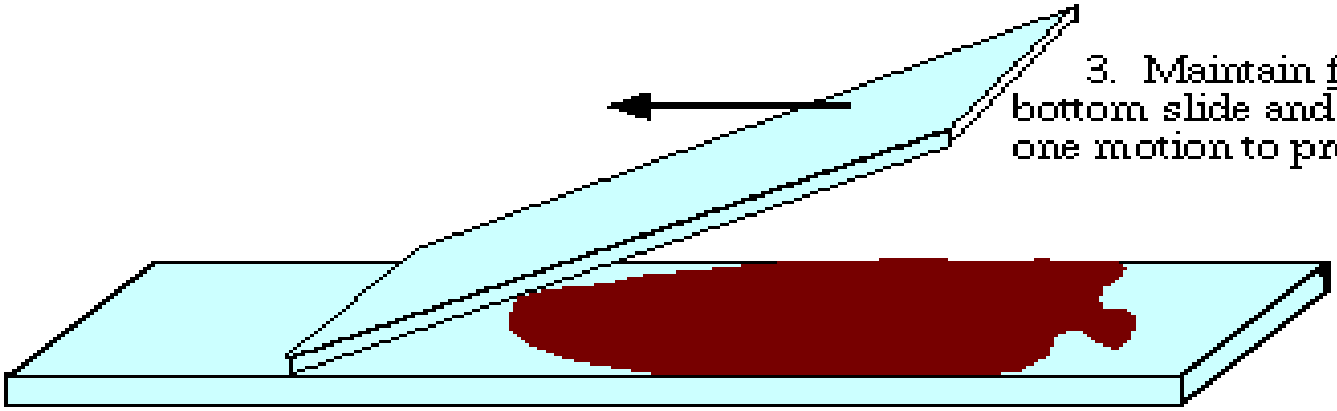
1. Place a small drop of whole blood on a VERY CLEAN slide. Hold a second slide at the angle shown.



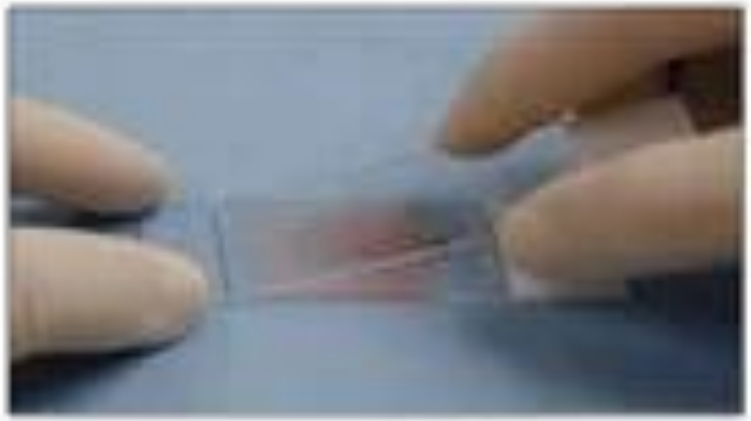
2. While maintaining contact with the bottom slide, pull the top slide back to contact the drop, which will spread by capillary action.



3. Maintain <u>firm</u> contact with the bottom slide and push the top slide in one motion to produce the smear.



8. Push the spreader forward with one light, smooth, and fluid motion



9. A thin film of blood in the shape of tongue or a bullet with a feathered edge will remain on the slide.

10. Allow the blood film to air-dry completely before staining. (Do not blow to dry. The moisture from breath will cause RBC artifact.)

