

## Literature Review

# A Review on Intestinal and Blood parasitic (Haemoparasite) protozoans

Said Muhammed Hussen\*

Salale University, College of Agriculture and Natural Resource, Ethiopia

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\*Corresponding author: Said Muhammed Hussen, Salale University, College of Agriculture and Natural Resource, Ethiopia, E-mail: [saidmuh2011@gmail.com](mailto:saidmuh2011@gmail.com)

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## Abstract

Parasites are a major cause of disease of man and domestic animals. More than half the human population lives in misery and pain and suffers vast economic losses due to parasites. Protozoa are ubiquitous throughout aqueous environments and the soil, and play an important role in their ecology. Optimal recovery and microscopic identification of protozoa from patients with intestinal infections is dependent on proper collection and preservation of fecal specimens. The most reliable way to diagnose a parasitic infection is by detection and identification of the infecting organism. All methods for the direct identification of parasites fail if the parasite density in the specimen is below the sensitivity of the method employed, or if the parasite cannot be directly demonstrated due to the life cycle in the host. In such cases indirect methods must be used. Ideally, serology should allow differentiation between recent and latent infections and should be able to demonstrate whether an animal is carriers as well as the elimination of the parasite after therapeutic measures have been applied.

## Introduction

Parasites are a major cause of disease of man and domestic animals. More than half the human population lives in misery and pain and suffers vast economic losses due to parasites. A possible solution to these problems lies in the development of new agricultural technologies for expanding food production. One of the ways in which livestock production can be increased is by reduction of losses due to disease. Estimates suggest that only a 6% reduction in disease could provide food for an additional 250 million people [1]. The situation is most serious in developing countries where 75% of the world's population resides. According to the Food and Agriculture Organization, it is estimated that up to 70% of the world's livestock resources exist in these regions, yet they account for only 30% of the world's meat output [1].

The effective control and treatment of parasitic diseases requires rapid, reliable and highly sensitive diagnostic tests, which can also serve to monitor the effectiveness of the therapeutic and prophylactic protocol. The usual immunological tests do not always meet these requirements and are not able reliably to distinguish between closely related species [2].

Protozoa are ubiquitous throughout aqueous environments

and the soil, and play an important role in their ecology. Protozoa occupy a range of trophic levels. They also play a vital role in controlling bacteria population and biomass. Farm animals are usually infected with several species of parasites and they are also confined to pasture or pens. Parasite eggs, larvae, and cysts are intense in soil. One of the most important aspects of animal protozoology is transmission to humans. These are called zoonosis. Some of zoonotic diseases are common and important to public health [3].

Documented in recent studies, multiplex PCR assays are both more sensitive and specific than microscopy for the detection and identification of pathogenic protozoa [4]. However, despite a rapidly growing field of molecular and genetic technologies for the clinical microbiology laboratory, diagnostic developments for intestinal protozoan parasites have remained relatively stagnant. Some veterinarian and physicians interpret the presence of nonpathogenic protozoa as indicative of patient exposure to contaminated food or water, although there are no studies that have clearly demonstrated this to be fact. Adequate diagnosis of intestinal protozoa by the clinical laboratory is limited by many factors. There is increasing demand for low-complexity, high-throughput, and cost-effective complements to (or replacements for) the labor-intensive microscopy-based approaches to protozoan diagnosis.

## Haemoparasitic protozoan

**Babesiosis:** Babesiosis is an infectious tick-borne disease of livestock that is characterized by fever, anemia, haemoglobinuria and weakness. The disease also is known by such names as bovine babesiosis, piroplasmosis, Texas fever, red water, tick fever, and tristeza [5]. The disease also is a haemoparasitic disease caused by protozoa of the genus *Babesia* (Phylum: Apicomplexa), which infects mainly ruminants [6]. Infection of a vertebrate host is initiated by inoculation of the sporozoite form of parasites into the blood stream during the taking of a blood meal [7].

