DIAGNOSTIC METHODS IN PROTOZOLOGY

Faeces or Intestinal Contents

Where motile organisms are to be searched for (e.g. Hexamita, trichomonas etc), material should be examine as soon as possible since the organisms lose their motility in cold material. The preparation should be kept warm until examination and a simple smear is made, mixing the specimen with warm saline. Direct examination is useful for the detection of motile organism, and the use of a phase contrast or dark field microscope greatly facilitate this.

Intestinal contents or faeces may need to be stained for more accurate identification of intestinal protozoa. Cover glass preparations are the most suitable and these should be coated with albumen fixative to facilitate adhere of faeces etc to the glass. A thin layer of faeces or intestinal contents is spread on the cover glass, and this is fixed before it is allowed to drip. The most satisfactory fixative is Schadinn's fluid and cover glasses should be gently dropped into this or floated on it, smear down, and left for about 10 - 15 minutes.

Blood or Tissue Fluids

Wet blood smear can be examined for living trypanosomes, the search for organisms been greatly facilitated by phase contrast or dark field illumination. Blood smears should be stained by one of the Romanowsly stains (methylene blue-eosin combination). Where organisms are plentiful, a thin blood smear is satisfactory examination.

Slide should be absolutely clean and the blood smear should be absolutely clean and the blood smear should be spread evenly and thinly. Thick blood smears cannot be used with avian or camel blood because of the nucleated erythrocytes. Thick blood smears need to be dehaemoglobinized before staining and this may be done by placing them in distilled water until the colour has disappeared. This smear or dehaemoglobinized smears are fixed in absolute methanol and may then be stained by Leishman, Giemsa, Wright's or Field's stain.

Tissue

Usually, the most satisfactory method of examination of tissue is for sections to be cut and stained with haematoxylin and eosin, Giemsa or other appropriate stain. Frozen sections provide a rapid means of examination. A diagnosis may often be made by mixing a scraping, or a small sample, of the tissue with a little saline and examining the preparation in the fresh state (e.g. schizonts of coccidia, toxoplasma, pseudocysts, sarcosporidia etc). Such preparation may also be stained to achieve a more critical examination.

TREATMENT AND CONTROL OF PARASITES

Arthropods

Although ticks/insects are in themselves important parasites, and should be combated for this reason, control measures are, as a rule, directed against the disease of which the ticks are the vectors and therefore based on the epizootiology of these diseases as well as on the habits of the ticks/insects.

Because ticks attach to various parts of the bodies of animals, treatment has to be applied to the whole body and may be carried out by dipping the animals in a suitable tank containing the dip in an aqueous solution, suspension or emulsion, however, spray races, showers etc are replacing conventional plunge dips since they are labor-saving and economical. In some cases ticks attached to the legs and under-sides of the bodies of animals and consequently shallow dips, through which hosts are made to walk, may be sufficient to give control.

These modern forms of apparatus drain the dipping fluid and filter it after it has been sprayed on the animals and return it for use again. The various stages of ticks may stay on their host for only a few days during each year and are often on the hosts only at certain times of the year.

Dipping for control of ticks/insects is therefore planned with knowledge of the biology of each species of tick, the duration of each of its stages and its feeding times and the duration of the whole life-history. An important consideration is whether the tick is one-host tick, a two-host species which uses one individual host for the larvae tick, each stages of which require a separate individual host. The one-host tick is obviously much easier to control than the other. Acaricides/Insecticides may act differently on the different stages of the life-cycle.

Other measure which have more limited value, are useful more specially against two-and threehost ticks, which spend relatively long period of their lives off the host and on the pastures. These methods include:

- 1. Burning of pasture, this may kill large number of the larvae and other stages, especially if it is done during the times of the year when these stages may be expected to be off the hosts.
- 2. Cultivation of land: this undoubtedly tend to reduce tick life by controlling the movement of domestic and wild animals, as well as by creating conditions unsuitable for ticks, for instance exposing of eggs to sunlight, or burying them decreasing the humidity on which the tick depend.
- 3. Repellant may be useful in certain circumstances

Tick control on your pet

Keeping pets out of grasses and woods helps to reduce their exposure to ticks. But any animal outside can quite easily have a tick crawl on board. Products that kill and repel ticks are needed.