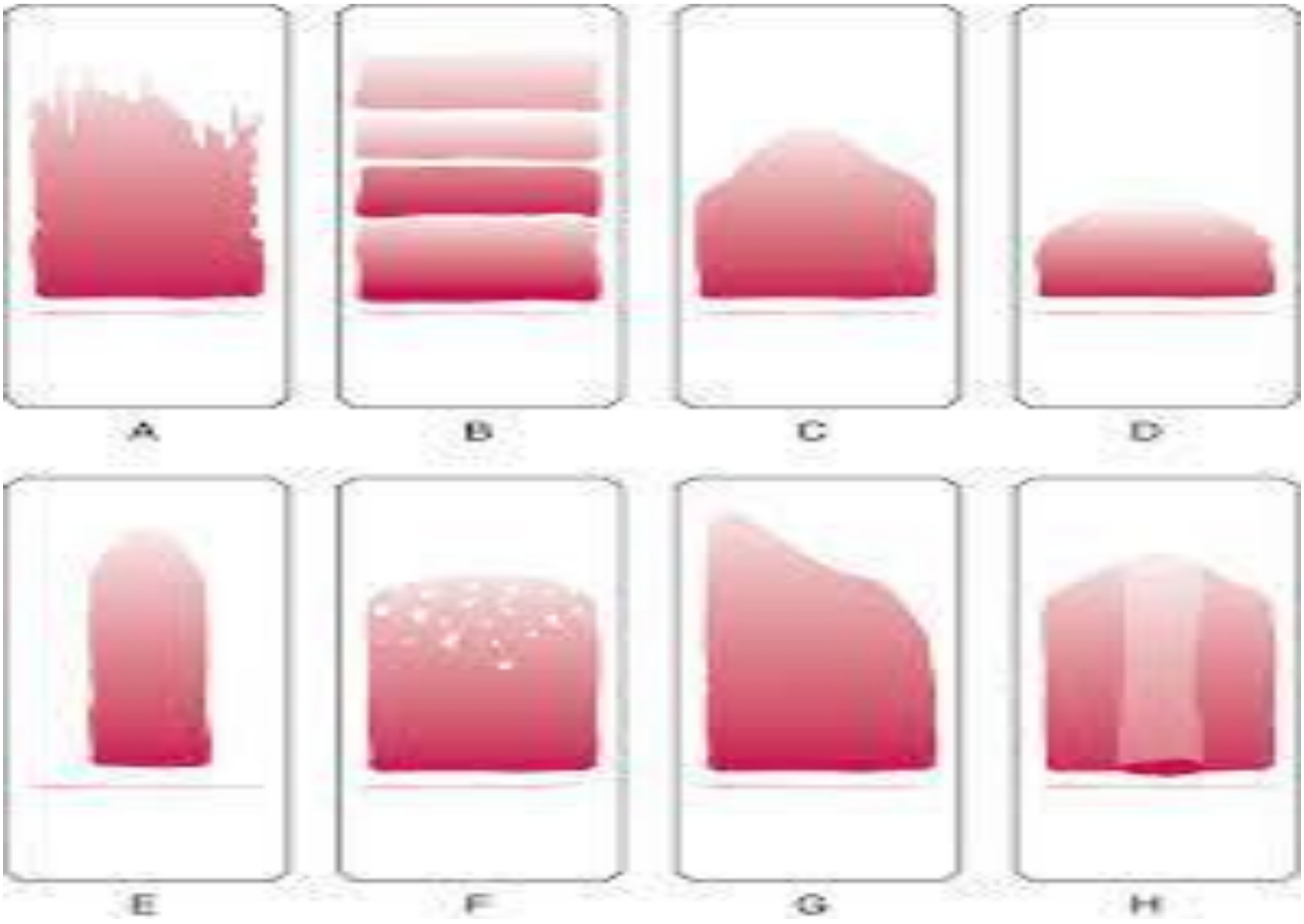
Thin Film

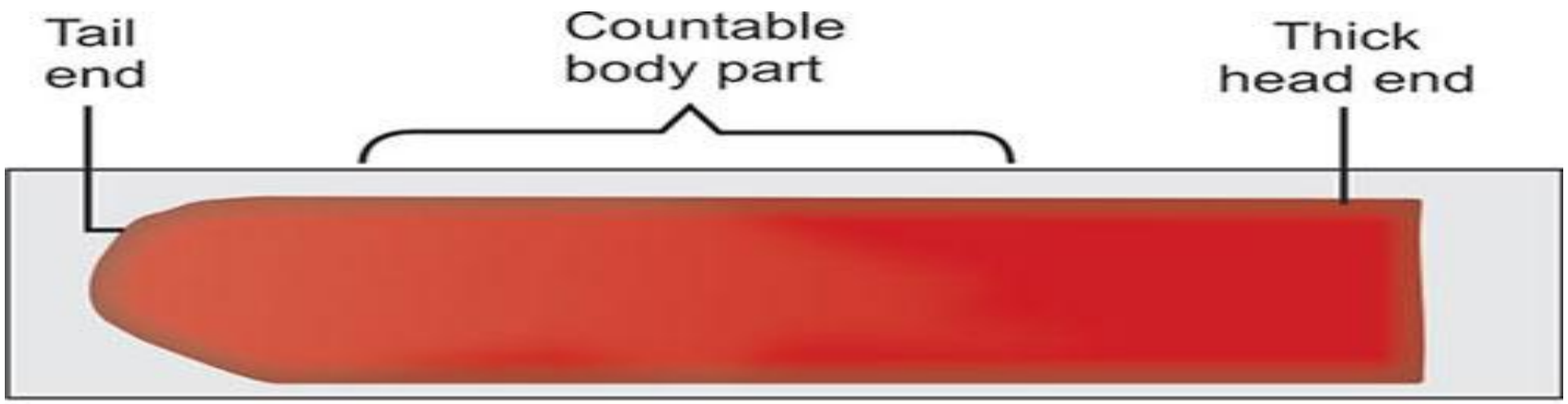
- Good preparation - feathered end of the film should be centrally located on the slide with free margins on both sides,

  when properly prepared, it will be only one cell layer thick at this end.

- Badly prepared smears can cause presence of streaks - as a result of chipped spreader

- holes in the film indicate faulty preparation and dirty or greasy slides, respectively.