Bovine babesiosis disease is caused by at least six *Babesia* species. Bovine babesiosis associated with *B. bigemina* and *B. bovis* is the most important disease of tropical and subtropical regions between 40°N and 32°S. Both species are transmitted transovarially by *Boophilus* ticks, but only tick larvae transmit *B. bovis*, whereas nymphs and adults transmit *B. bigemina* [7]. In Europe, babesiosis is caused by *Babesia divergens*, an intraerythrocytic parasite that can persist for >13 months in the organs of infected cattle. The distribution of *B. divergens* reflects its triphasic telotrophic tick vector, *Ixodes ricinus*, which is widespread across Western Europe and North Africa [8]. *B. major* occurs in Europe, North Africa and South America. *B. major* is transmitted by the three-host tick *Haemaphysalis punctata* [9].

### Clinical examinations

Incubation period is 2–3 weeks. *B. bigemina* and *B. bovis* produce acute syndromes which are clinically indistinguishable, and are characterized by high fever (41°C), anorexia, depression, weakness, cessation of rumination, and a fall in milk yield. Haemoglobinuria can be seen, the color of urine is dark-red to brown. Respiratory and heart rates are increased, and the red conjunctivae and mucous membranes change to the extreme pallor of severe anemia. Abortion occurs in pregnant animals [10]. Subacute syndrome also occurs in young animals, but fever is mild and haemoglobinuria is absent [7].

In cerebral babesiosis, hyperexcitability, convulsions, opisthotonos, coma, and death, may be observed in cattle infected with either *B. bigemina* or *B. bovis*, but especially with the *B. bovis*. Central nervous system signs are caused by brain anoxia resulting from severe anemia [5].

In sheep develop fever and parasitemia within 2 to 4 days; the clinical signs of the disease include anorexia, listlessness, anemia, moderate jaundice and haemoglobinuria. In intact animals, hyperthermia returned to normal on the fourth day after the peak pyrexia, and parasitemia is eliminated within the course of the disease [11].

### Diagnosis

Blood smears and clinical findings are useful in acute cases of piroplasmosis, but are not sufficient in subclinical cases. The complement fixation test is used as a serological test for bovine babesiosis. The most commonly used tests are ELISA, PCR and a DNA probe, which can detect specific parasitemia

at very low levels of infection [7]. Recently, the 'reverse line blot (RLB) is a versatile technique for simultaneous detection and identification of small ruminant piroplasm species, based on the recognition of specific gene regions by oligonucleotide probes [12–14].

### Treatment

After the haemoglobinuria or cerebral signs, prognosis is not well. In acute cases that PVC values are above 12%, treatment will be successful. Supportive therapy such as blood transfusions (4 L of whole blood per 250 kg of body weight), fluids, hematinics, and prophylactic antibiotics are important [5]. Babesiosis can be treated using

Diminazene aceturate (3–5 mg/kg)

Phenemidine diisethionate (8–13 mg/kg)

Imidocarb dipropionate (1–3 mg/kg)

Micarbalide diisethionate (5–10 mg/kg) [5,7,9,15,16].

The control of the disease depends on effective quarantine to prevent the introduction of the vector tick. The control of ticks by dipping or spraying animals at risk with recommended acaricides. In routine surgery, care should be taken to prevent accidental transfer of blood from one animal to another (e.g., castration, dehorning). In addition, in cattle, the selection and breeding of cattle which acquire a high degree of resistance to ticks is practiced. Widespread use of tick vaccines may also have a significant influence on the incidence of infection in cattle [5,7,9].

### Theileriosis

Theileriosis is caused by *Theileria* spp. in cattle, goats, sheep and wild and captive ungulates [10]. Theileriosis is a haemoparasitic disease caused by protozoa of the genus *Theileria* (Apicomplexa). *Theileria* species affect domestic and wild ruminants, especially in Africa, Europe, Australia, and Asia [17]. The parasites are transmitted by tick. These parasites undergo repeated merogony in the lymphocytes ultimately releasing small merozoites, which invade the red cells to become piroplasms. Theileriosis, have a variety of tick vectors which cause infections ranging from clinically inapparent to rapidly fatal [9].

