Anthelmintic resistance can be described as a heritable change in the ability of individual parasites to survive the recommended therapeutic dose of an anthelmintic drug. According to Prichard et al. (1980) anthelmintic resistance is present when there is a greater frequency of individuals within a population that are able to tolerate doses of a drug than in normal population of the same species. Tolerance describes a situation where a population of worms, previously unexposed to an anthelmintic, is not removed completely by it. Resistance describes a situation where a population of nematodes, originally sensitive to an anthelmintic, inherits the ability to survive treatment after repeated exposure to the drug (Taylor and Hunt, 1989). When a nematode becomes resistant to one product of a class of compounds, it becomes resistant to all of them. Where anthelmintic resistance nematodes occur, there is invariably a reduction in anthelmintic efficacy. Resistance varies from farm to farm based on anthelmintic used, frequency of use, pasture stocking rates, use of closed herd, plane of nutrition and use of bio-security, etc. Hence, a product that works on one farm may not be effective on another farm for reasons stated above.

Development of anthelmintic resistance

Frequent use of the same class of anthelmintics and underdosing are reported to be the main factors responsible for development of anthelmintic resistance in worm population of livestock. Use of a sub-therapeutic dose of anthelmintic may permit survival of some worms showing partial resistance to that drug. When these partially resistant worms interbreed they may produce offspring that are more resistant to the anthelmintic. It has been identified that there is a difference in anthelmintic metabolism between sheep and goats, which indicates that if goats receive the same therapeutic dose as sheep, under dosing occurs and the drug may fail to control a susceptible population of nematodes leading to development of resistance in goats. The general rule of thumb is that goats need twice the anthelmintic dosage (concentration on a body weight basis) as sheep or cattle. It appears that resistance develops first in goats and then spreads into sheep flocks (Sangster and Gill, 1999).
Poor administration of oral drenches also lead to development of resistance. Administration of oral drenches into the mouth rather than over the back of the tongue may stimulate oesophageal groove closure which can lead to the drug bypassing the rumen and thus reduce drug uptake and efficacy. The rate of movement of digesta and drug through the gastrointestinal tract can also influence drug uptake and efficacy. Hence administration of some anthelmintic drenches after feeding impairs drug levels and only reduced levels of drug are absorbed.

**Drenches (Anthelmintics)**

When anthelmintics are divided by mechanism of action, there are three classes of modern broad-spectrum anthelmintics available for use in small ruminants. These classes are benzimidazoles, imidazothiazoles, and macrocyclic lactones. The white drenches (group 1 - benzimidazoles /probenzimidazoles) affect the normal structure and function of the worm cells that leads eventually to the starvation of the worm. Although drugs within the group II (clear drenches - levamisoles/morantels) and group III families (macrocyclic lactones or ivermectins) both cause paralysis in worms the two drug families have different muscle and nerve targets within the worm. The usefulness of any anthelmintic is limited by the intrinsic efficacy of the drug itself, its mechanism of action, its pharmacokinetic properties, characteristics of the host animal (e.g., operation of the oesophageal groove reflex), and characteristics of the parasite (e.g., its location in the body, its degree of hypobiosis, or whether it has developed anthelmintic resistance). The “ideal” anthelmintic should have a broad spectrum of activity against mature and immature parasites (including hypobiotic larvae), be easy to administer, inhibit reinfection for extended periods of time, have a wide margin of safety and be compatible with other compounds, not require long withholding periods because of residue(s), and also be cost effective.

**Detection of Resistance**

Anthelmintic resistance is determined by a fecal egg count reduction test (FECRT). Animals are weighed and treated with the anthelmintics and fecal egg counts are conducted 10 days after treatment. If the anthelmintic kills 90 percent or more of the worm eggs, it is considered to be effective. If it kills 60 to 90 percent of worm eggs, it is considered to have a moderate level of resistance. Anthelmintics killing less than 60
percent of worm eggs are considered to have severe resistance. A wide range of *In-vitro* assays have been developed to detect resistance.