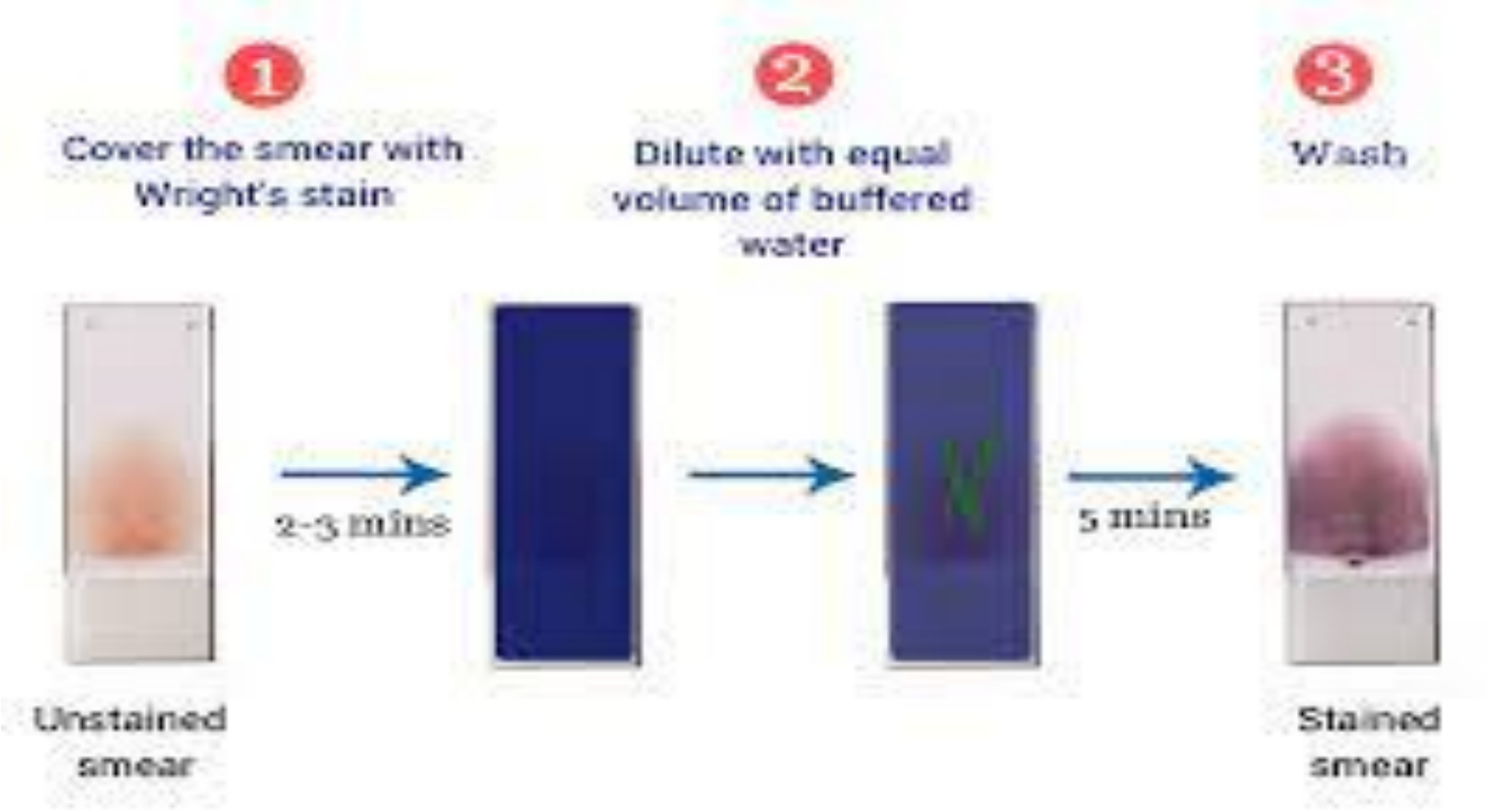

A
B
C
D
E
F
G
H

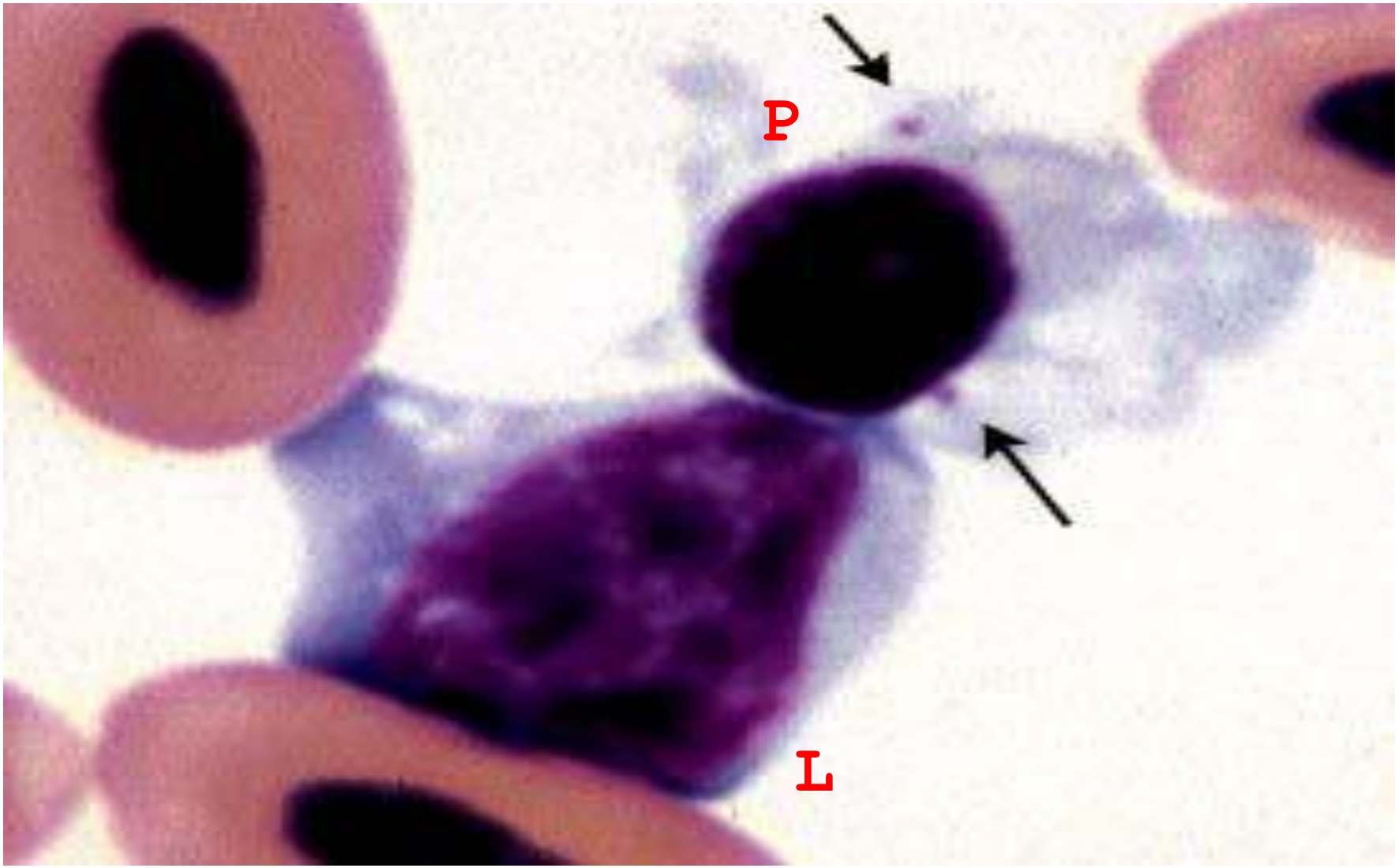


Ideal toungue shaped film

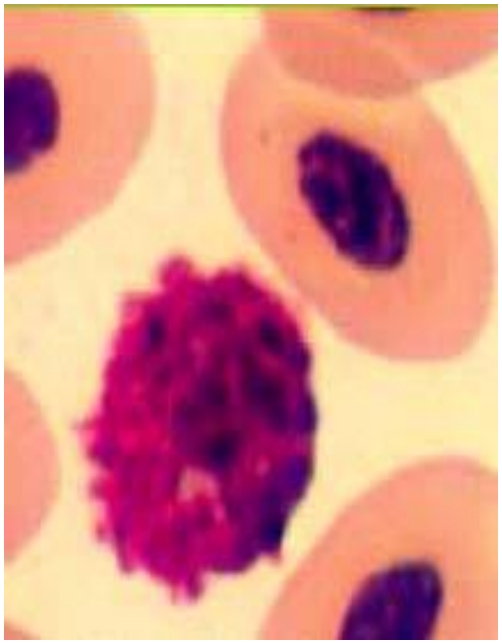
Striations at tail end

Film with uneven thickness

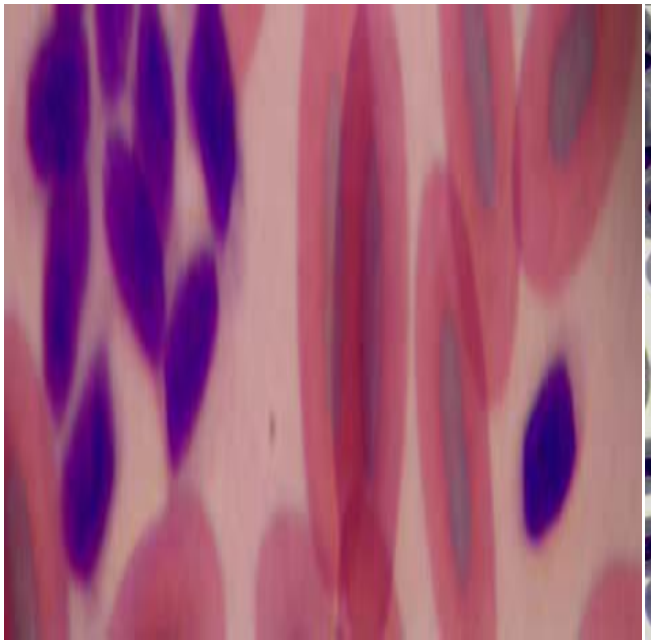

1
Cover the smear with Wright's stain
2
Dilute with equal volume of buffered water
3
Wash
2-3 mins
5 mins
Unstained smear
Stained smear



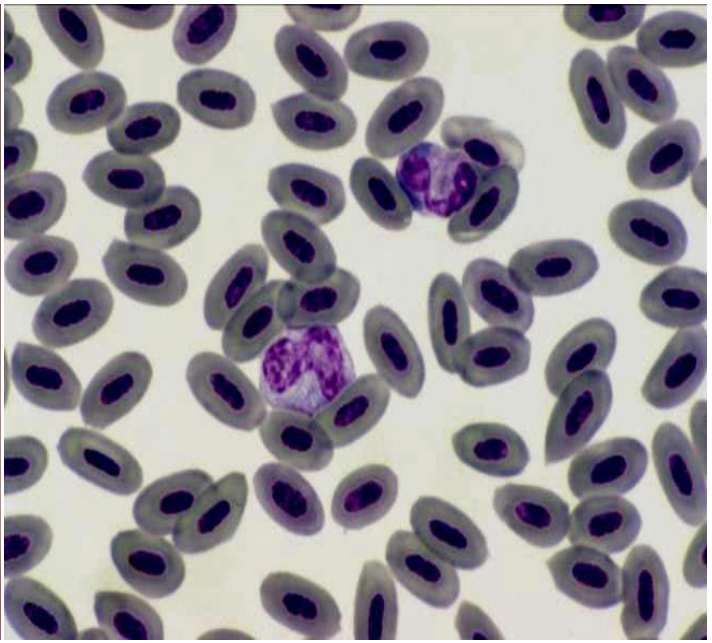
Small Lymphocyte with Platelet



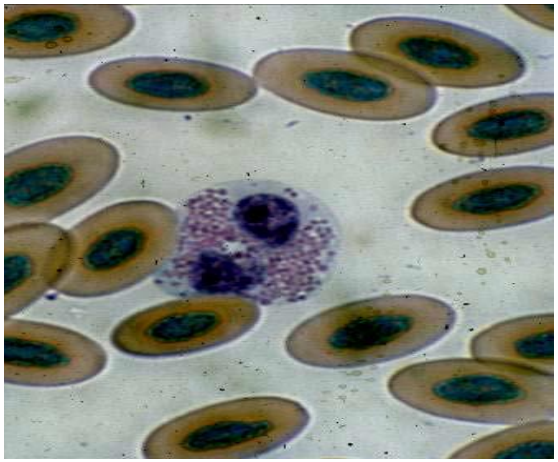
Basophil



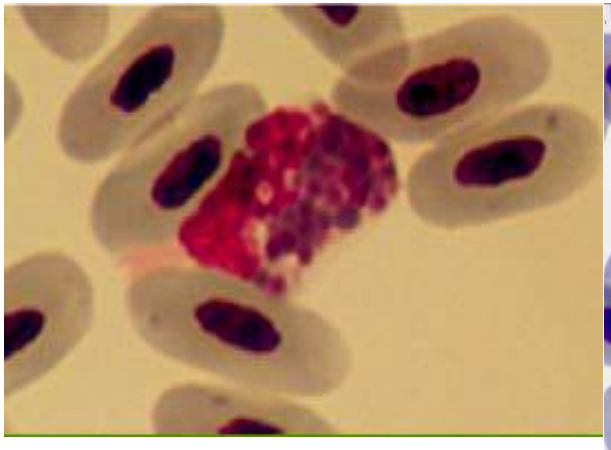
Platelet



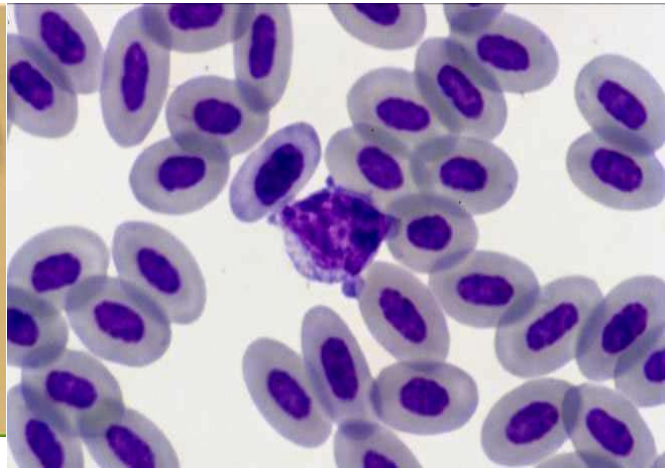
Monocyte



Heterophil



Eosinophil



Lymphocyte

**Species identification in the blood smear: Morphology of the gametocyte has been used to identify the Haematosporidia found in the blood to the genus and species level.

