The Epidemiology, Diagnosis and Control of

GASTRO-INTESTINAL PARASITES OF RUMINANTS IN AFRICA

Jørgen Hansen and Brian Perry
THE EPIDEMIOLOGY, DIAGNOSIS AND CONTROL OF GASTRO-INTESTINAL PARASITES OF RUMINANTS IN AFRICA

A Handbook

Jørgen Hansen, DVM, PhD
Assistant Professor of Parasitology
Department of Pathobiology
Virginia Maryland Regional College of Veterinary Medicine
Virginia Polytechnic Institute and State University
Blacksburg, Virginia

Brian Perry, BVM&S, DTVM, MSc, DVM&S, MRCVS
Head, Epidemiology and Socioeconomics Unit
International Laboratory for Research on Animal Diseases
Nairobi, Kenya
Formerly Associate Professor of Veterinary Epidemiology
Virginia-Maryland Regional College of Veterinary Medicine

International Laboratory for Research on Animal Diseases
FOREWORD

Gastro-intestinal parasites of ruminants are ubiquitous in Africa, where many environments provide near-perfect conditions for their survival and development. Although these parasites are widely prevalent, the clinical signs they cause in infected animals can be less obvious than signs of other livestock diseases. Partly for this reason, gastro-intestinal parasites are one of the most neglected areas of veterinary care in much of Africa. This handbook has been written to help redress this imbalance.

The handbook, in a simple style, reviews the epidemiology of gastro-intestinal parasites of ruminants in sub-Saharan Africa, and presents procedures and techniques for their diagnosis, survey and control. The book is designed for routine use in all types of animal health institutions, including universities, research institutes and field laboratories where diagnostic parasitology is performed. It is hoped that the widespread availability of this handbook will help to improve and standardize diagnostic capabilities, as well as contribute to the collection and use of basic epidemiological data, the foundation for effective disease control programmes.

It is clear that in order to optimise the benefits from improved control of protozoan diseases, the main focus of ILRAD's research, it will be of paramount importance to address the problems presented by other endemic livestock diseases in Africa, including those caused by gastro-intestinal parasites. ILRAD is therefore very pleased to be associated with the production of this handbook.

A.R. Gray MA, VetMB, PhD, DSc, MRCVS
Director General
ILRAD
PREFACE

Intestinal parasites are found in cattle, sheep and goats in all the countries and regions of Africa. These parasites are commonly associated with poor production and unthriftiness and can produce acute disease and even death. Their presence in an animal, however, does not mean that they are necessarily the cause of any overt disease in that animal, so it is important to assess the type and level of parasitism in a herd or flock in order to be able to determine the significance of parasite infections and to recommend the most cost-beneficial control measures.

We were stimulated to prepare this handbook for several reasons. We have both lived and worked in various parts of Africa for several years, where we became conscious that gastro-intestinal parasites play a controversial role on the continent. Sometimes their presence and identity are confirmed, after which they erroneously receive the blame for the ill health of an animal or a herd; sometimes they are identified but are perceived rightly or wrongly as unimportant and are ignored; and many times, of course, their presence and identity remain unknown. Superimposed on this is a system of parasite diagnosis in use over much of the continent that places great emphasis on parasite identification procedures at the expense of quantitative and economic assessments of infections. In the few circumstances where quantitative assessments are made, they are usually confined to parasite egg counts, which have severe limitations when carried out in isolation.

Most of the procedures for the diagnosis of gastro-intestinal parasites are simple and require simple equipment. Much of this equipment is available in even the smallest of veterinary diagnostic laboratories in Africa. This handbook therefore presents procedures and techniques for the identification, diagnosis, survey and control of intestinal parasites of ruminants in sub-Saharan Africa that can be applied in a variety of circumstances and institutions, including universities, national laboratories and the more rudimentary veterinary outposts. The epidemiology of gastro-intestinal parasites is reviewed, and procedures for their diagnosis are given in a simple cookbook style. Guidelines for the design and interpretation of field investigations, as well as the principles of control based on the results of such investigations, are presented. We hope, therefore, that the handbook will have a wide application on the continent.

We are grateful to the many people who helped us translate our ideas into reality. The original preparation of photographs of diagnostic techniques was supported by a Title XII grant from the Office of International Development, Virginia Polytechnic Institute and State University. We are grateful to Ms. Lee Bishop and Ms. Derry Hutt for the assistance in the preparation.
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BDP
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1 INITIAL SURVEYS FOR DETERMINING THE PARASITE SPECIES PRESENT

1.1 INTRODUCTION

As a first step in the investigation of gastro-intestinal parasitism of ruminants, it is important to establish what parasite species are present in an area. This may already be well documented, in which case this step is not necessary. However, the dominant parasites in an area can change, particularly as livestock management practices change, so existing parasite inventories as well as distribution data based on old studies may require updating.

Initial surveys should be kept extremely simple. They are intended to identify parasites present rather than to determine their importance, a later procedure.

One of the difficulties in identifying gastro-intestinal parasites of ruminants is that many of the intestinal nematodes look alike. They can, however, be identified down to a species level by microscopical examination. In addition, most of them live in specific sites in the intestinal tract, which helps in the identification process. Since different species have different pathogenic effects, it is important to know which broad groups are present in a herd or area. Furthermore, some of these parasites have very different development times, both outside and inside the host, a knowledge of which is important for effective control measures.

1.2 PARASITE GROUPINGS

Gastro-intestinal parasites can be classified into four broad groups.

1.2.1 Nematodes

*Haemonchus*
*Ostertagia*
*Trichostrongylus*
*Cooperia*
*Nematodirus*
*Bunostomum* (hookworms)
*Strongyloides*
*Oesophagostomum*
*Chabertia ovina*
*Trichuris* (whipworms)
1.2.2 Cestodes

*Monezia*

1.2.3 Trematodes

*Paramphistomum*

1.2.4 Protozoa

Coccidia* (*Eimeria*)

1.3 IDENTIFICATION PROCEDURE

The identification of parasites present in an area can be carried out in the following two ways:

- a post-mortem examination of animals

  (a) that have died from acute or chronic diseases or
  (b) that have been slaughtered at a slaughterhouse/slaughter place, and

- the identification of parasite eggs present in faecal samples from live animals.

1.3.1 Post-mortem Examination

The entire gastro-intestinal tract (rumen to rectum) should be obtained from slaughterhouses/places, butchers, veterinary diagnostic centres, etc., for the purpose of calculating the total number of parasites present (see a description of this procedure in section 4.3), as well as identifying the species found. The most useful data will be acquired from young animals and those that have not recently been dewormed. This procedure should be performed towards the end of the rainy season.

1.3.1.1 Sites in the intestinal tract

Most gastro-intestinal parasites live in distinct sites of the intestinal tract. The location of parasites in the intestinal tract of different ruminant species is shown in Table 1.1. Parasites vary also in their geographical distribution

*Members of this family Eimeriidae are referred to here as Coccidia.
which depends particularly on climate (especially rainfall), vegetation and livestock density.

Table 1.1 LOCATION OF PARASITES IN THE INTESTINAL TRACT

<table>
<thead>
<tr>
<th>Site</th>
<th>Host species</th>
<th>Parasites</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen</td>
<td>Cattle</td>
<td>I'aratmiphistomn</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td>Abomasum</td>
<td>Cattle, sheep, goats</td>
<td>Haemonchus</td>
<td>Blood sucking</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ostertagia</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichostrongylus axei</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Cattle, sheep, goats</td>
<td>Trichostrongylus</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bunostomum</td>
<td>Blood sucking</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cooperia</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nema:oides</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strongyloides</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paramphistomum</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>larva</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coccidia</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monezia</td>
<td>Minimal</td>
</tr>
<tr>
<td>Large intestine</td>
<td>Cattle, sheep, goats</td>
<td>Trichuris</td>
<td>Blood sucking</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oesophagostomum</td>
<td>Mucosal damage, nodules</td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td>Coccidia</td>
<td>Mucosal damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chabertia ovina</td>
<td>Minimal</td>
</tr>
</tbody>
</table>

The identification of parasite species is described in Chapter 4.