In bovine, Tropical Theileriosis (Mediterranean coast fever), occurs in Mediterranean countries, Middle East, India and China, and is caused by *T. annulata* [7]. The tick vectors are *Hyalomma dentritum* in North Africa and in the Mediterranean countries, *H. dentritum* and *H. dromedarii* in central Asia, and *H. marginatum* in India [7,9]. In endemic areas indigenous cattle are relatively resistant while modified cattle, especially European breeds, are highly susceptible [9]. *Theileria orientalis* complex is a milder disease than East coast fever and Tropical Theileriosis, and called benign theileriosis in cattle [7]. The tick vectors are *Ambylomma variegatum*, *A. cohaerens*, *A. haebraeum*, *Haemaphysalis bispinosa* that these vectors are the probable vectors in Australia [9]. *Theileria*



velifera has been described in cattle and zebu and is very mild theileriosis in Africa [7,9]. The tick vectors are *Ambylomma variegatum*, *A. lepidum* and *A. haebraeum* [9]. *Theileia taurotragi* has been described in cattle, antelope and eland. Geographical distribution of this disease is in Africa. The tick vectors are *Rhipicephalus appendiculatus* and *Rhipicephalus pulchellus* [9]. *Theileria mutans* has been seen in cattle and deer [18]. *Theileria hirci* is malignant theileriosis of small ruminants [19], and is enzootic in North Africa, Middle East, China and India. The tick vectors are *Rhipicephalus bursa* and *Hyalomma anatolicum* [9].

*Theileria ovis* is benign theileriosis in Africa [7]. But in China *Theileria ovis* is a serious disease [20]. The incidence and mortality rates of *Theileria ovis* in adult sheep and goats were found 17.12% and 65.78%; 8.06% and 73.33% respectively in China [21]. In Turkey, prevalence of infection of *T. ovis* in sheep and goats were having been 64.19% and 12.36%, respectively [22]. The tick vectors of disease are *Rhipicephalus bursa* in the Mediterranean and *Rhipicephalus evertsi* in Africa [9].

### Clinical examinations

*Theileia* species are classified in to 2 groups. In first group (*T. parva* and *T. annulata*), proliferate is seen in lymphocytes but in the second group (*T. orientalis*) it is seen in erythrocytes that causes hemolytic anemia [23].

In *Theileria parva* incubation period is approximately 1–3 weeks [7]. One or two days later, the first clinical sign is generalized swelling of the superficial lymph nodes, eyes, ears and submandibular regions. After few days there is anorexia, decreased milk production, loses condition, ceases rumination, rapid heartbeat, petechial haemorrhages under the tongue and on the vulva [9]. In cerebral theileriosis there are localized nervous signs and convulsions, tremor, salivation and head pressing [7].

In *Theileria annulata*, pyrexia, anorexia, enlargement of superficial lymph nodes, nasal and ocular discharges and salivation is the most common signs. Constipation is recorded in some cases. Respiratory distress, coughing and pulmonary oedema are seen [24].

In *Theileria orientalis* clinical signs are associated with anemia, jaundice and lymphadenopathy [9]. Clinical signs in *Theileria velifera* are not reported but in *Theileria taurotragi* mild fever and anemia are reported [9].

In *Theileria hirci* which is very pathogenic in sheep and goats, in the acute form there is fever, inappetence, ceases rumination, weakness, enlargement of superficial lymph nodes, diarrhea, jaundice, hemorrhage in submucous may occur. In chronic form there is fever, inappetence, emaciation, anemia and jaundice [9]. *Theileria hirci* is not reported in Turkey [22]. *Theileria ovis* that is benign theileriosis in sheep and goats clinically is mild [9].

### Diagnosis

Investigation of giemsa-stained blood smears and lymph

node biopsy will reveal piroplasms in erythrocytes and schizonts in lymphocytes. For diagnosis, indirect fluorescent antibody test (IFAT) and indirect enzyme-linked immunosorbent assay (ELISA) are the most commonly used techniques. The ELISA tests are more sensitive than IFAT [7,9].

### Treatment

Buparvaquone is the most effective drug and the recommended dose in cattle, sheep and goat is 2.5mg /kg BW [7,9]. In control of the disease use of genetically resistant breed, a judicious and selective application of acaricides at 3-week intervals and the use of vaccines are recommended [7].