***Leucocytozoon* gametocytes are large and distort the cell that they infect to the point that it is unrecognizable.

***Plasmodium* is the only haematosporidia that will undergo schizogony in red blood cells, therefore if schizonts are seen in a blood smear, a *Plasmodium* infection can be confirmed.**

**Detection of subclinically infected birds:

Subclinically infected birds can be detected by examining blood smears for cells containing the gametocytes or by using PCR assays on DNA extracted from blood.

** Both PCR assays and examination of the blood smear are highly specific for infection.

**Disease caused by these parasites is predominately the result of damage done to internal organ systems in the early phase of infection.

Anemia is a common finding in birds infected with *Plasmodium* and a less frequent finding in birds infected with *Leucocytozoon* and *Haemoproteus*.

** **Gross pathological changes:**

The most common changes are enlargement of the liver and spleen.

**Microscopic changes:

1-Impression stained smears of infected organs (kidney, liver, spleen, lung, heart, skeletal muscle, brain, spinal cord and eye) will often demonstrate cells containing schizonts. They are found within the cytoplasm of the infected cell, are multinucleate and severely distort the cells.

2- Infected cells include: vascular endothelial cells, macrophages, histiocytes, hepatocytes, renal tubular epithelial cells and myocytes .

3- Hemorrhage: Infected vascular endothelial cells can block capillaries (thrombus and embolus formation) and damage.

3- Hemorrhage: Infected vascular endothelial cells can block capillaries (thrombus and embolus formation) and damage.

4- Multifocal necrosis of the liver, spleen, and kidney that may range from mild to severe. Degeneration of the heart muscle and skeletal muscle may occur .

5-Pulmonary edema and release of fibrin into the airways and interstitial pneumonia may develop in severe cases .