**Management of Resistance**

**Dosage** It is critically important that all members of the flock being treated receive the full therapeutic dose recommended by the manufacturer. If the stock is overdosed, only the most resistant worms survive. On the other hand, if the stock is underdosed, heterozygotes for resistance will survive, leading to development of resistance. To avoid underdosing, it has been suggested that a representative group of animals from the same age or age class is weighed to produce an accurate therapeutic dose. The dose should be aimed at the heaviest sheep in any one age or sex class (Coles, 1986 and Coop, 1991).

**Strategic drenching** aims to reduce the number of treatments by timing the treatment to complement the environmental control of parasites. Strategic deworming will help to control parasite burdens in the animals and on pastures. Treatment at strategic times (August, November, and February) and movement to pastures with low numbers of infective larvae will control *H. contortus*. The most important time to deworm a sheep or goat is prior to lambing/kidding. This will help to prevent the "periparturient rise" in worm eggs that generally occurs around lambing/kidding time. It will also reduce the number of eggs that the ewe/doe sheds into her environment that could potentially infect her newborn lambs/kids. Other strategic times to treat with anthelmintics is prior to moving animals to a safe or "cleaner" pasture, at the start of the grazing season in monsoon seasons when the grass first starts to green up and when worm larvae numbers are typically the highest. Anthelmintic treatments should be targeted to the most susceptible animals in the flock. This would include lambs/kids, lactating ewes/does, and high producers. Leaving some animals untreated and focusing treatments on susceptible animals will slow down resistance. The maintenance of worm populations *in refugia* (not exposed to anthelmintics) is one of the strategies for slowing the development of anthelmintic resistance. The recommended tactic is to leave some sheep undrenched, as whole or part flocks, when routine strategic treatments are given, to reduce the intensity of selection for drench resistance in environments where there is a high risk (Van Wyk, 2001).
Tactical drench refers to use of anthelmintics at a time when most of the total worm population is within the host and not on the pasture, such as when livestock are moved from a contaminated pasture to a parasite free or nearly free pasture. Treatments when weather conditions have been favorable for the transmission of parasites, eliminates worms from the gastrointestinal tract before they have the opportunity to reproduce and further contaminate the environment. The timing of tactical deworming may be based on increasing fecal egg counts.

Smart drenching Anthelmintics should be administered orally, over the tongue of the animal. Research has shown that benzimidazoles are more effective when the animals are fasted 12 to 24 hours before treatment or when two treatments are given 12 hours apart. Goats metabolize anthelmintics differently (it clears their system faster) than sheep and cattle and require higher doses. Administering divided doses has also been shown to increase efficacy against benzimidazole resistant populations. In ruminants, the benzimidazoles are most effective if deposited directly into the rumen. Administration directly into the abomasum, via the esophageal groove, may shorten the duration for drug absorption and increase the rate of excretion in the feces, which may reduce efficacy. The rumen acts as a drug reservoir from which plasma concentrations can be sustained for long periods; it also slows the passage of unabsorbed drug through the GI tract. The absorption and excretion of levamisole is rapid and not affected by the route of administration or ruminal bypass because it is highly soluble (van Wyk, 2001 and Kaplan et al., 2004).

Alternative approaches to nematode parasite control The FAMACHA system is a system whereby the lower eyelid of the sheep is examined and treatment administered only if signs of anemia are present (Malan and Van Wyk, 1992). FAMACHA® is a technique developed in South Africa in which a colour eye chart depicting varying degrees of anemia is used to determine the need for anthelmintic treatment. It was developed as a tool for anthelmintics resistance management and integrated parasite management. It only works for the barber pole worm. It was developed for sheep, but should work with goats with slight modifications. The tool is a simple chart that allows individuals to determine the degree of anemia by comparing the color of an animal’s ocular mucous membranes to colors of eyes on a chart. The level of anemia is a telltale sign of the degree of parasitic infection with *H contortus* (van Wyk and Bath, 2002).
The FAMACHA© technique reduces the number of treatments because only animals showing physical signs of infection are dewormed. It identifies worm susceptible animals for culling and slows anthelmintics resistance, as worms have less exposure to the drugs. Only those sheep found to have pale mucous membranes, and where subsequent determinations showed haematocrit of 15% or below, were drenched to prevent death. This system not only saves anthelmintic usage, but animals could also be identified for culling. Such an approach dramatically reduces the selection for anthelmintic resistant parasite populations, as nearly three quarters of the flock remained untreated, with the result that their worm populations were not exposed to drug selection. The FAMACHA system can be used to control *H. contortus* throughout its endemic region where worm control is troubled owing to prevalence of anthelmintic resistance (Kaplan et al., 2004).