### Trypanosomosis

Member of the genus *Trypanosoma* are lived in the bloodstream and tissues of cattle, sheep, and goats in the entire world. These are flagellated protozoal agents that produce a variety of very serious disease of human and animals, but many of them are nonpathogenic [25]. Trypanosomosis is one of the most important diseases of animals and human. Most African species are transmitted by the tsetse flies [9]. There are 7 species of *Trypanosoma* species in cattle, sheep, and goat but all of them may not be pathogenic.

### Clinical examinations

Clinical findings are based on the speed of onset of anemia and the grade of organ impairment. Trypanosomosis can be acute, subacute, or chronic. In acute form abortion, drop in milk, depression, anorexia can be seen. Hyperemic mucous membranes and lacrimation also can be occurring. In subacute form clinical signs include weight loss, enlargement of lymph nodes and dry hair coat. In chronic form dull, dry hair coat, inelastic skin, lethargy, pale mucous membranes and exercise intolerance may be seen [16]. *T. theileri* rarely causes clinical signs, but sometimes parasitemia may develop [16].

### Diagnosis

Diagnosis can be based on the clinical findings, presence of vectors, appearance of trypanosomes on a fresh blood smear, or a Giemsa-stained blood smear. Indirect fluorescent antibody test (IFA) and the enzyme-linked immunospecific assay (ELISA) test are used for diagnosis [16].

The first nucleic acid-based detection and characterization of trypanosomes was done by using the genes coding for trypanosome variable surface glycoprotein (VSG's) [26–28]. These probes did not cross-hybridize to other trypanosome species and even distinguished groups within the species *Trypanosoma congolense* [29]. Nucleic acid probes have thus been developed that can distinguish between closely-related trypanosome species [30–32] and that can detect the parasite in the tsetse fly vector [33,34]. These probes are all based on repetitive DNA sequences and can detect as few as 100 trypanosomes by dot-blot hybridization [35].

### Treatment

The most common drugs that are used for treatment of

trypanosomosis are shown below:

- Diminazene aceturate 3.5-7 mg/ kg BW.
- Homidium bromide and chloride 1mg /kg BW  
-Pyrrithidium bromide 2mg / kg BW.
- Isometamidium 0.25-1mg / kg BW [7].

Vector control can help to control or prevent trypanosomosis. Insecticides can be used for prevent bites by tsetse flies and other flies [16].

### Intestinal protozoan parasites

**Coccidiosis:** Coccidiosis is a protozoan parasitic (genus *Eimeria*) disease that cause diarrhea in calves, lambs and kids [7]. There are 12 species of *Eimeria* in cattle but all of them may not be pathogenic. the disease is dependent on the destroy of the crypt cells of the intestinal mucosa because in the ruminant small intestine is very long and providing a high number of host cells and is a potential for parasite replication with minimal damage. Some *E.* species that invade the large intestine cause pathological changes, especially when large numbers of oocysts are ingested in a short period of time [9].

In healthy nonimmune animals when number oocysts ingested is low, the animals show no clinical signs of disease but in many oocysts condition, rupture and exfoliation of intestinal cells triggers intestine function and causes loss of blood, fluid, albumin and electrolytes into the intestine. Disaster of mucosal capillaries of intestine can cause to hypoproteinemia and anemia. Secondary bacterial infection may cause severe enteritis [36].

**Clinical examination:** In most clinical cases the temperature is normal or subnormal, Disaster of epithelial cells of intestine cause sometimes bloody diarrhea. Dehydration may occur but anemia occasionally may be seen. In severe infections, diarrhea which may be mucoid or bloody, tenesmus, abdominal pain, anorexia, dehydration and weight loss are the common clinical signs in Coccidiosis [7,36].

In the calves that infected with high number of oocysts may developed dysentery. Clinical Coccidiosis occurs rarely in adult cattle [7].

Coccidiosis in lambs is widely similar to that in calves, but dysentery does not usually occur. In infected kids clinical signs are associated with watery diarrhea, dehydration, anorexia, and weight loss [36].