1.3.2 Identification of Parasite Eggs in Faecal Samples from Live Animals

Fresh faecal samples should be taken from a small number of animals. These samples should be preferably taken towards the end of the rainy season from young animals and those that have not recently been dewormed.

Collected faecal samples should be subjected to a flotation procedure for separating and concentrating parasite eggs (see section 3.3) and examined microscopically. The eggs of some parasites are easy to differentiate. The following are examples of these parasites.
Trichuris

Strongyloides
Coccidia oocysts
The eggs of other parasites, however, are similar in size and structure and cannot easily be differentiated. These include the Trichostrongyles, *Oesophagostomum* and *Bunostomum*. To differentiate and identify these nematode eggs, faecal cultures may be set up for each faecal sample or group of samples (see section 3.5). Faecal cultures allow parasite eggs present in the faeces to develop into larvae. The third-stage larvae (L₃) are then isolated from the faecal cultures and used to identify definitively the parasites at the species or genus level. This is described in Chapter 3.
THE EPIDEMIOLOGY OF GASTRO-INTESTINAL PARASITES

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2.2.2 Egg Production
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2.4 TREMATODES

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2 THE EPIDEMIOLOGY OF GASTRO-INTESTINAL PARASITES

2.1 INTRODUCTION

This section is devoted primarily to the epidemiology of the nematodes, which are the intestinal parasites of greatest economic importance.

2.2 NEMATODES

2.2.1 Life Cycles

The most important and widely prevalent nematodes are the Trichostrongyle group (Haemonchus, Ostertagia, Trichostrongylus, Cooperia and Nematodirus), Bunostomum and Oesophagostomum. The life cycles of most Trichostrongyles, Oesophagostomum and Bunostomum are similar: the cycles are direct, that is, these nematodes do not require other animals to complete their life cycles.

FIGURE 2.1

The life cycle of gastro-intestinal nematodes

1. L₁ → L₂ → L₃ → Adult
   (Haemonchus sp., Trichuris)
2. L₁ → L₂ → L₃ → Adult
   (Ostertagia sp.)
3. L₁ → L₂ → L₃ → Adult
   (T. colubriforme, Cooperia sp., Nematodirus)

1-3 weeks
4-6 weeks
7-8 weeks
Adult nematodes inhabit the gastro-intestinal tract. Eggs produced by the female are passed out in the faeces. The eggs embryonate and hatch into first-stage larvae (L₁) which in turn moult into second-stage larvae (L₂), shedding their protective cuticle in the process. The L₂ larvae moult into third-stage larvae (L₃), but retain the cuticle from the previous moult. This double-cuticled L₃ is the infective stage of these nematodes. The time required for the eggs to develop into infective larvae depends on temperature. Under optimal conditions (high humidity and warm temperature), the developmental process requires about 7 to 10 days. In cooler temperatures the process may be prolonged. Ruminants are infected by ingesting the L₃. Most larvae are picked up during grazing and pass to the abomasum, or intestine, ex-sheathing the extra cuticle in the process. The L₃ of the Trichostrongyle group penetrate the mucous membrane (in the case of Haemonchus and Trichostrongylus) or enter the gastric glands (Ostertagia). During the next few days the L₃ moult to the fourth stage (L₄) and remain in the mucous membrane (or gastric glands) for about 10 to 14 days. They then emerge and moult into a young adult stage (L₅). Most Trichostrongyli mature and start egg production about 3 weeks after infection.

The parasitic part of the life cycle of Oesophagostomum requires about 6 weeks to complete. The infective L₃ penetrate the lamina propria of the intestinal wall of the host; the infection which surrounds the L₃ results in the formation of fibrous nodules. The larvae emerge into the lumen of the intestine after about 2 weeks and mature in the following 4 weeks. In animals previously infected, the larvae may spend a prolonged period of time (3-5 months) in the nodules. Eventually many of the larvae will die and the nodules may become calcified.

The L₃ larvae of Bunostomum infect ruminants when they are ingested or penetrate the host’s skin. The larvae are carried in the venous blood through the heart to the lungs, they penetrate the alveoli, are coughed up and then swallowed, and so pass to the small intestine. Here they moult and mature 8-9 weeks after infection.

The infective larval stage of Trichuris is contained within the egg. The larva is released after the egg is ingested by the host.

2.2.2 Egg Production

Adult female nematodes produce eggs. The period between the infection of an animal by ingestion of infective L₃ larvae and the first egg production by the adult female parasite is called the prepatent period. This period is different for different species of parasites, as shown in Table 2.1.
Table 2.1  PREPATENT PERIODS OF SOME GASTRO-INTESTINAL NEMATODES

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Prepatent period</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemonchus placei</em> (cattle)</td>
<td>3–4 weeks</td>
</tr>
<tr>
<td><em>Haemonchus contortus</em> (sheep)</td>
<td>2 weeks</td>
</tr>
<tr>
<td><em>Ostertagia</em> (sheep and cattle)</td>
<td>3 weeks</td>
</tr>
</tbody>
</table>

For most other gastro-intestinal parasites, the prepatent period is about 3–4 weeks.

Different species of nematodes have different egg-producing capacities. The individual female *Cooperia*, for example, produces many eggs but is not very pathogenic. Females of *Trichostrongylus* are quite pathogenic but produce few eggs. This means that the number of nematode eggs in a faecal sample is not an accurate indication of the amount of damage being done by a parasite.

The number of eggs produced by an adult female nematode will also depend on the level of immunity to the intestinal parasites the host possesses. In addition, adult female nematodes may increase their egg output around the time the host gives birth (parturition), especially in sheep and goats.

The number of eggs detected in the faeces also depends on the consistency of the faeces. Diarrhoeic faeces often contain lower numbers of eggs per gram than formed faeces, due to the effect of dilution.

In summary, the number of parasite eggs found in the faeces is influenced by:

- Number of adult parasites established in the gastro-intestinal tract
- Level of host immunity
- Age of the host
- Species of parasite
- Stage of infection
- Parturition
- Consistency of the faeces

2.2.3  Development and Survival of Infective Larvae in the Environment

The development of larvae in the environment depends upon warm temperature and adequate moisture. In most of tropical and sub-tropical Africa, temperatures are permanently favourable for larval development in the
environment. Exceptions to this are the highland and mountainous regions, such as parts of Ethiopia and Kenya, and the winters of southern Africa, where temperatures may fall below those favourable for the development of *Haemonchus* larvae.

The ideal temperature for larval development of many species in the microclimate of the tuft of grass or vegetation is between 22° and 26°C. Some parasite species will continue to develop at temperatures as low as 5°C, but at a much slower rate. Development can also occur at higher temperatures, even over 30°C, but larval mortality is high at these temperatures.

The ideal humidity for larval development in this microclimate is 100%; the minimum humidity required for development is about 85%.

The survival of larvae in the environment depends upon adequate moisture and shade. Desiccation from lack of rainfall kills eggs and larvae and is the most rapidly lethal of all climatic factors. Larvae may be protected from desiccation for a time by the crust of the faecal pat in which they lie or by migrating into the soil. The development of infective larvae ingested by an

**FIGURE 2.2**

*Survival and dissemination of larvae on pasture*

Protection of eggs and larvae from desiccation for several weeks by crust of faecal pat.

