About author

Dr. Prem Sagar Maurya had completed his B.V.Sc & A.H. in the year 2009 from Bombay Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India. He got admission in a master program in the subject of Veterinary Parasitology at Indian Council of Agricultural Research-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India after securing 23rd rank in All India ICAR-JRF examination. He had completed his Masters in the year 2011 and carried out research on Molecular characterization of *Cryptosporidium* spp. Isolated from domestic animals. He completed his Ph.D. in the year 2016 from the Council of Agricultural Research-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh. He had worked on Molecular diagnosis *Cryptosporidium parvum* infection in calves during his Ph.D. degree program. He had been selected as Assistant Professor in the department of Veterinary Parasitology, College of Veterinary and Animal Science, Sardar Vallbhbhai, Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India in the year 2013. He has in his credit 39 research papers and 1 success stories. He is a member of 03 professional societies and attended conferences/ symposiums/ workshops. He has remained on a panel of experts for framing question papers for various Universities.

Description

This lecture note on "Diagnosis of Veterinary importance haemoprotozoan parasites" were prepared and delivered to my BVSc.&A.H students studying Veterinary Parasitology courses. This course was offered during the academic year 2022-23 in the third professional year at College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India. This lecture provides basic Giemsa staining techniques. I had tried my level best to extract the contents simplify the facts in easy to memories in very short time. Further constructive suggestions to improve this lecture note are always welcome its users on my email and whatsapp.

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Address Correspondence to:

Dr. Prem Sagar Maurya
Assistant Professor
Department of Veterinary Parasitology.
COVAS, SVPUAT, Meerut- 250110 (U.P.) India
vet.drpremm@gmail.com

Diagnosis of Veterinary importance haemoprotozoan parasites

Introduction: Haemoprotozoa present in the circulatory/ hematopoietic system of infected animals. Haemoparasites present either in cells (intracellular) or plasma (intercellular). Obviously, for diagnosis of these protozoan parasites the blood sample is collected from the animals showing clinical signs and symptoms of anemia, high fever, swelling of lymph node, splenomegaly, hepatomegaly, hemoglobinuria and corneal opacity.

Collection of blood samples: For the diagnosis of haemoprtozoan infections blood samples must be collected from blood circulations. In the majority of species of domesticated animals blood sample is collected from the ear tips. The tips ears pinnae must be clipped of with scissors/ blades then these areas sterilized with spirit, now allow evaporate spirit then pricked out. The first drop of blood discarded because it contains less number of cells and more plasma. If blood does not oozes out in case of severe anaemia/ hypovolemia nthen apply xylol to increase blood flow to these area. For right diagnosis of Veterinary importance haemoprotozoan parasites suspected blood sample must be collected in a vial containing EDTA (1mg for 1ml blood) anticoagulants.

Preparation of blood smears: In the laboratories three different types of blood can be prepared for diagnosis of number of haemoprotozoan infections. In order to get good smears the glass slides must be clean and grease free. For cleaning of slides they soaked in 1:1 mixture of alcohol and ether at least for 11-12 hours. Subsequently the slides are cleaned by good quality cotton clothes and clean slides must be handled by putting fingers at margin.

Three different types of blood smears:

1. Wet blood smears
2. Thin blood smears
3. Thick blood smears

1. Wet blood smears: A drop of blood is taken on a glass slide to which 1-2 drops of physiological normal saline is added. The cover slip is applied to prepare blood smear. Then smear is examined under 10X or 40X objective of compound microscope to detect the motile extracellular parasites like Trypanosomes. This method can't be used for the diagnosis of intracellular haemoprotozoan parasites.

2. **Thin blood smears:** A very small drop of blood smear is placed on a glass slide and is spread with the help of other clean slide at the angle of 30 to 45^0 by one soon action. In this way blood smear contain single monolayer then naturally allow to air dry. Don't put smear in front of hot blower otherwise smear become black and lead to misdiagnosis of cases.

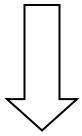
3. **Thick blood smears:** 1-2 big drop of blood is taken on a glass slide and is spread on slide with the help of a clean glass rod by moving fingers in circular manner so that to cover area of 1.5-2 centimeter in diameter then naturally allows to air dry.

Staining methods: Two type's stains (Giemsa& Leishman) are commonly used in the field of Veterinary Parasitology

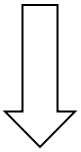
1. **Giemsa stain:** Stock Giemsa stain prepared from mixing of 54ml of glycerol and one gram (1gm). Then mixed properly and kept at 60-70 ^{0}C on magnetic stirrer for overnight. Next morning take away from magnetic stirrer and kept at platform, and wait till come on room temperature. Now in solution mixed with 84 ml of absolute methanol and filter it with whatman filter paper and store in amber colour bottle and keep at dark place. Working solution of stain prepared by adding one part of stock with nine parts of normal water/ staining buffer of pH6.4-.6.8. Working Giemsa's stain is aqueous stain, therefore fixation and staining needed separately. The nucleus of blood protozoa takes reddish colour & cytoplasm of its take bluish.

2. **Giemsa's staining procedure for thin blood smear:**

Prepare tongue shape thin blood smear on grease free clean slide

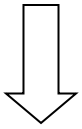


Air dries the smear and fixes it in absolute methanol



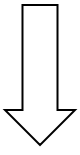
(1 minute)

Flood the smear with Giemsa stain (Dilution 1:9)



(30-40 minutes)

Wash the stain under gentle flow of tap water



(After drying, the smear will be examined under the high power and oil immersion microscopy)

Advantage of thin blood smear: Details of morphological characters of haemoparasites can be studied beautifully. This method can be used for the diagnosis of haemoparasites infection of avian species& camels.