### Diagnosis

Diagnosis is based on history, clinical and necropsy findings, and microscopic examination of feces. Acute Coccidiosis can be diagnosed by direct examination of feces but in chronic Coccidiosis that very low oocysts number are seen in feces, direct examination of feces may not be adequate [37].

### Treatment

Coccidiostats drugs are used for treatment. All animals in

the flock should be treated. Trimetoprim+Sulfadoxine (16-24 mg/kg) can be used for three days by intramuscularly in lambs and kids [38] Amprolium (10 mg/kg BW) for 5 days or 65 mg/kg BW one dose can be effective in calves [7]. Diclazuril and toltrazuril are also coccidiocidal drugs [39,40]. Suggested that toltrazuril is more effective than sulphadimine and amprolium in *E. Bbovis* and *E. zuernii* oocysts shedding in buffalo calves. Because of the low serum vitamin C levels in lambs with Coccidiosis, administration of vitamin C is suggested to be useful combined with the classical treatment [41].

Hygiene in the house, minimization of predisposing factors, avoiding the overcrowding of animals in the pens, control of the pastures for parasite are important factors for the control of Coccidiosis.

### Avian coccidiosis

One of the most common & expensive diseases of poultry production

#### Chickens

- 9 species of *eimeria* involved
- *Eimeria acervulina* - Most frequently encountered
- *Eimeria tenella* - Most pathogenic (high mortality)
- Site of infection ranges from small intestine to cecum depending on species involved
- Intestinal / cecal lesions range from minor to round, white/grey plaques to severe necrotic cores and hemorrhage with significant blood loss

#### Clinical signs

- Bloody droppings and hemorrhagic diarrhea beginning 4 days post infection
- Acute death (high mortality rates)
- Emaciation, pallor & inappetence.

#### Control and treatment

- Isolate sick birds; treat healthy birds with anti-coccidian medications (treating sick birds is futile)
- Almost all poultry flocks receive preventative medication
- Ionospheres (monensin, salinomycin, lasalocid)
- Amprolium
- Decoquinatate
- Resistance is becoming widespread
- Drugs must be rotated or use Ashuttle@ program (one drug in starter feed, another in grower feed)
- Vaccines have been developed but problems with administration

- Raise young chickens separate from older birds (segregation of industry), All-in-All-out strategy
- Environment as in general section.

## Giardiasis

Giardiasis is the infection caused by the flagellate intestinal protozoan, *Giardia intestinalis*, which is also known as *G. duodenalis*, or *G. lamblia*. Infection occurs in a wide range of domestic and wild animals and also humans. *G. intestinalis* is a potential pathogen in livestock, causing diarrhea, weight loss, poor condition and lethargy [42]. The parasite is a noninvasive and colonizes the mucosal surface of the small intestine.

## Clinical examinations

Clinical signs of giardiasis include acute or chronic diarrhea, dehydration, weight loss, abdominal pain and reduction in weight gain. The diarrhea may be continuous or intermittent.

## Diagnosis

Diagnostic methods for introduce of giardia include direct microscopy, fluorescent antibody staining, ELISA, and PCR [43].

Requires multiple fecal samples (3 consecutive or 3 over 5 days) as cyst shedding is intermittent.

## Fecal flotation

- Gold Standard: fecal flotation with centrifugation in ZnSO<sub>4</sub> (with or without Lugol's iodine stain)
- Cytoplasm within cysts collapses producing a crescent shaped refractile osmotic artifact.

## Direct smear

- Saline smear of fresh diarrhea, trophozoites movement (via flagella) – 'falling leaf'
- Trophozoites die quickly so sample must be observed soon after collection (i.e. per rectum) or within ~ 20-30 minutes of 'deposit' & kept at body temperature in humid environment to avoid desiccation.
- Cysts may be observed in high numbers
- Lugol's iodine can be used to stain both trophozoites & cysts

## Antigen detection

- Detect *Giardia*-specific antigen
- Pro Spec T Fecal ELISA (micro plate - multiple samples) - detect infections in cats & dogs (100 & 96%, sensitivity & specificity respectively)
- IDEXX SNAP *Giardia* test (lateral flow ELISA - individual samples)
- Based on cyst wall protein released into the feces during encystation

- Approved for cats & dogs (> 90% sensitivity & specificity)

\*\*\* No current test is 100% reliable; therefore, best to use a combination of centrifugal fecal flotation & antigen testing. Accuracy increases with more than one fecal sample analyzed per animal re: intermittent shedding of cysts.