Vertical migration by larvae

Horizontal migration by larvae

Protection by migration to lower herbage and soil to avoid desiccation
animal during adverse environmental conditions may be temporarily arrested in the abomasal or intestinal mucosa. This suspension of development helps some nematode parasites survive the dry seasons. Of the three larval stages in the environment (L1, L2, and L3), it is the L1, which has a protective sheath, that is the most resistant to variations in moisture, temperature and sunlight.

2.2.4 The Dissemination of Infective Larvae

The parasite's eggs develop into third-stage, infective larvae (L3) in faecal material. To make themselves accessible to ingestion by ruminants, the larvae have to migrate or be transported from the faeces in which they were deposited on the ground to any nearby herbage. Such movement occurs in two ways: horizontal migration/transport and vertical migration/transport.

The horizontal distance L3 will actively migrate does not usually exceed 5-10 cm. Suitable conditions for larval migration occur when rainfall or moisture disintegrates the crust of faecal material and larvae in this material are washed onto herbage. Invertebrates such as dung beetles may also play a role in the transport of larvae onto herbage.

Once on the herbage, infective larvae migrate up and down blades of grass according to the amount of moisture on the grass. During rainfall and when dew is on the grass, larvae migrate to the top of the herbage. Following evaporation, the larvae migrate to the base of the herbage and even down into the soil. Heavy rain may wash larvae off the herbage and onto the ground. Larvae in water pools may infect drinking animals.

2.2.5 Effect of Climate on Survival and Development of Infective Larvae

The development and survival pattern of infective larvae in the environment differs according to the climate. Three broad types of climate are found in tropical and sub-tropical Africa:

- humid tropical climate
- savannah-type tropical and sub-tropical climate with a long dry season
- arid tropical climate

The humid tropical climate characterizes much of West Africa as well as the regions surrounding Lake Victoria and parts of coastal eastern Africa. This climate provides a more or less permanently favourable environment for the survival and development of parasitic larvae.

The savannah-type tropical and sub-tropical climate with a long dry season
is found in much of eastern, central and southern Africa. As the dry season progresses, the environment for larval development and survival changes from unfavourable to hostile, with populations of surviving larvae declining rapidly in open pastures and more slowly in wooded areas where ample shade is available. At the start of the rains, of course, this unfavourable environment is transformed rapidly into a favourable one for the larvae.

Arid tropical climate characterizes parts of lowland Ethiopia, parts of Somalia and Sudan and much of the Sahel. This climate, with its sparse vegetation cover, is often permanently unfavourable for parasitic larval survival. Where vegetation exists, however, short periods of rainfall or irrigation can transform the environment rapidly into a favourable one for the nematode larvae, particularly the highly pathogenic *Haemonchus*.

2.2.6 Factors Affecting the Size of Nematode Infections

The size of any gastro-intestinal nematode infection depends on the following five main factors:

- The number of infective larvae (L₃) ingested by the host, which in turn is influenced by the climate, the amount of protection of larvae provided by vegetation, the livestock density (i.e., pasture contamination level) and the grazing pattern of the ruminants present.

- The rate at which acquired resistance develops in the host, which is influenced by the species of the parasite and host, genetic factors, nutrition and physiological stress (e.g., parturition).

- The intrinsic multiplication rates of the species of parasites present which are controlled by the fecundity, pre-patent period and environmental development and survival rates of these species.

- Management, particularly grazing patterns.

- Use of anthelmintics, including the timing and frequency of administration.

2.2.7 Pathogenesis of Nematode Infections

2.2.7.1 Effect of larval stages on the host

Considerable damage is caused by fourth-stage larvae (L₄) of abomasal parasites (*Haemonchus*, *Ostertagia* and *T. axei*). The L₃ enter the mucous membrane or the glands in the wall of the abomasum within six hours of entering the host, and will usually stay in the mucous membrane or the
glands for about two to three weeks. If large numbers of *Haemonchus*, *Ostertagia* and *T. axei* larvae enter the abomasum, the host will be affected by:

- reduced appetite
- reduced digestive capability of the abomasum

The larvae of *Trichostrongylus* in the small intestine may cause severe damage to the intestinal mucous membrane with similar effects. Under certain circumstances, larvae ingested at the end of a rainy season (in savannah-type climates) may remain inhibited in the abomasal glands during the dry season until the next rainy season or until the animal experiences stress, such as that produced when the animal is calving or sick. The inhibition will then cease, and the L₄ will develop into an adult worm. This development may be accompanied by destruction of the mucous membrane, the extent of which depends on the numbers of inhibited larvae.

The L₄ of *Haemonchus* is a blood sucker in the abomasum. Animals infected with large numbers of larvae therefore may suffer from anaemia before the parasite eggs can be detected in the animal’s faeces.

2.2.7.2 Effect of adult worms on the host

Infections with gastro-intestinal nematodes usually involve several different species of parasites, which may have an additive pathogenic effect on the host. Mixed infections comprising *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Bunostomum*, *Cooperia*, *Nematodirus*, *Oesophagostomum* and *Trichuris* are common. The pathogenic effect of gastro-intestinal parasites may be sub-clinical or clinical. Young animals are most susceptible. The effect of these parasites is strongly dependent on the number of parasites and the nutritional status of the animals they are infecting. The following clinical signs may be seen:

- weight loss
- reduced carcass quality
- reduced wool production/quality
- reduced feed intake
- diarrhoea
- blood and protein losses to the gut
- mortality

Severe protein loss into the abomasum and intestine due to damage caused by the parasites often results in oedema in the submandibular region (a condition called bottle jaw). Some nematode species, especially those that suck blood, such as *Haemonchus*, *Bunostomum* and *Oesophagostomum*, are
responsible for specific clinical signs. *Haemonchus* is the most pathogenic of the blood suckers and infections with large numbers of this parasite often result in severe anaemia in the host. Diarrhoea may not be a feature of *Haemonchus* infections. Blood losses from *Bunostomum* and *Oesophagostomum* infections may add to the severity of the anaemia.

### 2.3 CESTODES

Species of the genera *Moniezia*, *Avitellina* and *Thysaniezia* have been described in cattle, sheep and goats. Of these, *Moniezia* are the most common. The life cycle of these tapeworms is indirect; herbage mites are the intermediate host. Ruminants become infected by ingesting herbage in which lie mites carrying the infective stage of the parasite. Lambs, kids and calves under six months of age are most susceptible to infection. Light to moderate infections are considered non-pathogenic. Heavy infections have been reported to cause poor growth and diarrhoea in lambs.

### 2.4 TREMATODES

A number of trematode species have been described from the rumen and reticulum of cattle, sheep and goats. Of which *Paramphistomum cervi* is the most common. The life cycle of this species is indirect; a water snail is the intermediate host. The final host becomes infected by ingesting the infective stage with the herbage. The young immature paramphistomes attach themselves to the mucous membrane of the duodenum. The adult parasites are found attached to the mucous membrane of the rumen. Large numbers of immature stages in the duodenum may cause destruction of the mucous membrane resulting in diarrhoea. The mature parasites in the rumen are considered non-pathogenic, even when large numbers are present. Outbreaks of clinical disease may occur during the dry seasons when large numbers of animals and snails concentrate around waterholes.