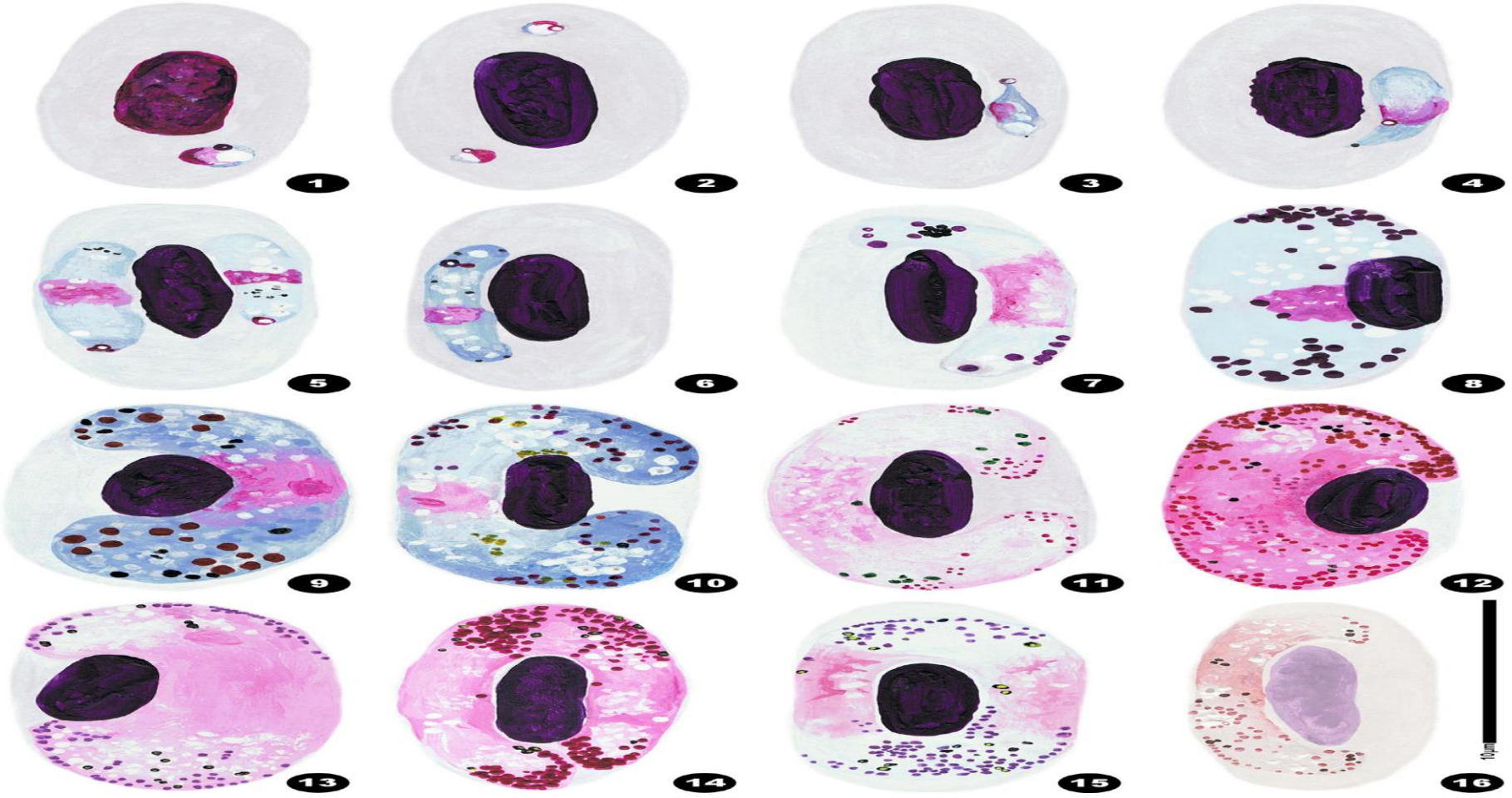
Laboratory diagnosis

**In the live animal: blood smears from fresh blood .

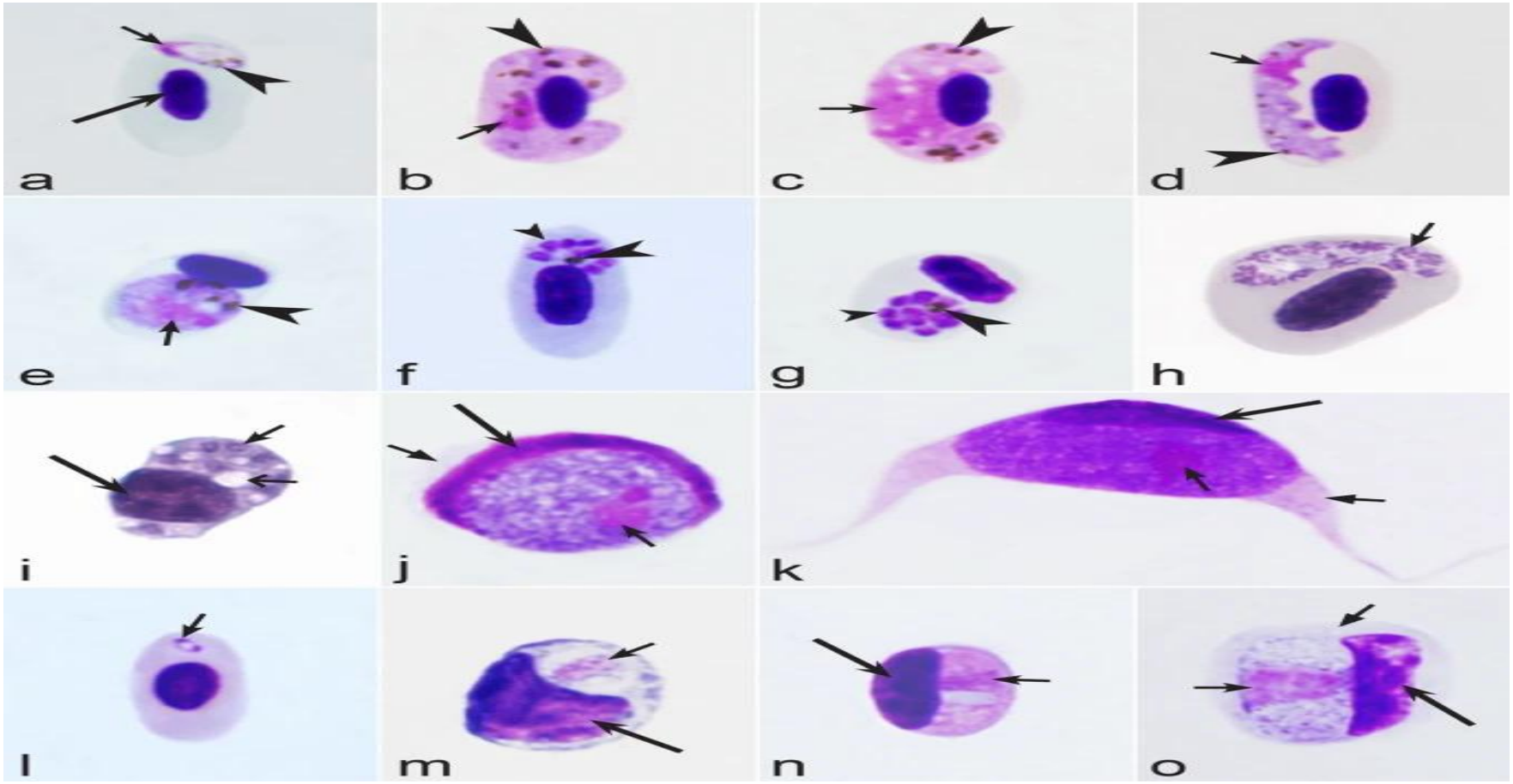
**PCR assays included dried blood smears and blood with anticoagulant EDTA . Depending on the primer sets ,the identification down to the species level.

**Schizonts can be found in impression smears made from liver, kidney, spleen, lung and brain and PCR can be done on any of these tissues if they are fresh or have been frozen.

** Histopathology : Haematoxylin and eosin stained tissues sections (brain, heart, skeletal muscle, liver, kidney, spleen, and lung).

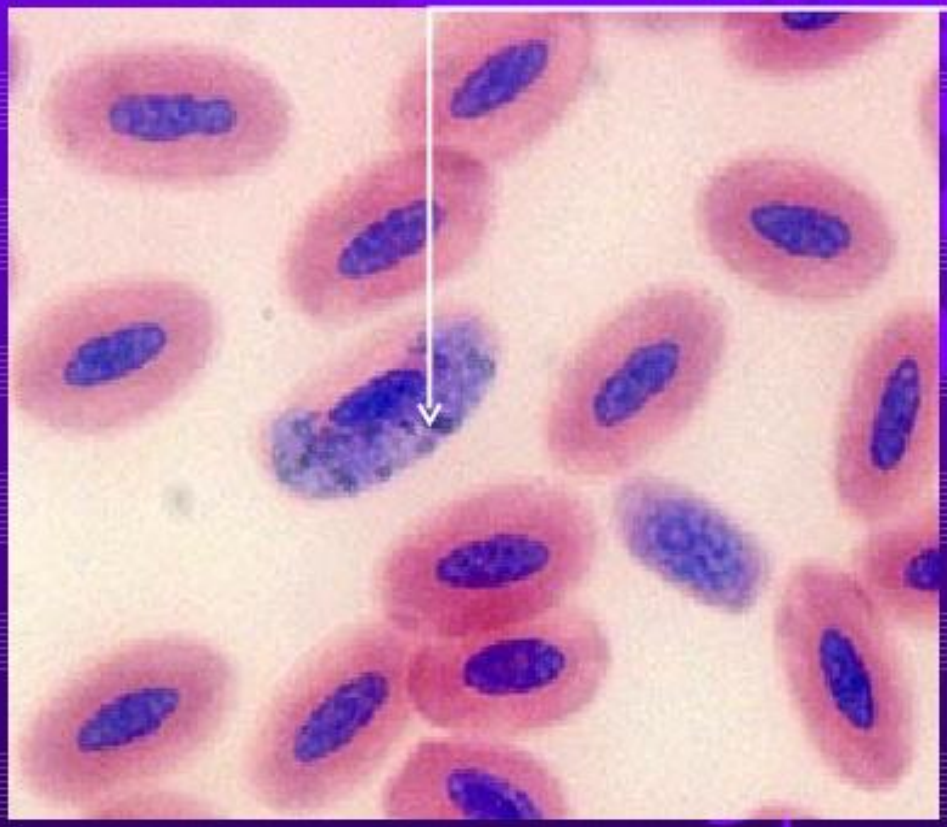


Drawings of erythrocytic stages of *Haemoproteus syrnii* in the blood of *Strix aluco*. 1–4: young trophozoites with an initial volutin granule; 5: two young gametocytes with 1 or 2 volutin granules attached to the initial granule; 6–7 young gametocytes; 8–10 mature macrogametocytes; 11, 13: mature microgametocytes; 12: old microgametocyte; 14: two microgametocytes within the same RBC; 15: amicrogametocyte spread beneath the RBC nucleus; 16: microgametocyte with faded staining and where the volutin granules are still visible.



Main morphological features of blood stages, which are helpful to distinguish Haemoproteus parasites (a–c) from other avian intracellular protists (d–o). Young (a) and fully grown (b, c) gametocytes of Haemoproteus species. Fully grown gametocytes (d, e) and mature erythrocytic meronts (f, g) of Plasmodium species. Growing meronts of Garnia (h) and Fallisia (i) species. Gametocytes (j, k) in roundish host cell (j) and fusiform host cell (k) of Leucocytozoon species. Growing meront (l) of Babesia species. Merozoite (m) of Isospora species. Sporozoite (n) of Lankesterella and gamont (o) of Hepatozoon species. Note presence of malarial pigment (haemozoin) in species of Haemoproteus (a–c) and Plasmodium (d–g) and its absence in species of other avian blood parasites (h–o). Elongate gametocytes of malaria parasites belonging to the subgenera Giovannolaia and Huffia (d) are similar to gametocytes of Haemoproteus species in forms, but the gametocytes of malaria parasites usually are more irregular in shape (d) and the outline of their macrogametocyte nuclei often is not so well indistinct (compare b and d). Presence of merogony in blood cells (f, g) clearly shows malaria infection. Long simple arrows—host cell nuclei. Short simple arrows—parasite nuclei. Simple arrowheads—pigment granules. Simple small arrowheads—merozoites. Simple wide short arrows—vacuoles. Triangle arrows—remnants of host cell cytoplasm.

Morphology



- Gametocyte partially surrounds the cell's nucleus
- Multiple, refractile, golden-brown particles of hemozoin pigment.