## Treatment

Infected sheep and goats can be treated with fenbendazole (5-10 mg /kg) for 3 days [37]. Fenbendazole (10 mg /kg BW daily for 3 days) and albendazole (20 mg /kg BW daily for 3 days), have been shown also to be effective in calves [7,44,45] demonstrated the efficacy and safety of a 5 day treatment with paromomycin against an experimental *Giardia* infection in calves. [46] Suggested that five day treatment with Trimethoprim + Sulfadoxine and multivitamin complexes can be effective in the prevention and treatment of *Giardia intestinalis* in lambs and kids. Re-infection frequently occurs if the sources of contamination are not eliminated and the frequency of transmission is high [44].

## Cryptosporidiosis

*Cryptosporidium* are protozoan parasite with at least 13 species occurring in mammals, birds, and fish, but only 2 of these species are important in livestock animals [7,9]. *Cryptosporidium* species are intracellular protozoan parasites causing gastrointestinal disease and diarrhea, and they are important infection in young ruminants.

## Clinical examinations

Infections that caused by *Cryptosporidium andersoni* are usually asymptomatic, although depressed weight gain in calves and milk yields in milking cows have been reported [9]. In *Cryptosporidium parvum* infections, clinical signs are characterized by anorexia, depression, weight loss, diarrhea, dehydration, high morbidity and possibly death because of dehydration. *Cryptosporidium parvum* can cause diarrhea in calves 5to15 and in lambs and kids 5to10 days of age [7,37]. Relapses of diarrhea are quite common, and *Cryptosporidium parvum* usually occurs as a component of mixed infections [37].

## Diagnosis

Oocysts can be detected using Ziehl-Nielsen stained fecal smears *Cryptosporidia* can be diagnosed by fecal flotation. A number of molecular and immunological assays are available for diagnosis for example Immunofluorescence (IF) or Enzyme-Linked Immunosorbent Assays (ELISA) [9]. *Cryptosporidiosis* is a zoonotic disease, and human can become infected from handling infected animals or feces.

## Treatment

There is no effective drug for treatment of *cryptosporidiosis* in ruminants [37]. Halofuginone has efficacy in calves with diarrhea due to *Cryptosporidium parvum*. Halofuginone is reported to reduce oocyst shedding and the intensity of diarrhea. Oral dose of 0.1mg / kg BW daily for 7 days can reduce oocyte excretion [46].

Decoquate, 2.5 mg/ kg, can be effective in prevention of cryptosporidiosis in kids and goats [37]. Cryptosporidiosis in young animals should be treated with fluid therapy and correction of acid-base disturbance.

The control of disease is based on the minimize transmission between the source of the organism and young animals. During the course of the diarrhea, diarrheic young animals should be isolated from healthy animals [16]. The best control of cryptosporidiosis comes from calves, lambs, and kids getting adequate immunity through colostrum after birth.

### Specimen collection

Optimal recovery and microscopic identification of protozoa from patients with intestinal infections is dependent on proper collection and preservation of fecal specimens. Well-recognized factors that influence the sensitivity of parasite examinations include patient medications, specimen collection interval, and the preservation of stool prior to testing [47]. The diagnostic yield of the O&P is also significantly impacted by the number of stool specimens collected and submitted to the laboratory for testing. Many intestinal protozoa are irregularly shed, and data suggest that a single stool specimen submitted for microscopic examination will detect 58 to 72% of protozoa present [48,49]. However, alternative approaches have been proposed to help curtail unnecessary testing, including application of an algorithm that requires a negative specimen and persistence of symptoms before a second or third specimen is analyzed by the laboratory [48]. Specimens may also be pooled prior to screening based on microscopy. In contrast, the enhanced sensitivity of molecular detection methods may require only 1 specimen for testing to achieve sensitivity equal to, if not greater than, microscopy. One study demonstrated a 14% increase in yield for gastrointestinal protozoa when a real-time PCR was performed on a single stool specimen, compared to microscopy on three specimens [50].