### 2.5 Protozoa

Coccidia are protozoan parasites; most species infecting cattle, sheep and goats belong to the genus *Eimeria*. All *Eimeria* species parasitize the intestinal epithelium of infected animals. Older animals usually become immune to infection but often remain carriers of coccidia and continue to pass oocysts in the faeces. Young animals become infected by ingesting sporulated oocysts in contaminated food and water. The parasites usually migrate into the intestinal mucous membrane, where oocysts are produced which pass out in the faeces. Successive infections in young animals may cause the animals to excrete large numbers of oocysts and this excretion will heavily contaminate kraals and watering places. The multiplication of the
parasite in the intestine causes damage to the mucous membrane. The severity of this damage depends on the number of oocysts ingested. Clinical signs are usually seen only in young animals. A prominent sign of clinical coccidiosis is diarrhoea, which is sometimes bloody. Affected animals have poor growth rates; severely affected animals may die.
TECHNIQUES FOR PARASITE ASSAYS AND IDENTIFICATION IN FAECAL SAMPLES

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### 3.6 ISOLATING AND IDENTIFYING INFECTIVE LARVAE (BAERMANN TECHNIQUE)

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3 TECHNIQUES FOR PARASITE ASSAYS AND IDENTIFICATION IN FAECAL SAMPLES

3.1 INTRODUCTION

To diagnose gastro-intestinal parasites of ruminants, the parasites or their eggs/larvae must be recovered from the digestive tract of the animal or from faecal material. These are subsequently identified and quantified. This chapter presents diagnostic techniques within the reach of most laboratories to identify and quantify parasite infections from the examination of faecal material. The following are the main tasks involved in this process.

- Collection of faecal samples
- Separation of eggs/larvae from faecal material, and their concentration
- Microscopical examination of prepared specimens
- Preparation of faecal cultures
- Isolation and identification of larvae from cultures

It is important to understand the following basic limitations of faecal examination in the diagnosis of gastro-intestinal parasitism.

(a) The demonstration of parasite eggs or larvae in the faeces provides positive evidence that an animal is infected but does not indicate the degree of an infection.

(b) The failure to demonstrate eggs or larvae does not necessarily mean that no parasites are present; they may be present in an immature stage or the test used may not be sufficiently sensitive.

(c) There is generally no correlation between the numbers of eggs/larvae per gram of faeces and the number of adult nematodes present in the host. An exception to this may occur in a primary infection in a young grazing animal.

Various factors can limit the accuracy and significance of a faecal egg count.

(a) There is a fairly regular fluctuation in faecal egg output.

(b) Eggs are not evenly distributed throughout the faeces.

(c) The quantity of faeces passed will affect the number of eggs per unit weight.
(d) The egg output is influenced by the season of the year (large infections may be acquired during rainy seasons).

(e) The resistance of the host can depress or entirely inhibit the egg production of parasites.

(f) Immature worms do not indicate their presence by producing eggs.

(g) Immunity may result in a marked extension of the prepatent period and a lower egg output by female parasites.

(h) An egg count often refers to the total number of eggs of a mixture of species, which differ widely both in their biotic potential and their pathogenicity.

(i) Eggs may not be detected due to low numbers of them or to a low test sensitivity.

3.2 COLLECTION OF FAECAL SAMPLES

Faecal samples for parasitological examination should be collected from the rectum of the animal.
If rectal samples cannot be obtained, fresh faecal samples may be collected from the pasture.

Several samples should be collected. Samples should be dispatched as soon as possible to a laboratory in suitable containers such as:

- screw cap bottles
- plastic containers with lids
- disposable plastic sleeves/gloves used for collecting the samples
- plastic bags
Each sample should be clearly labelled with animal identification, date and place of collection.

Samples should be packed and dispatched in a cool box to avoid the eggs developing and hatching. If prolonged transport time to a laboratory is expected, the following may help to prevent the eggs developing and hatching.

(a) Filling the container to capacity or tightening the sleeve/glove as close to the faeces as possible. This is to exclude air from the container.

(b) Adding 3% formalin to the faeces (5–20 ml, depending on the volume of faeces). This is to preserve parasite eggs. (N.B. Formalin-fixed faeces cannot be used for faecal cultures.)

3.3 QUALITATIVE TECHNIQUES FOR SEPARATING AND CONCENTRATING EGGS/LARVAE

A number of different methods are available for separating, concentrating, demonstrating and quantifying eggs, oocysts and larvae in faecal samples. Three methods are described:
3.3.1 Simple Test Tube Flotation

3.3.1.1 Principle

The simple test tube flotation method is a qualitative test for the detection of nematode and cestode eggs and coccidia oocysts in the faeces. It is based on the separating of eggs from faecal material and concentrating them by means of a flotation fluid with an appropriate specific gravity.

3.3.1.2 Application

This is a good technique to use in initial surveys to establish which groups of parasites are present.

3.3.1.3 Equipment

- Beakers or plastic containers
- A tea strainer (preferably nylon) or double layer cheesecloth
- Measuring cylinder or other container graded by volume
- Fork, tongue blades or other type of stirring rod
- Test tube
- Test tube rack or a stand
- Microscope
- Microslides, coverslips
- Balance, teaspoon
- Flotation fluid (see the Appendix to this handbook for formulation)
3.3.1.4 Procedure

(a) Put approximately 3 g of faeces (weigh or measure with a precalibrated teaspoon) into Container 1.

(b) Pour 50 ml flotation fluid into Container 1.
(c) Mix (stir) faeces and flotation fluid thoroughly with a stirring device (tongue blade, fork).

(d) Pour the resulting faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.
(e) Pour the fecal suspension into a test tube from Container 2.
(f) Place the test tube in a test tube rack or a stand.

(g) Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully place a coverslip on top of the test tube.

(h) Let the test tube stand for 20 minutes.
(i) Carefully lift off the coverslip from the tube, together with the drop of fluid adhering to it, and immediately place the coverslip on a microscope slide.

3.3.2 Simple Flotation Method

3.3.2.1 Principle

The principle for the simple flotation method is the same as for the simple test tube flotation method.

3.3.2.2 Application

This is another good technique for use in initial surveys. In addition, it can be used in conjunction with the McMaster technique to detect low numbers of eggs (when present below the McMaster sensitivity of 50 eggs per gram of faeces).

3.3.2.3 Equipment

- 2 beakers or plastic containers
- A tea strainer or cheesecloth
- Measuring cylinder or other container graded by volume
- Fork, tongue blades or other type of stirring rod
- Test tube (dry)
- Microscope
- Microslides, coverslips
- Balance or: teaspoon
- Flotation fluid (see the Appendix to this handbook for formulation)
3.3.2.4 Procedure

(a) Put approximately 3 g of faeces (weigh or measure the faeces with a precalibrated teaspoon) into Container 1.

(b) Pour 50 ml of flotation fluid into Container 1.
(c) Mix (stir) the contents thoroughly with a stirring device (tongue blade, fork).

(d) Pour the resultant faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.
(e) Leave the container to stand for 10 minutes.

(f) Press a test tube to the bottom of the filtrate, lift it quickly and transfer a few drops adhering to the surface to a microslide.

(g) The test tube ought to touch the microslide for at least 2-4 seconds for the drops to run off.
(h) Mount the coverslip on the microslide for microscopical examination.

3.3.3 Sedimentation Technique (for Trematode Eggs)

3.3.3.1 Principle

The sedimentation technique is a qualitative method for detecting trematode eggs (*Paramphistomum*) in the faeces. Most trematode eggs are relatively large and heavy compared to nematode eggs. This technique concentrates them in a sediment.

3.3.3.2 Application

This is a procedure to assess the presence of trematode infections. It is generally run only when such infections are suspected (from previous post-mortem findings on other animals in the herd/flock area), and is not run routinely.
The procedure can also be used to detect liver fluke (*Fasciola*) eggs.