Once-a-month Topicals: Once-a-month topical insecticides which are applied to the back of the pet, are probably the easiest product to use, and generally, last the longest. Some kill fleas and ticks, and others just fleas, so check the label carefully. Ingredients generally include permethrin, pyrethrin, imidacloprid, or fipronil.

Sprays: Flea and tick control sprays can come as aerosols or pump bottles. When using a spray, you do not have to soak the pet with the spray, but be sure to spray all parts of the animal. Spray a small amount on a cotton ball to apply the product around the eyes and ears. Do not get any of these products in the eyes.

Powders: Powders are generally easy to apply but can create a mess. If you or your pet has asthma, powders may not be the best choice of product since the powder could be inhaled. Be sure to use powders in well-ventilated areas. Powders often contain pyrethrin.

Dips: Dips and rinses are applied to the entire animal. They generally have some residual activity. It is helpful to put cotton balls in the pet's ears and ophthalmic ointment in the pet's eyes. Even with these precautions, be very careful not to get any of the product in the pet's ears or eyes. Dips and rinses may contain permethrin, pyrethrin, or organophosphates.

Shampoos: Shampoos help to primarily rid the pet of the ticks it already has on it, although some have residual activity. To properly use a flea & tick shampoo you must be sure to work the shampoo in over the entire body and then leave it on at least 10 minutes before you rinse it off. This is true of almost any medicated shampoo. Again, remember to protect the eyes and ears of the pet.

Collars: Collars can be effective, but must be applied properly. To get the right degree of snugness, you should just be able to get two fingers between the collar and the neck of your pet. Be sure to cut off any excess portion of the collar after you have properly applied it. Otherwise, that animal or other pets may try to chew on the end.

<u>Helminth</u>

Differences in epidemiology under different climatic conditions require different approaches for control. Control based on management incorporates the knowledge of the life-cycle, larvae ecology and epidemiology of the parasites. It is influenced by grazing management provision of clean pastures alternate by immunologically resistant hosts of the same species, stocking rate, and timing of reproductive events. Control based on management factor will be aided by the

strategic use of anthelmintic. Alternatively, particularly where permanent pastures are utilized, formers may rely solely on anthelmintics for control, treatments being given as often as every three to four weeks, but this is likely to be uneconomical.Intermediate host of the trematode could be removed to break the life cycle of the parasites.

The principle of a parasite control is to keep the challenge to young livestock by the pathogenic parasites at a minimum rate. This is achieved in the following ways.

(a) Controlling the density of livestock (stocking rate). Overstocking forces the animals to graze closer to faecal material and closer to the ground, and may result in the consumption of a higher number of infective larvae.

(b) Periodic deworming.

(c) Strategic deworming when conditions are most favorable for larval development on the pasture.

(d) Separating age groups in the more intensive production systems.

(e) Reducing the effects of gastro-intestinal parasites by ensuring an adequate plane of nutrition.

(f) Using grazing management to minimize the uptake of infective larvae and to create safe pastures.

The development of such programme requires a thorough knowledge of the types of parasites present (including their biology and epidemiology), herd structure and grazing management, parasite seasonal availability and survival and the weather conditions in particular areas.

Control of gastro-intestinal nematodes

The ideal approach is an integration of:

- · adjusting stocking rate
- · optimum use of safe pastures
- · strategic use of anthelmintics
- · use of resistant breeds or genotypes

Overstocking is a major problem in large parts of the world particularly in Africa outside the tsetse-infested areas. In addition to contributing to pasture degradation and soil erosion in certain marginal areas, it also forces the animal to graze closer to faecal material which inevitably results in the uptake of higher number of infective larvae. Reducing the stocking rate can significantly reduce the parasite burden of grazing livestock.

Improving grazing management and introducing the safe pasture concept can reduce the use of anthelmintics, minimizing the risk of developing anthelmintic resistance. Ungrazed pastures are parasitologically safe at the end of a prolonged period of dry weather (10 weeks or more). Other types of safe pasture are those used for hay/silage production and those previously grazed by other species. In some countries safe pastures are created by letting cattle graze pasture first, and following with sheep/goats. Grazing different species of livestock together may reduce the overall parasite burden of the species in question but this will not usually be sufficient for efficient parasite control. Fields of harvested cereal crops are also safe. If safe pastures are available, treat young stock with an anthelmintic at the onset of the rains and place them on the safe pastures entirely separated from the older animals.