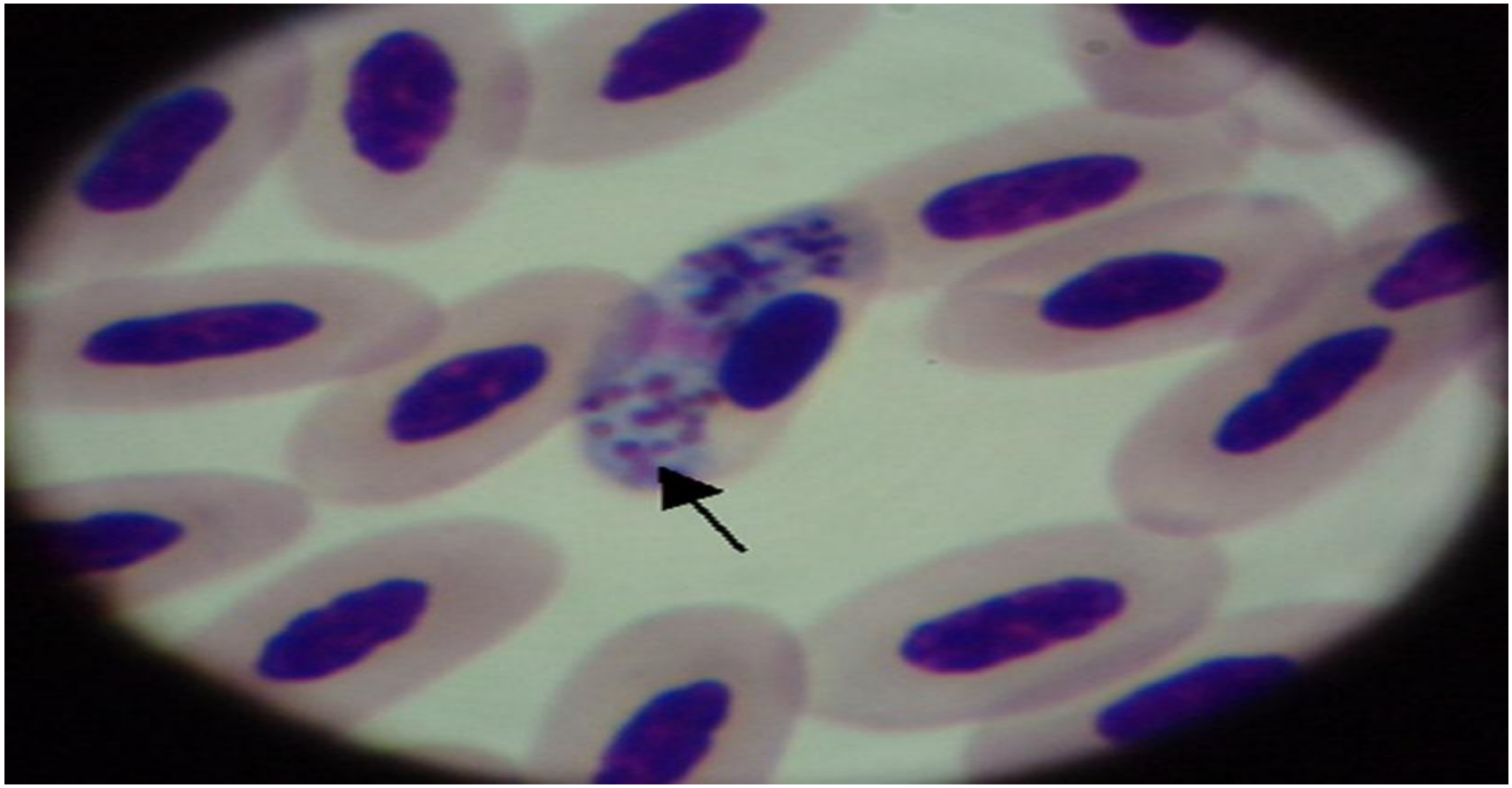


Figure :Giemsa stained thin blood smear showing gamont stage of *Haemoproteus columbae* (arrow) inside the RBC (X100).

Figure 2

A) Male *haemoproteus columbae* gametocyte in the upper left cell stains with more pink (Giemsa staining) than the female parasite in the lower left, bluer in color. Rock Pigeon red blood cells are nucleated, stained dark purple in this photograph.

B) A mosquito bites the face of an apapane—species of Hawaiian honeycreeper endemic to the Hawaiian Islands.

A

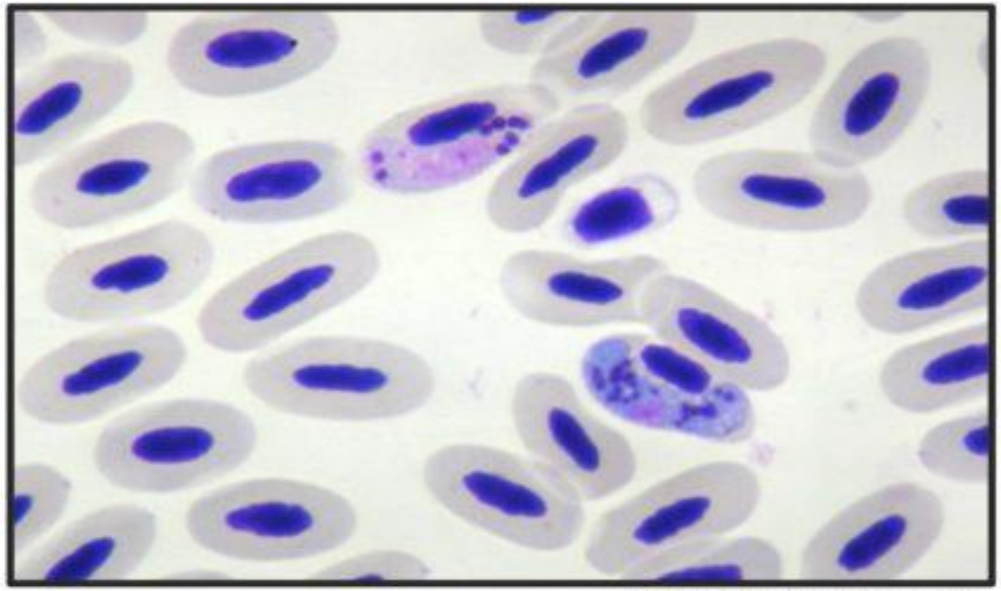


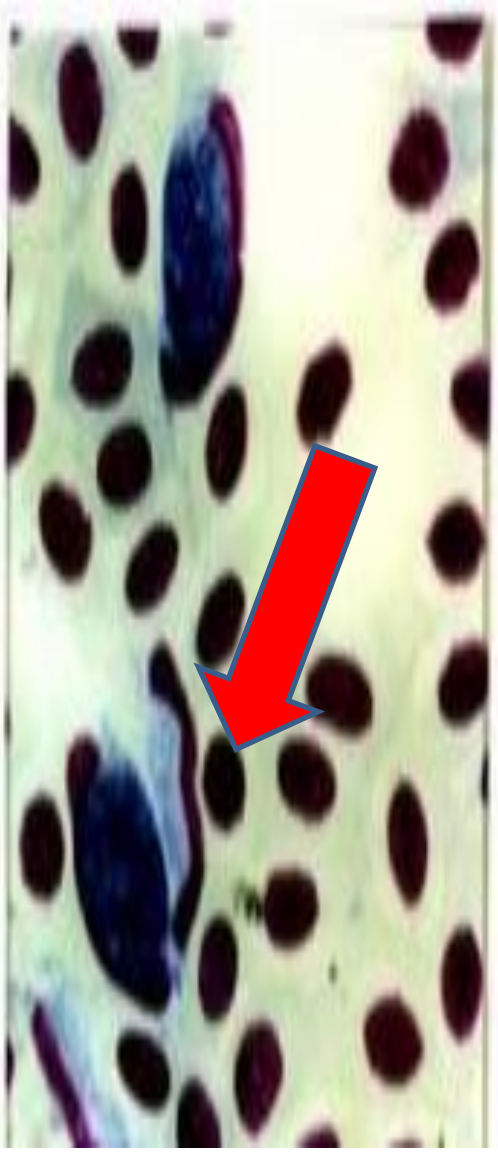
Photo by PlasmodiumLady.