3.3.3.3 Equipment

- Beakers or plastic containers
- A tea strainer or cheesecloth
- Measuring cylinder
- Stirring device (fork, tongue blade)
- Test tubes
- Test tube rack
- Methylene blue
- Microslide, coverslips
- Microscope

3.3.3.4 Procedure

(a) Weigh or measure approximately 3 g of faeces into Container 1.
(b) Pour 40–50 ml of tap water into Container 1.

(c) Mix (stir) thoroughly with a stirring device (fork, tongue blade).
(d) Filter the faecal suspension through a tea strainer or double-layer of cheesecloth into Container 2.

(c) Pour the filtered material into a test tube.
(f) Allow to sediment for 5 minutes.

(g) Remove (pipette, decant) the supernatant very carefully.

(h) Resuspend the sediment in 5 ml of water.
(i) Allow to sediment for 5 minutes.

(j) Discard (pipette, decant) the supernatant very carefully.
(k) Stain the sediment by adding one drop of methylene blue.

(l) Transfer the sediment to a microslide. Cover with a coverslip.
3.3.4 Microscopical Examination of Prepared Samples

The prepared samples on microslides from the simple test tube flotation method, the simple flotation method and the sedimentation method are examined under a microscope at the magnifications listed in Table 3.1.

Table 3.1 MAGNIFICATION LEVELS FOR EXAMINING PREPARED SAMPLES

<table>
<thead>
<tr>
<th>Magnification</th>
<th>Parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 x 10</td>
<td>Nematode and cestode eggs</td>
</tr>
<tr>
<td>10 x 40</td>
<td>Coccidia oocysts</td>
</tr>
<tr>
<td>10 x 4</td>
<td>Trematode eggs</td>
</tr>
</tbody>
</table>

3.4 QUANTITATIVE TECHNIQUES FOR SEPARATING AND CONCENTRATING EGGS/LARVAE

The simplest and most effective method for determining the number of eggs or oocysts per gram of faeces is the McMaster counting technique described below.

3.4.1 McMaster Counting Technique

3.4.1.1 Principle

The McMaster counting technique is a quantitative technique to determine the number of eggs present per gram of faeces (e.p.g.). A flotation fluid is used to separate eggs from faecal material in a counting chamber (McMaster) with two compartments. The technique described below will detect 50 or more e.p.g. of faeces.

3.4.1.2 Application

This technique can be used to provide a quantitative estimate of egg output for nematodes, cestodes and coccidia. Its use to quantify levels of infection is limited by the factors governing egg excretic...
3.4.1.3 Equipment

- Beakers or plastic containers
- Balance
- A tea strainer or cheesecloth
- Measuring cylinder
- Stirring device (fork, tongue depressor)
- Pasteur pipettes and (rubber) teats
- Flotation fluid (see the Appendix to this handbook for formulation)
- McMaster counting chamber*
- Microscope

* Suppliers

Baird & Tatlock Ltd.
P.O. Box 1, Romford RM1 1HA
Essex, England
Tel: 01-590-7700

Olympic Equine Products
50004, 228th Avenue S.E.,
Issaquah, Washington 98027, USA

3.4.1.4 Procedure

(a) Weigh 4 g of faeces and place into Container 1.
(b) Add 56 ml of flotation fluid.

(c) Mix (stir) the contents thoroughly with a stirring device (fork, tongue blade).
(d) Filter the faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.

(e) While stirring the filtrate in Container 2, take a subsample with a Pasteur pipette.
(f) Fill both sides of the McMaster counting chamber with the subsample.

(g) Allow the counting chamber to stand for 5 minutes (this is important).
(h) Examine the subsample of the filtrate under a microscope at 10 × 10 magnification.

(i) Count all eggs and coccidia oocytes within the engraved area of both chambers.

(j) The number of eggs per gram of faeces can be calculated as follows:

Add the egg counts of the two chambers together. Multiply the total by 50. This gives the e.p.g. of faeces. (Example: 12 eggs seen in chamber 1 and 15 eggs seen in chamber 2 = (12 + 15) × 50 = 1350 e.p.g.)
(k) In the event that the McMaster is negative (no eggs seen), the filtrate in Container 2 can be used for the simple flotation method, steps f, g and h.

3.4.1.5 Guideline to the interpretation of Faecal Egg Counts in Young Animals

Table 3.2 below provides guidelines to aid in interpreting faecal egg counts in young animals.

Table 3.2 FAECAL EGG COUNTS IN YOUNG ANIMALS

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Degree of infection (eggs per gram of faeces)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>50–200</td>
</tr>
<tr>
<td>Mixed infection</td>
<td>200</td>
</tr>
<tr>
<td>Pure <em>Haemonchus</em> infection</td>
<td>50–100</td>
</tr>
<tr>
<td>Pure Trichostrongylus infection</td>
<td>200–300</td>
</tr>
<tr>
<td>SHEEP</td>
<td></td>
</tr>
<tr>
<td>Mixed infection with <em>Haemonchus</em> absent</td>
<td>300–800</td>
</tr>
<tr>
<td>Pure <em>Haemonchus</em></td>
<td>100–2000</td>
</tr>
<tr>
<td>Pure Trichostrongylus</td>
<td>100–500</td>
</tr>
<tr>
<td>Pure <em>Nematodirus</em></td>
<td>50–100</td>
</tr>
<tr>
<td>Pure <em>Oesophagostomum</em></td>
<td>100–800</td>
</tr>
</tbody>
</table>

3.5 PREPARATION OF FAECAL CULTURES

3.5.1 Principle

Many nematode eggs are alike and species such as *Haemonchus, Ostertagia, Trichostrongylus, Cooperia, Bunostomum,* and *Oesophagostomum* cannot be clearly differentiated from the eggs in faecal samples. For these parasites, differentiation can be achieved by the use of faecal cultures. They provide a suitable environment for the hatching and development of helminth eggs into the infective stage (L3).
3.5.2 Application

The identification of parasite species present is an important component of initial surveys and of the investigation of clinical disease caused by gastrointestinal nematodes.

3.5.3 Equipment

- Fork, spoon, tongue depressor, spatula
- Water
- Jars, containers
- Charcoal (Dried, sterile bovine faeces may be used if charcoal is unavailable. This is prepared as follows. Faeces should be sterilised to remove any helminth eggs present, completely dried by heating to 70°C and ground to a fine powder.)

3.5.4 Procedure

(a) Break up collected faeces finely using a stirring device.
(b) Faeces should be moist and crumbly.

If faeces are too dry, add water.

If faeces are too wet, add charcoal (or sterile bovine faeces) until the correct consistency is obtained.
(c) Transfer the mixture to jars or other containers.

(d) Leave the culture at room temperature for 14–21 days, by which time all larvae should have reached the infective stage.

(e) If an incubator is available, the culture can be placed at 27°C and left for 7 to 10 days.
Add water to cultures regularly (every 1–2 days).

Larvae are recovered using the Baermann technique (see section 3.6).

3.6 ISOLATION AND IDENTIFICATION OF INFECTIVE LARVAE (THE BAERMANN TECHNIQUE)

3.6.1 Principle

The Baermann technique is used to isolate infective larvae from faecal cultures. It is based on the active migration of larvae from faeces suspended in water and their subsequent collection and identification.

3.6.2 Application

This is a procedure for harvesting infective larvae for identification purposes. (The technique can also be used for isolating lungworm larvae [L.1] from fresh faeces.)