Based on the seasonality of development and survival of (L_3) on the pasture, the timing of strategic anthelmintic use can be determined and integrated into control programmes

Anthelmintics

An anthelmintic is a compound which destroys or removes helminths from the gastro-intestinal tract and other tissues and organs they may occupy in their hosts.

Currently a good selection of safe anthelmintics is available, some with broad spectrum activity and others with activity against specific helminth infections. Many modern anthelmintics are effective against both adults and larval stages and an increasing number are efficacious against arrested or dormant larvae.

Due to their cost and their tendency to delay or interfere with natural host immunity mechanisms, anthelmintics may not be the most desirable method of managing helminth problems. However, in many circumstances the sensible use of anthelmintic drugs is likely to be the only available method of controlling helminth parasites. They should not be used indiscriminately.

Characteristics and selection of anthelmintics

The ideal anthelmintic has the following properties:

(a) A broad spectrum activity against adult and larval helminth parasites.

A number of factors influence the efficacy of an anthelmintic drug. Animals often harbour several different species of helminths, which may not have the same sensitivity to a given anthelmintic. In addition, there is usually a difference in sensitivity between adults and larval stages, with immature stages being less sensitive than the adult parasites.

Very few if any of the anthelmintics are completely effective at the recommended doses under field conditions. Some anthelmintics may be very effective in sheep but not in cattle, or *vice versa*.

(b) A rapid metabolism in the body and short-lived presence at low levels in the milk and/or tissues.

Animals should not be slaughtered for human consumption and milk not distributed to consumers until the drug residues have reached acceptably low levels. The withdrawal period of the drug should be considered before its use.

(c) A low toxicity in the target species. The ratio of the therapeutic dose to the maximum tolerated dose should be as large as possible.

It is desirable that an anthelmintic has a safety margin of at least six-fold.

(d) No unpleasant side-effects to the animal or to the operator.

Drugs may cause vomiting, or pain at the injection site. Some drugs irritate the skin of humans.

(e) Suitable for practical and economical integration into various management systems.

The selected drug(s) should be competitively priced and ready to use in a simple way. They should be stable and not decompose on exposure to normal ranges of temperature, light and humidity, and have a long shelf life.

Administration of anthelmintics

It is important to first identify the nature of the parasitic problem in order to select the appropriate drug to treat the infection. The optimal time and mode of administration of the drug should then be considered.

A wide variety of formulations and preparations have been developed to provide methods of dosing animals, which are convenient for a wide range of species and circumstances.

Dosing by mouth

The majority of anthelmintics are given by mouth as liquid preparations, pastes, boluses and tablets.

Liquid preparations are usually sold ready to use. Several devices such as syringes, bottles and drenching guns can be used for delivering the dose. Drenching guns are generally preferable and a wide variety, including single dose, multi-dose and automatic types, are available. It is important to keep the drenching equipment clean after use. The dose to be delivered should be checked before-and several times during-dosing to ensure that the correct dose is given to all animals. A graduated cylinder should be included in the field equipment for calibration purposes. It may be necessary to fit a short piece of rubber tubing on the end of the dosing nozzle to protect the mouth and pharynx of dosed animals.

Pastes are relatively easy to administer if a proper dispenser is available. If that is not the case, care should be taken to ensure the animal receives a full dose.

Boluses and tablets can be placed deep in the mouth of the animal by using a dosing gun or a pair of long-handled forceps, both of which can be manufactured locally. Bolus and tablet formulations have the advantage that if the dose is rejected, it is usually the total dose and a replacement can then be administered.

Prolonged protection of grazing livestock can be achieved by incorporating anthelmintics into medicated salt-molasses blocks and prepared mineral mixes, but animals do not always consume the amount required for an efficient treatment. Controlled-release preparations, such as slow release boluses allow the effective delivery of anthelmintics over several months.

Dosing by injection

A number of anthelmintics are available for injection. The size of needles should be appropriate for the formulation and the site of injection. In order to avoid local reactions (such as abscess formation at the injection site) the highest possible hygienic standards should be maintained.

Dosing by external application

Several dewormers are now available in a formulation for external application, termed "pouron" preparations. The active ingredient of the drug is absorbed through the skin reaching its target via the circulatory system. This application form, which is particularly convenient for animals kept under range conditions, has the advantage that only minimum restraint of animals is needed, as the dose is applied to their back while passing through a crush or standing at a feeding trough.