Disadvantage of thin blood smear: Chronic infections can be overlooked during thin blood smear examination.

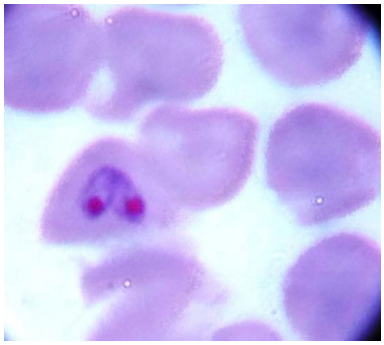
Giemsa's staining procedure for thick blood smear: First step is dehemoglobinization in distill water followed by fixation with absolute methanol and then slide kept in working solution of Giemsa stain in coplin jar for 30-40 minutes.

Advantage of thick blood smear: In thick blood smear large number of blood cells (RBCs, WBCs & platelets) are concentrated in a small circular area, thus large number of cells can be examined at a time and hence chronic or lightly infected cases can be examined.

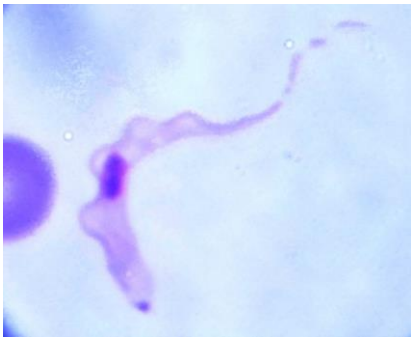
Disadvantage of thick blood smear: Details of morphological characters of haemoparasites cannot be studied nicely. This method can't be used for the diagnosis of haemoparasites infection of the host having nucleated RBCs (Avian species& camels)

Interpretation of results: During the examination of the blood smear should be view under 10X objective to locate the smear showing monolayer of cells (at tongue of smear). Subsequently that location is examined under high power (100X) oil emersion objective. The stained smear nuclei of WBCs take purple/violet colour while cytoplasm of WBCs &RBCs takes pink colour. The nucleus of blood protozoa takes reddish colour & cytoplasm of its take bluish.

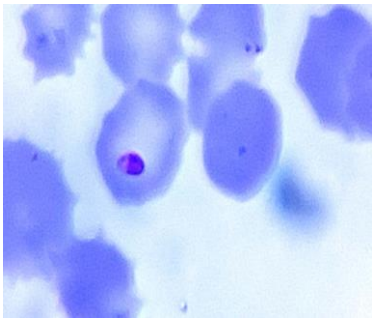
Bellow all these slides are stained with Giemsa stain



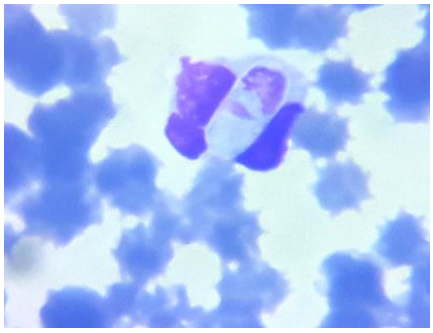
Babesia canis **(In RBC)**



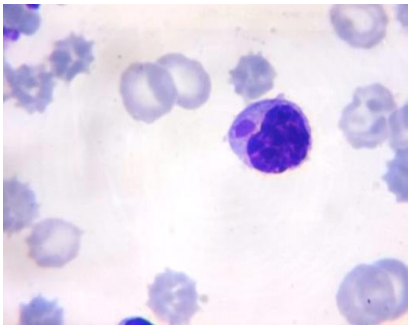
Trypanosoma evansi **(Extracellular)**



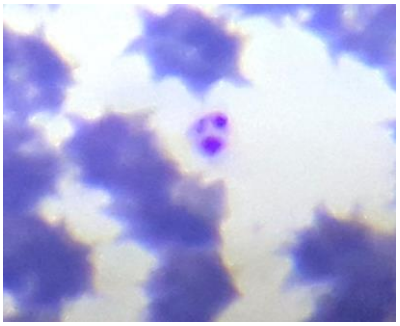
Babesia gibsoni **(In RBC)**



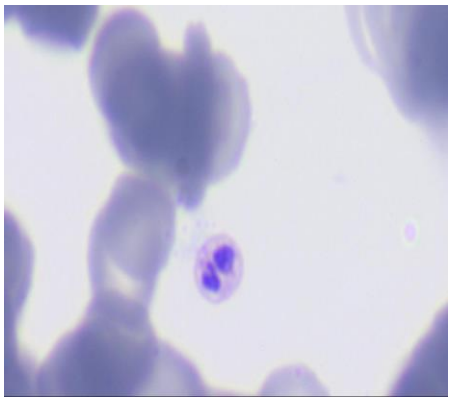
Hepatozoan canis **(In neutrophil)**



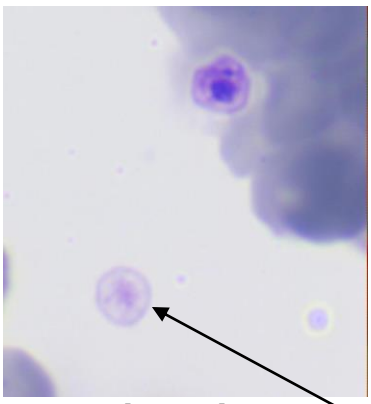
Ehrlichia canis **(In monocyte)**



Anaplasma playts **(In platelets)**



Anaplasma playts



Anaplasma playts

Normal platelet