3.6.3 Equipment

- Funnel (size according to need)
- Funnel stand
- Rubber or plastic tubing
- Rubber bands
- Clamp or spring clip
- Cheesecloth or screen
- Simple thin stick (about 15 cm long)
- Strainer
- Microscope
- Test tube
- Pasteur pipette
3.6.4 Procedure

(a) Fit a short piece of tubing which is closed at one end with a clamp or spring clip, to the stem of a funnel of appropriate size. 

(b) Support the funnel by a stand.

(c) Weigh or measure about 5-10 g of faecal culture faeces and place it on a piece of double layer cheesecloth.
(d) Form the cheesecloth around the faeces as a “pouch”.

(e) Close the pouch with a rubber band.
(f) Fix a supporting stick under the rubber band.
(g) Place the pouch containing faecal culture material or faeces in the funnel. Trim the surplus cheesecloth off.

(h) Fill the funnel with lukewarm water, covering the faecal material.
(i) Leave the apparatus in place for 24 hours, during which time larvae actively move out of faeces and ultimately collect by gravitation in the stem of the funnel.

(j) Draw 10-15 ml of fluid from the stem of the funnel into a test tube or other container.

(k) Leave the tube to stand for 30 minutes. Remove the supernatant with a Pasteur pipette.
(l) Transfer a small aliquot of the remaining fluid using a Pasteur pipette to a microslide, add a drop of iodine and cover with a coverslip.

(m) Examine under 100 × 10 magnification. (See Tables 3.3 and 3.4 below for larval identification).

(n) Repeat steps l and m until 100 larvae have been identified.

(o) The counts for each species provide an estimate of the composition (%) of the parasite population of the host.
3.6.5 Identification of Infective Larvae

Table 3.3 provides a key to the infective larvae of some common nematodes of cattle.

**Table 3.3 KEY TO THE INFECTIVE LARVAE OF SOME COMMON NEMATODES OF CATTLE (after Keith [1953])**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sheath absent, oesophagus more than 1/3 the length of the body.</td>
<td>Strongyloides</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Sheath present, oesophagus short.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Length, including sheath, less than 600 μ.</td>
<td>Bunostomum</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Length, including sheath, more than 600 μ.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Tail of sheath less than 200 μ.</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Tail of sheath more than 200 μ.</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>Two conspicuous oval bodies at anterior end of oesophagus.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No such structures at anterior end of oesophagus.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Length including sheath more than 1000 μ, tail of larva with dorsal and ventral lobes with a rod-like process between.</td>
<td>Nematodirus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length including sheath less than 1000 μ, tail of larva ending in a simple point.</td>
<td>Oesophagostomum</td>
<td>radiatum</td>
</tr>
<tr>
<td>6.</td>
<td>Length, including sheath, usually more than 850 μ; tail of sheath usually more than 150 μ long, tapering gradually to end bluntly.</td>
<td>Cooperia onecphera</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length, including sheath, usually less than 850 μ; tail of sheath tapering rapidly to a point or short fine filament less than 150 μ long.</td>
<td>Cooperia punctata</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. pectinata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Tail of sheath short and conical, less than 110 μ long.</td>
<td>Trichostrongylus</td>
<td>axeí</td>
</tr>
<tr>
<td></td>
<td>Tail of sheath at least 126 μ long.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Tail of sheath ending bluntly.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tail of sheath ending in a fine whip-like filament</td>
<td>Ostertagia</td>
<td>ostertagií</td>
</tr>
<tr>
<td></td>
<td>Haemonchus</td>
<td>contortus</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.4 provides a key to the infective nematode larvae of sheep and goats.

<table>
<thead>
<tr>
<th>Total length of larva (μ)</th>
<th>Length, end of larva to end of sheath (μ)</th>
<th>Species, with range of total length (μ)</th>
<th>Other differential features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short 500–700</td>
<td>No sheath</td>
<td><em>Strongyloides</em> 570–700</td>
<td>Slender body with oesophagus, 1/3 to 1/2 total length of larvae.</td>
</tr>
<tr>
<td>Medium 650–900</td>
<td>Short 20–40</td>
<td><em>Cooperia curticei</em> 710–850</td>
<td>Oval bodies at anterior end of larva. Tail of larva rounded.</td>
</tr>
<tr>
<td>Medium 650–900</td>
<td>Medium 30–60</td>
<td><em>Haeomochus</em> 650–750</td>
<td>Tail sheath is usually &quot;kinked&quot;. Pointed tail of larva.</td>
</tr>
<tr>
<td>Medium 650–900</td>
<td>Medium 30–60</td>
<td><em>Cooperia oncophora</em> 800–920</td>
<td>Oval bodies anterior end of larva. Tail of larva rounded.</td>
</tr>
<tr>
<td>Long 900–1200</td>
<td>Long 60–80</td>
<td><em>Chabertia</em> 710–790</td>
<td>Stout body with 24 to 32 rectangular intestinal cells.</td>
</tr>
<tr>
<td>Long 900–1200</td>
<td>Extremely long 250–290</td>
<td><em>Nematodirus</em> 922–1180</td>
<td>Tail of larva is forked.</td>
</tr>
</tbody>
</table>
# POST-MORTEM DIFFERENTIAL PARASITE COUNTS

## 4.1 INTRODUCTION

## 4.2 EQUIPMENT

## 4.3 METHODS FOR POST-MORTEM DIFFERENTIAL PARASITE COUNTS

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## 4.4 INTERPRETING ADULT NEMATODE COUNTS

## 4.5 IDENTIFYING GASTRO-INTESTINAL PARASITES OF SHEEP AND GOATS

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4 POST-MORTEM DIFFERENTIAL PARASITE COUNTS

4.1 INTRODUCTION

Post-mortem parasite counts provide a more precise assessment of parasite burdens than parasite egg counts. For parasite counts, the intestinal tract from abomasum to rectum is required. The adult and larval nematodes are carefully washed out, counted and identified. In addition, a complete post-mortem examination of all organs should be done, bearing in mind alternative causes of ill health or death. It is important to record all abnormalities and lesions observed. A number of parasites will be found in almost every grazing animal, irrespective of the state of its health. To assess the significance of parasite infections in field mortalities, it is therefore necessary not only to determine the species present, but also to assess the number of each species.

Methods suitable for differential parasite counting under field or laboratory conditions using simple, easily obtainable and inexpensive equipment are described below.

4.2 EQUIPMENT

(a) One or two deep trays of about $30 \times 45 \times 15$ cm. The precise size is not important. Suitable plastic trays are easily procurable. A rectangular shape facilitates pouring from them.

(b) One or two large, wide-mouthed plastic jars or buckets of about 3–5 litres capacity. These are used to collect the contents of each organ examined and hence are called the "total contents" jars. Calibrate the sides of the "total contents" jar in litres.

(c) A large kitchen ladle or similar utensil with about a 40-ml capacity and with a handle about 12 inches long.

(d) A smaller, wide-mouthed glass or plastic jar of about 500–1000 ml capacity. This jar must have a close-fitting screw-top lid. Make a hole in the top of the lid as large as possible, without interfering with proper sealing between the edge of the lid and the top of the jar.
Cut and fix a piece of brass wire mesh (40 mesh per linear inch) neatly inside the lid.

Calibrate the sides of the "wash jar" in 100 ml gradations.
This jar is used to wash the colouring matter out of the faeces and is called the “wash jar”.

(e) Two glass petri dishes about 9 cm in diameter.

(f) An aqueous solution of iodine.

(g) A saturated aqueous solution of sodium thiosulphate.

(h) A light box or some white background material. A large white tile is very suitable. Paper will suffice, or the bottom of a petri dish can be painted white.