References


IMPROVED PARASITOLOGICAL TECHNIQUES FOR DIAGNOSIS OF
HAEMOPROTOZOAN AND RICKETTSIAL DISEASES

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Diagnosis remains the cornerstone for control of diseases and accurate
diagnosis is a prerequisite for successful treatment of diseases. The old adage
says, “Correct diagnosis is half cure”. Haemoprotozoan and rickettsial diseases
cause mortality and morbidity in livestock and companion animals. Among the
haemoprotistan diseases, theileriosis, babesiosis, trypanosomiosis,
anaplasmosis and ehrlichiosis are important. Demonstration of parasites in the
appropriate clinical material is important to make a correct diagnosis. This paper
focuses attention on new and standard techniques that are employed for
diagnosis of haemoprotozoan and rickettsial diseases of animals.

Trypanosomiosis

In the acute phases of infection, the parasite can readily be observed by
microscopic examination of a wet-mount or stained blood films under a
microscope. *T. evansi* is a motile organism that can be easily seen under X40
objective in wet films. Thin blood films stained with Giemsa, Leishman or Wright
are usually employed for detecting trypanosomes, but in thin fixed blood films,
the parasites may be hard to demonstrate in cases where parasitaemia is low.
The usual cryptic form of the infection of bovines cannot be detected in thin
smears. In such cases, thick blood smears are 120 times more successful than
thin films as they make possible examination of a larger volume of blood. Dry
thick films are stained with 0.5% aqueous methylene blue for 1 second, then
lysed in distilled water, fixed in methyl alcohol and stained with Giemsa stain.
Micro haematocrit centrifugation technique (MHC) is a useful test for detecting
various trypanosomal infections including *T. evansi*. Stained lymph node smears
are also equally good for diagnosis. In chronic infections, the parasites localize in
the microcirculation of the lymph nodes and in other capillary beds, allowing
diagnosis by examination of lymph node smears.
**Animal Inoculation tests:** Albino mice and rats are most suitable hosts while guinea pigs and rabbits are less suitable for detecting sub clinical and latent infections of Surra. In white mice, 0.5 ml of suspected blood with an anticoagulant is inoculated intra-peritoneally while 2-4 ml of blood is inoculated in other experimental animals. In mice, trypanosomes appear in its blood 2-3 days after inoculation while in other animals, 7-8 days are required for parasitaemia. Peripheral blood collected from the infected experimental animals are either seen as wet mount films or stained and examined for the presence of parasites. The animal inoculation test is more reliable than the direct microscope infection in chronic and sub clinical cases. It is useful in the detection of latent infection of bovines and cryptic infection of goats.

**Babesiosis**

Demonstration of babesia in peripheral blood still remains the best method. In haemoproteozoa diseases and babesiosis in particular, the parasites are trapped in the small capillaries (sludging) and therefore, the first drop of capillary blood may contain more parasites than the succeeding drops. Hence, whenever blood is collected from the auricular vein for making a blood smear, it is imperative that the first drop of capillary blood is not missed. By using 18 mm² cover slips rather than slides to smear the blood, homogenous thin films can be made. Efficiency is more than doubled by using a 40X instead of a 100X oil immersion lens. The sensitivity of a thin film examination therefore ranges between 10⁻⁵ to 10⁻⁶. Thick films are needed for detection of *Babesia* when parasitaemia is usually lower than 10. The procedure should be standardized by using a wire loop of 2.5 mm external diameter (30 gauge wire twisted around a 14 gauge needle). The loop will deliver about 1.4 μl of blood containing up to 9 X 10 RBC’s. The blood should be spread within a circle of 6mm. The air-dried thick film is fixed at 100 °C for 15 minutes before staining with 8% Giemsa solution in buffer. The sensitivity of thick film examination ranges from 1 to 10 parasites/μl of blood.
Tropical Bovine Theileriosis