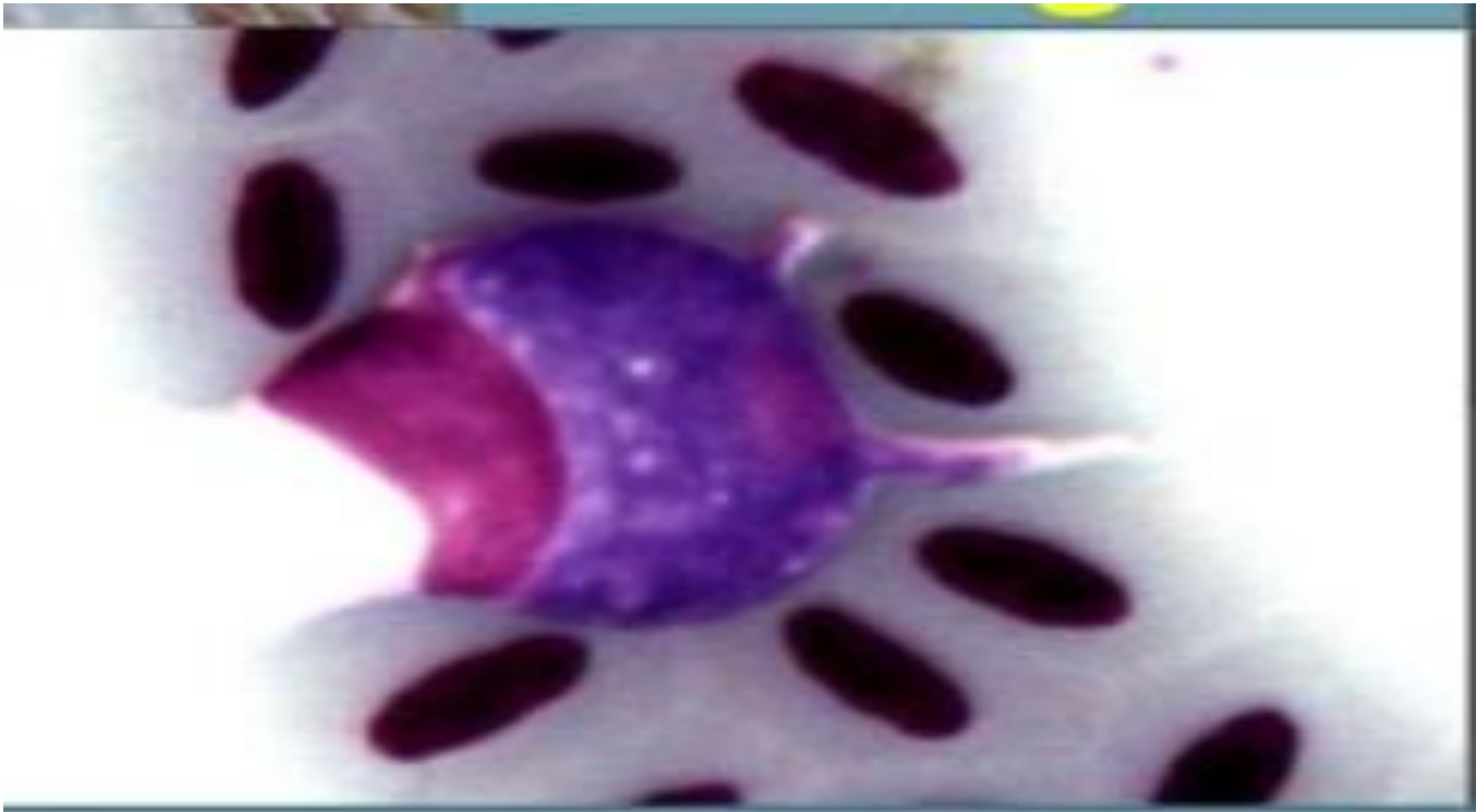
B

Photo by Jack Jefferey.

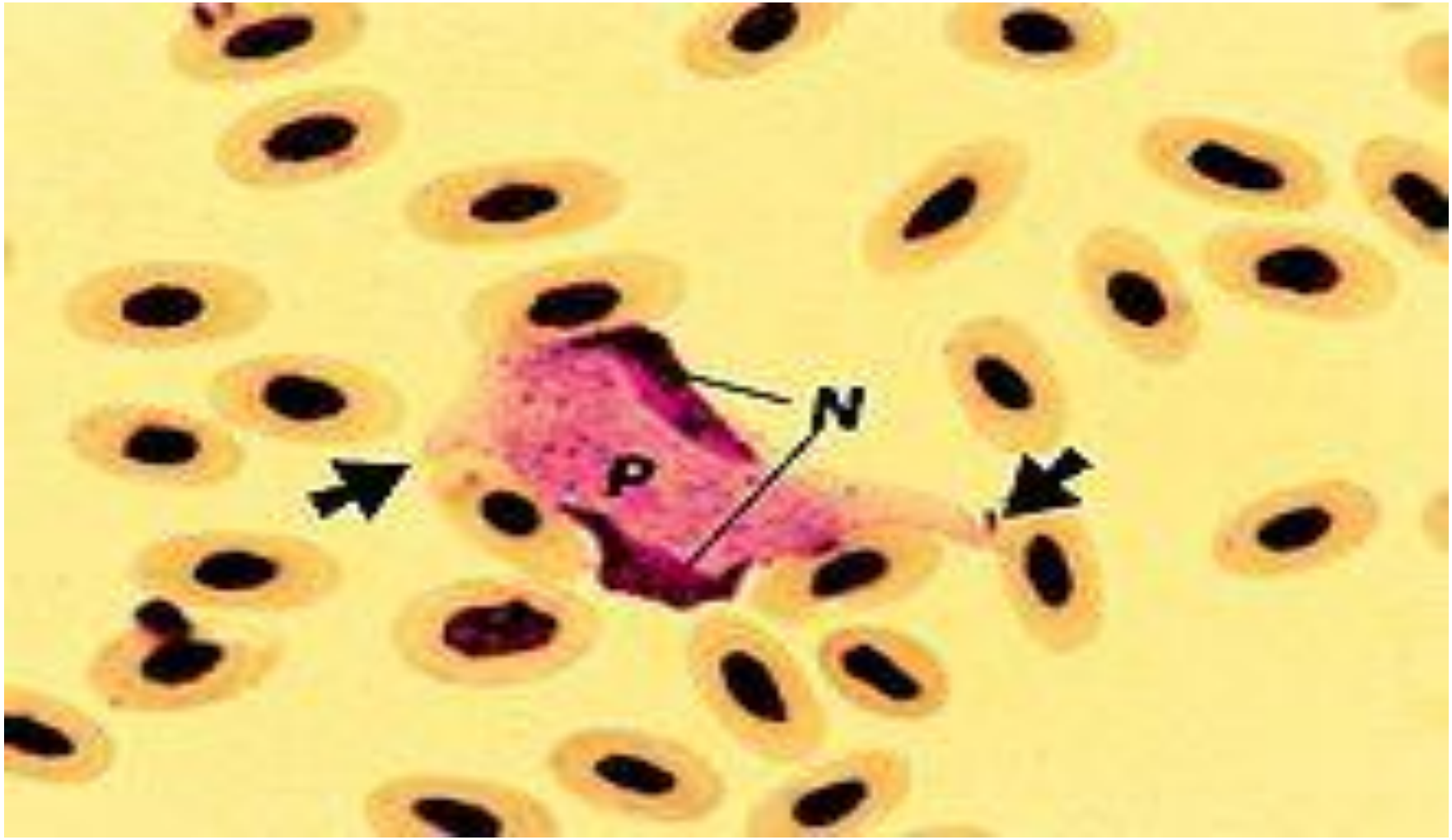
Leucocytozoon smithi - gametocyte

This slide shows a typical gametocyte which has distorted the white blood cell into an elongate, elliptical body. Little evidence of the white blood cell morphology remains. Note that no schizonts appear in the blood.