### Specimen (Feces) preservation

Preservation in 10% formalin is necessary when faeces cannot be examined within the prescribed time interval. These specimens can be stored for several months. However it should be noted that it has its drawbacks which include its interference with PCR especially after extended fixation time, its inadequate preservation of morphology of the protozoan trophozoites as well as its non-suitability for some smears stained with trichrome stain [51]. There are many microscopic artifacts that can be found in faeces which may be confused with trophozoites.

Visualization of motility in unpreserved specimens may facilitate diagnosis; this technique is impractical for most laboratories, as transport of fresh feces to the laboratory for testing is rarely within the requisite time frame for examination (i.e., 30 to 60 min). A variety of fecal fixatives have been developed and modified in recent decades for use with traditional microscopic examination. Those that remain widely used and commercially available include formalin, sodium acetate-acetic acid-formalin (SAF), Schaudinn's fluid,

polyvinyl alcohol-containing fixatives (mercury, copper, or zinc based), and mercury-free/formalin-free fixatives. A two-vial collection system, consisting of one vial containing 5 to 10% buffered formalin for use in concentrated wet mounts and a second vial containing a polyvinyl alcohol-based preservative for permanent stained smears, is considered the "gold standard." However, concern over working with toxic formalin in the laboratory and the environmental impact and disposal costs associated with the use of mercury-based fixatives have led many to consider alternate preservatives and single-tube collection systems [52]. SAF may be used to achieve this goal, if coupled with iron hematoxylin for the permanent stained smear; however, for laboratories desiring to maintain the trichrome stain, SAF is not a valid option, as poor-quality results have been documented with this combination.

### Specimen transportation, storage and processing

Use aseptic technique. Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags, with the exception of Sellotape slide/perianal swab for *E. vermicularis* ova which should be transported in a sealed plastic bag [53]. In the case of CSF, any inoculated plates should also be transported in a robust, CE marked leak proof container. Compliance with postal, transport and storage regulations is essential. Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet [54].

Disposable gloves should be worn for all Parasitology investigations.

### Parasite diagnostic techniques

Epidemics among animals are still one of the main obstacles to the increase in production of animal protein and contact with vector-borne diseases often results in considerable losses [55]. The most reliable way to diagnose a parasitic infection is by detection and identification of the infecting organism. Laboratory diagnosis is a basic step in the evaluation of the disease process, at times confirming a presumptive diagnosis or providing evidence of an unsuspected agent of disease. However, failure to demonstrate or recover a parasite does not exclude the possibility of infection. Many of these parasites, especially the protozoa, can be identified only by microscopic examination. This requires considerable skill and apart from being time consuming and labour-intensive the method has limitations. This also applies to most of the commonly used serological techniques.

### Direct identification

Parasitic infections are usually diagnosed from samples of faeces, urine, blood and tissue.

**Faeces:** Evidence of intestinal parasitism, apart from the general clinical signs, is obtained from faecal or post-mortem examination. There is no "general" technique, nor is there an "ideal" technique for the microscopic examination of faeces. In fact, a reliable diagnosis can usually be made only by using a combination of several techniques [56], such as:



**Direct saline smear:** This procedure provides only an indication of the parasites present and cannot be used quantitatively. To prepare a direct faecal smear a drop of saline is placed in the centre of a microscope slide and a 2 mg faecal sample is suspended in this drop without spreading it. This is then covered with a cover slip and examined [57].

**Stained smears:** This type of smear is essential for accurate diagnostic detail and is also suitable for long-term storage for record purposes. The two stains generally used are haematoxylin and trichrome [56,57].

**Parasite concentration in faeces by flotation:** This is used for the identification of oocysts of coccidia [56] and helminth eggs. One drawback of this technique is that there is not always a direct relationship between the numbers of eggs in faeces and the number of parasites present.

**Urine:** Examination of urine sediment is used mainly for the identification of *Encephalitozoon cuniculi* and *Schistosoma* eggs [58].