Microscopic detection of schizonts and piroplasms of *Theileria sp.* in stained smears is important in the detection of the parasite associated with clinical disease. Examination of thin blood smear is a remarkably sensitive technique, where a single parasite can also be detected. In early *T. annulata* infections, where schizonts are actively multiplying in lymph nodes, the intra-erythrocytic piroplasms may not be seen in the peripheral blood smear but in the advanced stages of the disease, only the intraerythrocytic piroplasms are seen. Therefore, clinical theileriosis should be diagnosed in the laboratory, preferably by examination of lymph node smears for the presence of schizonts. However, if blood smears are examined, the percentage of piroplasm parasitaemia should be taken into consideration.

Clinical laboratory reports should preferably indicate the percentage of piroplasm parasitaemia in the blood smear to rule out past infections. A laboratory report may indicate the presence of *Theileria annulata piroplasms*, whose number might have been very few and the clinician invariably concludes that the parasite mentioned in the report, could be the cause of the clinical condition. The piroplasm parasitaemia and not the mere presence of few parasites in the blood are suggestive of a clinical disease. Animals, which are in enzootic areas are bound to show few parasites in their peripheral blood, but may not be suffering from Theileriosis (enzootic stability) and animals that are in a state of premunity following recovery from infection, may also show few parasites in their peripheral blood. Hence, it is erroneous to presume that the animal is suffering from Theileriosis, based on the laboratory report, which might have indicated their existence.

Canine ehrlichiosis
There are no pathognomonic signs of infection and confirmatory diagnosis of suspected cases is based upon demonstration of typical morulae in the cytoplasm of leukocytes. Careful and systematic examination of stained peripheral blood smears may reveal the presence of *Ehrlichia canis* morulae or granules in the cytoplasm of monocytes. Morulae are seen 11 to 20 days post infection, after the first rise in temperature. Examination of blood smears may reveal a relative abundance of monocytes, which are large, and irregular in shape with prominent vesicular nuclei and a foamy vacuolated cytoplasm. There may be evidence of reduced numbers of platelets and increase in the size of the platelets or platelets with densely stained centers and clear ragged peripheries. Concentrations of leucocytes, such as from the buffy coat, may be stained, but results are unreliable.

When examining blood smears, *E. canis* should be differentiated from phagocytozed *Babesia* parasites and bacteria, clusters of blood platelets, *H. canis, and L. donovani*, stain deposits or artefacts superimposed on white blood cells and nuclear materials. The parasite may also be demonstrated in smears of lung, lymph node and spleen aspirates of infected animals. Thrombocytopenia is the most consistent haematologic abnormality in both the acute and chronic stage of ehrlichiosis. Diagnosis of subclinical disease should be based on anamnesis, geographic location of the dog, and persistent antibody titers to *E. canis*, mild thrombocytopenia and hypergammaglobulinemia. The chronic disease is the end-stage of the disease process and its diagnosis is based on the anamnesis, the typical severe pancytopenia, antibody titers to *E. canis* and serum hypergammaglobulinemia and a lack of response to antibiotic therapy. This stage is usually easier to diagnose.

**Anaplasmosis**

Routine diagnosis of anaplasmosis is done by microscopic examination of thin blood smears stained with Giemsa or Leishman. This procedure, although convenient, becomes less reliable when the percentage of parasitized
erythrocytes is low and where artifacts may be mistaken for *Anaplasma marginale*. Use of Acridine orange (AO) stain can improve detection.

**Quantitative Buffy Coat Technique (QBC)**

The use of the Quantitative Buffy Coat (QBC) for diagnosis of haemoparasites has become the order of the day. QBC can detect very low parasitaemia. Venous blood is collected in a specially designed capillary tube containing potassium oxalate and Acridine orange stain. Following centrifugation in a microhaematocrit centrifuge, the interface between the buffy coat and erythrocyte layers is examined using a fluorescent microscope to detect stained organisms.

**References**