(i) A mounted needle or fine forceps to handle the worms during counting.

(j) A jug and a bucket for handling water are useful additions to field equipment, although suitable utensils may be readily procurable from the farmer.

(k) An illuminated background. Much eye-strain may result from doing large numbers of worm counts indoors where lighting is poor or variable. An illuminated background overcomes this. Electric lamps, preferably fluorescent, are fitted inside a wide shallow box. The top of the box is made from translucent white plastic or ground glass. Samples in clear glass petri dishes are placed on top. The diffuse white light shining up through the petri dishes provides a strong contrast for the stained worms and no shadows are cast.

4.3 METHODS FOR POST-MORTEM DIFFERENTIAL PARASITE COUNTS

Counts of gastro-intestinal parasites are most conveniently done by examining the abomasum, small intestines and large intestines separately.

The following techniques are quantitative procedures for isolating counting and identifying adult and larval nematodes in the abomasum and adult nematodes in the small and large intestines.
4.3.1 Differential Parasite Counts of the Abomasum

4.3.1.1 Procedure

(a) During the post-mortem examination, ligate the abomasum with string and separate it from omasum and duodenum.

(b) Place the abomasum in a tray. Open the abomasum along the greater curvature so that its contents fall into the tray: empty the abomasal contents into the total content jar.
(c) Wash the empty abomasum thoroughly in the tray several times, paying particular attention to cleaning between the folds of the mucous membrane. Add the washings to the total contents jar.
(d) For cattle, make the total volume of contents and washings in the total contents jar up to 4 litres with water. Occasionally it will be necessary to make the total volume up to 5 litres for cattle. For sheep and goats, make the volume up to 2 or 3 litres.
(e) Using the large ladle, stir vigorously until all food material, mucus and water are thoroughly mixed.

(f) Transfer a total of 200 ml of the contents to the wash jar in 5 steps of 40 ml per step, using the ladle container and stirring the mixture continuously.
(g) Fill the wash jar with water. Screw the lid on securely. Invert the jar and shake it till most of the fluid is shaken out. Repeat this process until all faecal colouring matter is removed.
(h) Add water to make the volume in the wash jar up to 50 ml (for convenience).

(i) Pour small volumes into petri dishes.
(j) Add a few drops of iodine solution to the sample in each petri dish. Mix the iodine with the sample and allow to stand for 3–5 minutes, during which time the worms will stain deeply with iodine.

(k) Count the number of each species of nematode present in the sample. Repeat the process for each petri dish, and add the species counts for all dishes.
NOTE: In the case of a field post-mortem, procedures (f)–(k) can conveniently be carried out on return to the laboratory.

For cattle, multiply the total count for each species by 20 to arrive at the total burden in the animal examined (assuming that an original volume of 4 litres was used). For sheep and goats, multiply the count for each species by 10 or 15 to arrive at the total burden (assuming that an original volume of 2 or 3 litres was used).

For *Haemonchus*, small differences in worm burdens may cause significant differences in their pathogenic effect. For this reason, a more accurate assessment of the burden should be obtained by carrying out a total abomasal count of *Haemonchus* as opposed to the sub-sampling procedure described above.

4.3.2 Isolating Inhibited/Immature Larvae from the Abomasum

4.3.2.1 Principle

This is a quantitative procedure for isolating, counting and identifying larvae from the abomasal mucus membrane.

4.3.2.2 Application

This technique is carried out in conjunction with the isolation of adult abomasal parasites. It can be used to determine:

- the number of abomasal nematodes present as immature larvae, and hence the ratio of immature larvae to adult nematodes
- the number and seasonal occurrence of inhibited larvae

To prevent immature larvae from being counted as inhibited larvae, the number of inhibited larvae should be determined only in animals kept isolated from reinfection for at least 21 days. This allows non-inhibited larvae to complete development.

4.3.2.3 Equipment

- A tray or bucket
- Normal physiological saline, 0.9% (see the Appendix at the end of this handbook for the formulation)
- Sieve or nylon net, 32-µm mesh
- Beaker
- Petri dishes
• Washbottle
• Pasteur pipette
• Microslides/coverslips
• Microscope/dissecting microscope

4.3.2.4 Procedure

(a) Place the opened and washed abomasum with the mucous membrane face down in the tray/bucket containing lukewarm normal saline solution.

(b) Leave the abomasum to soak overnight.
(c) Remove the abomasum, rinse well with saline solution and discard.

(d) Pour the saline solution left in the tray/bucket through the sieve/nylon net, which will retain the larvae.
(e) Flush the larvae from the sieve/nylon net into a beaker using the wash bottle. Make the total volume up to 200 ml.

(f) Using a dissecting microscope, examine an aliquot of 10 ml in a petri dish and count the larvae.
To identify the parasite species, transfer further subsamples by Pasteur pipette to microslides for examination under the microscope.

The total number of larvae is calculated as follows: number in 10 ml subsample × 20 = total abomasal larval count.

4.3.3 Differential Parasite Counts of the Small Intestine

4.3.3.1 Principle and application

The principle and application of the differential parasite counts of the small intestine are the same as those for parasite counts of the abomasum.

4.3.3.2 Procedure

(a) The procedure used for the small intestines is similar to that for the abomasum.

(b) When examining the small intestines it is convenient to “run” the intestines out, free from the mesentery, into one tray.

(c) Initially, the gut is washed by pouring water into one end of the gut and flushing it out into the total volume jar. For further washing and scraping, the intestine has to be opened.

(d) It is important to scrape the mucous membrane in some manner to recover the smaller parasites, especially *Trichostrongylus*.

(e) Opening and scraping can be done quickly, efficiently and easily in one operation using a simple instrument that can be made by any skilled metal fitter. The instrument is called a “gut-runner”.

The “gut-runner”
(f) When the small intestines have been opened, scraped and washed, place all of the contents plus all of the washings in the total contents jar.

(g) The procedure for sampling, washing, subsampling, staining and counting is the same as previously described for parasites of the abomasum.

NOTE: Even very large numbers of the smaller nematodes can be very easily overlooked unless some kind of washing procedure is used. They are very difficult to detect when mixed with faecal material.

4.3.4 Differential Parasite Counts of the Large Intestines

4.3.4.1 Principle and application

The principle and application of the differential parasite counts of the large intestine are the same as those for parasite counts of the abomasum.

4.3.4.2 Procedure

(a) Uncoil the large intestines into one tray. Open them with scissors, placing the opened portion into the second tray.

(b) The nematodes of the large intestines are easily seen. There are relatively few of them and they can be picked off with forceps as the gut is opened and can be placed in a petri dish containing water. Few parasites will be overlooked using this procedure.

(c) When the contents are fluid because of diarrhoea, or when a more precise count is required, the contents should be processed as described for the abomasum and small intestine. A large open sieve of 40 mesh/inch brass wire can be used.
4.4 INTERPRETING ADULT NEMATODE COUNTS

Table 4.1 below provides a guideline for interpreting adult nematode counts.