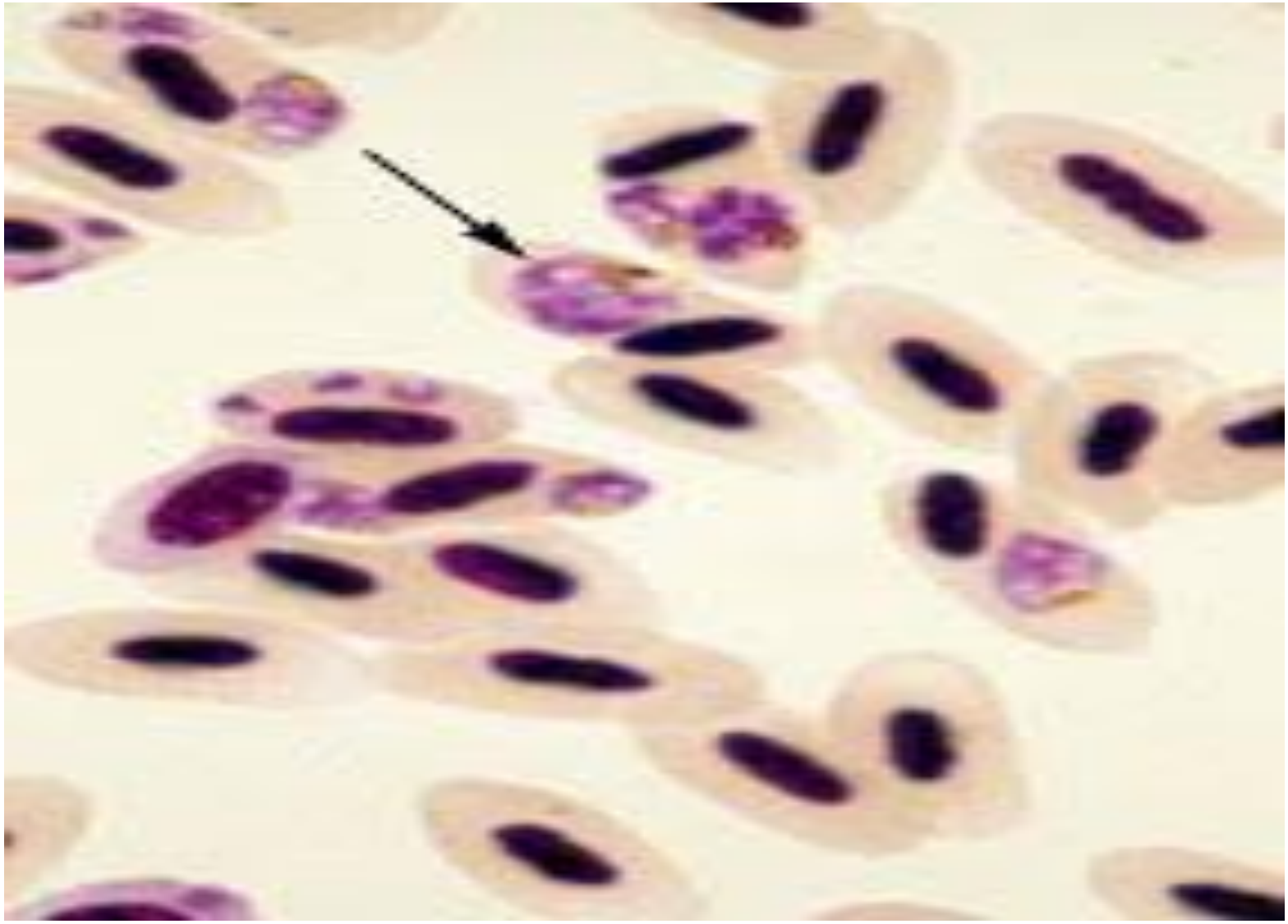




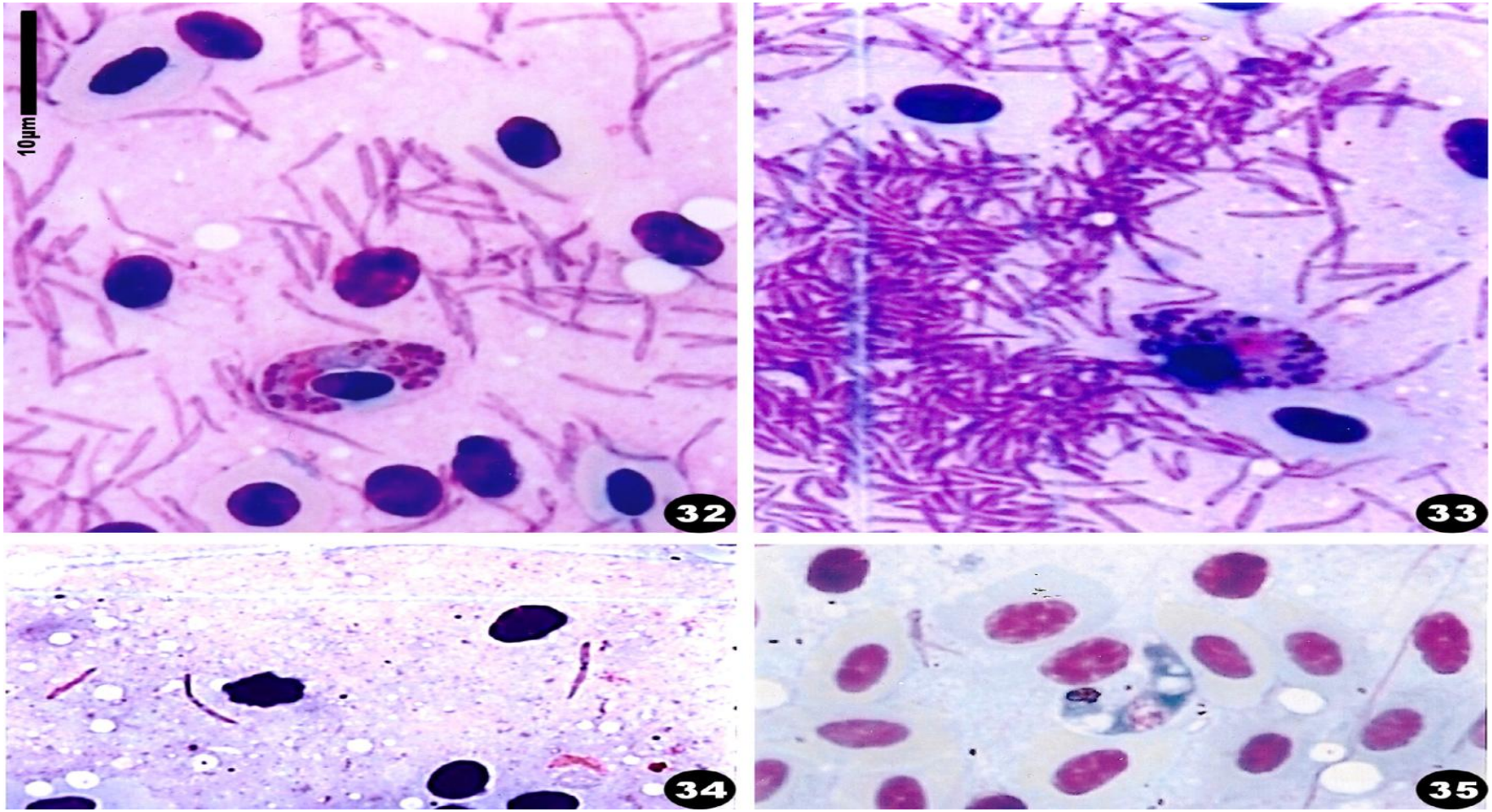
Leucocytozoon in leucocytes



Leucocytozoon smithi **in a stained blood smear from a turkey**



Plasmodium relictum **in the bird species**



Microphotographs of developmental stages of *Haemoproteus syrnii* in the hippoboscid fly. 32: sporozoites issued from a burst oocyst and a gametocyte; 33: burst oocyst with sporozoites still attached to the cytomeres, and a gametocyte; 34: two mature sporozoites; 35: ookinetes. Giemsa staining.

Treatment

**A drug regimen using both chloroquine and primaquine has been found to be effective against the infection with *Plasmodium* and *Haemoproteus*.

**Mefloquine hydrochloride has been used effectively to treat *Plasmodium* .

** Treatment of *Leucocytozoon* successfully has not been reported.