**Blood:** Testing is used to identify the various stages of blood parasites and is routinely applied to diagnose malaria, theileriosis, babesiosis, anaplasmosis, ehrlichiosis, trypanosomiasis and most types of filariasis [56]. Trypanosoma can also be diagnosed with wet smears. Depending on the application and purpose, two types of blood films are used.

**Thin blood films** [57] are useful for studying morphological changes of blood cells and blood parasites. The main disadvantage is that sample volume is small, making the detection of low parasitemia and carrier animals difficult.

**Thick blood films** [58] contain 6 to 20 times as much blood per unit area as thin films. The thick film is suited for rapid diagnosis of parasitemia that is too low to be detected with thin films. This method is not suitable for detailed morphological studies of parasites.

**Tissue:** Recovery of protozoa or helminths from biopsy material is often an important aid to diagnosis. Lymph node, spleen, liver, lung, bone marrow or spinal fluid biopsies are frequently used to diagnose a variety of diseases [57].

**Post-mortem:** Post-mortem examination is currently the most effective way to accurately diagnose helminth infection [59]. Brain-cortex smears are examined for babesiosis (*Babesia bovis*), turning sickness (cerebral theileriosis) and cowdriosis.

### Indirect identification

All methods for the direct identification of parasites fail if the parasite density in the specimen is below the sensitivity of the method employed, or if the parasite cannot be directly demonstrated due to the life cycle in the host (e.g. toxoplasmosis, echinococcosis and cysticercosis). In such cases indirect methods must be used. Ideally, serology should allow differentiation between recent and latent infections and should be able to demonstrate whether an animal is carriers as well as the elimination of the parasite after therapeutic measures have

been applied. In practice, serology seldom achieves this. There is a lack of dependable tests for the sero-diagnosis of parasitic disease in animals. Commercially available tests present problems in reliability and interpretation of results. Costly, specialized apparatus is often needed to perform the tests [60]. With the majority of tests, specificity is not satisfactory and cross-reaction seldom allows distinction between closely related organisms. However, monoclonal antibodies may overcome this problem and enable the identification of highly specific antigen sites.

Tests commonly in use include the Complement Fixation Test (CFT), Immunodiffusion (ID), Indirect Haemagglutination (IHA), Indirect Immunofluorescent Antibody test (IFA), Enzyme-Linked Immunosorbent Assay (ELISA) and Radioimmunoassay (RIA) [61-63]. Less frequently used tests include latex agglutination, capillary agglutination and card agglutination [61-63]. Most of these tests are based on the reaction of antibodies with antigenic parasite components (whole or soluble) resulting in antigen-antibody complexes. These complexes are detected by the addition of antiglobulins coupled to fluorescein and rhodamine dyes, radioisotopes or enzymes. Because antibodies can persist for a long time after elimination of the parasites, another drawback of serology is that the demonstration of a specific antibody does not indicate the present parasitological status of the host. The results of a serological test are therefore retrospective. Sero-diagnosis of helminth infections is even more difficult because cross-reactivity is more the rule than the exception. Only highly purified, defined antigens allow sero-diagnosis to the genus level; species-specific sero-diagnosis is unusual [61-63]. Antigen-capturing ELISA can be used for demonstrating infection with trypanosomes and *Cowdria* for instance.

### Antigen

The application and reliability of serology is in many cases dependent on the availability of sufficient amounts of high quality antigen. The inability to culture many parasites *in vitro* and the lack of suitable animal models often hamper antigen preparation.

### Conclusion and recommendation

Immunodiagnostic and molecular methods will become increasingly important for the detection and identification of blood parasites. However, light microscopy will still be required for the foreseeable future, and hematologists should maintain their proficiency in the morphological diagnosis of the common blood parasites.

Based on the above conclusion the following points are forwarded:

- Proper diagnosis and treatment should be given for sick animal.
- Awareness should be given for farmers about parasitic infections by government or other responsible bodies.
- Young animal should be kept separately from older animal.



- Grazing on marshy area should be avoided or cutting or feeding strategy should be followed.
- Regular eradication of flies (Tsetse) and ticks must be exercised.
- Newly introduced animal to the flock should be dewormed.

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