Table 4.1  A GUIDELINE TO THE INTERPRETATION OF ADULT NEMATODE COUNTS

<table>
<thead>
<tr>
<th>Nematode species</th>
<th>Degree of infection (total number of parasites)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
</tr>
<tr>
<td>CATTLE</td>
<td></td>
</tr>
<tr>
<td>Abomasal parasites</td>
<td>1–5000</td>
</tr>
<tr>
<td>Small intestinal parasites</td>
<td>1–8000</td>
</tr>
<tr>
<td><em>Haemonchus</em></td>
<td>1–400</td>
</tr>
<tr>
<td><em>Trichostrongylus</em></td>
<td>1–10000</td>
</tr>
<tr>
<td><em>Cooperia</em></td>
<td>1–5000</td>
</tr>
<tr>
<td>SHEEP</td>
<td></td>
</tr>
<tr>
<td><em>Haemonchus</em></td>
<td>≤500</td>
</tr>
<tr>
<td><em>Trichostrongylus</em></td>
<td>1–1000</td>
</tr>
<tr>
<td><em>Nematodirus</em></td>
<td>1–2500</td>
</tr>
<tr>
<td><em>Oesophagostomum</em></td>
<td>1–50</td>
</tr>
</tbody>
</table>

NOTE: These numbers should be considered only as a guideline in the interpretation of parasite burdens.
4.5 IDENTIFYING GASTRO-INTESTINAL PARASITES OF SHEEP AND GOATS

Tables 4.2 to 4.5 below provide simple keys for identifying some common gastro-intestinal parasites of sheep and goats, describing nematodes of the abomasum, small intestines and large intestines.

Table 4.2 A SIMPLE KEY FOR IDENTIFYING COMMON GASTRO-INTESTINAL PARASITES OF SHEEP AND GOATS

<table>
<thead>
<tr>
<th>Organ location</th>
<th>Characteristics of Parasite</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abomasum</td>
<td>Cervical papilla present</td>
<td>Haemonchus</td>
</tr>
<tr>
<td></td>
<td>Prominent excretory pore</td>
<td>Ostertagia</td>
</tr>
<tr>
<td></td>
<td>Very long oesophagus</td>
<td>Strongyloides</td>
</tr>
</tbody>
</table>
| Small intestines| Cephalic swelling with stria
tions | Cooperia       |
|                | Small, coiled              |                |
|                | Medium, tangled            | Nematodirus    |
|                | Head bent dorsally         | Bunostomum     |
|                | Large, stout               |                |
| TAPEWORMS      | Scolex with suckers        | Aoniezia       |
|                | Large, 2 genital pores/seg.|                |
|                | Small, indistinct segments | Avitellina     |
| Cæcum and large intestines | Very long thin neck | Trichuris     |
|                | Large, whip-like           |                |
|                | Cervical papillae level with oesophagus | Oesophagostomum columbianum |
|                | Cervical papillae behind oesophagus | Oes. venulosum |

Further differential features are given in Tables 4.3–4.5.
Table 4.3  NEMATODES OF THE ABOMASUM

<table>
<thead>
<tr>
<th></th>
<th>Haemonchus</th>
<th>Ostertagia</th>
<th>Trichostrongylus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mature</strong></td>
<td>Males 10 to 20 mm long. Females 18 to 30 mm.</td>
<td>Males 7 to 8 mm long. Females 9 to 12 mm.</td>
<td>Males 4 to 5 mm long. Females 5 to 7 mm.</td>
</tr>
<tr>
<td>size</td>
<td>Large, easily seen, mostly in fundus region of abomasum.</td>
<td>Mostly found at pyloric region of abomasum.</td>
<td>Very small, difficult to see without washing or staining.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Much the same thickness throughout length of worm.</td>
</tr>
<tr>
<td><strong>Head</strong></td>
<td>Prominent large cervical papillae.</td>
<td>Small cervical papillae set more posteriorly.</td>
<td>No cervical papillae. Prominent excretory pore.</td>
</tr>
<tr>
<td></td>
<td>Distance from anterior end about 3½ times diameter between papillae.</td>
<td>Distance from anterior end about 5 times diameter between papillae.</td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>Vulva covered by large vulval flap.</td>
<td>Small or no vulval flap.</td>
<td>Simple genital opening without vulval flap.</td>
</tr>
<tr>
<td></td>
<td>Red and white spiral striping visible in fresh specimens resembling &quot;Barber’s pole&quot;.</td>
<td>Under high magnification tip of tail shows annular rings.</td>
<td>Cuticle striations are annular.</td>
</tr>
<tr>
<td></td>
<td>Cuticle striations are longitudinal.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>Asymmetrical dorsal lobe in bursa.</td>
<td>Bursal lobes are symmetrical.</td>
<td>Bursal lobes are symmetrical.</td>
</tr>
<tr>
<td>tail</td>
<td>Spicules taper to barbed points.</td>
<td>Spicules vary with species.</td>
<td>Spicules vary with species.</td>
</tr>
</tbody>
</table>
### Table 4.4  NEMATODES OF THE SMALL INTESTINES

<table>
<thead>
<tr>
<th></th>
<th>Strongyloides</th>
<th>Coepria</th>
<th>Nemaodirus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mature size</strong></td>
<td>Usually only females are found, 3 to 6 mm long.</td>
<td>Males 4 to 6 mm long. Females 5 to 7 mm.</td>
<td>Males 10 to 15 mm long. Females 15 to 20 mm.</td>
</tr>
<tr>
<td></td>
<td>These worms lose the iodine stain quickly when de-colourised with hypo solution.</td>
<td>Usually coiled flat or in 1 or 2 tight coils.</td>
<td>Usually tangled shape due to twisting of the &quot;thin neck&quot;.</td>
</tr>
<tr>
<td><strong>Other features</strong></td>
<td>Very long oesophagus, one third to one half total length of worm.</td>
<td>Body of female swollen at region of vulva.</td>
<td>Female tail has prominent spine protruding from a blunt end.</td>
</tr>
<tr>
<td></td>
<td>Eggs expressed from females have a fully developed larva in them.</td>
<td>Male tail has short, stout spicules.</td>
<td>Male tail has very long, slender spicules usually extending beyond the bursa.</td>
</tr>
</tbody>
</table>

**Bunostomum**

**Mature size**  Male 12 to 17 mm long. Female 19 to 26 mm. A stout worm much thicker than any other round worms of the small intestine.

Large buccal cavity has prominent teeth.  
*B. trigonocephalum* of sheep and goats has one large and 2 small teeth.

**Head**  *B. phlebotomum* of cattle has 2 pairs of subventral teeth.

**Other features**  *B. trigonocephalum* has short, twisted spicules.  
*B. phlebotomum* has long, slender spicules.
### Table 4.5  NEMATODES OF THE LARGE INTESTINES

<table>
<thead>
<tr>
<th>CAECUM</th>
<th>Trichuris</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mature size</strong></td>
<td>Male 50 to 80 mm long. Female 35 to 70 mm long.</td>
</tr>
<tr>
<td></td>
<td>The anterior end is very thin, the posterior end is thick. It is called the “whip-worm” because of its shape.</td>
</tr>
<tr>
<td><strong>Other features</strong></td>
<td>Male has single spicule in spine-covered protrusible sheath. Female produces barrel-shaped eggs with a transparent plug at each end.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>COLON</th>
<th>Chabertia</th>
<th>Oesophagostomum venulosum</th>
<th>Oesophagostomum colombianum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mature size</strong></td>
<td>Male 13 to 14 mm long. Female 17 to 20 mm.</td>
<td>Male 11 to 16 mm long. Female 13 to 24 mm.</td>
<td>Male 12 to 16 mm long. Female 15 to 21 mm.</td>
</tr>
<tr>
<td><strong>Other features</strong></td>
<td>Chabertia has a large globular buccal cavity that is visible to the naked eye in fresh specimens. There are no teeth in the buccal cavity.</td>
<td>Small buccal cavity surrounded by leaf crown. Cervical papillae are situated posterior to the oesophagus.</td>
<td>Small buccal cavity surrounded by leaf crown. Cervical papillae are situated opposite anterior region of oesophagus.</td>
</tr>
</tbody>
</table>
# SUPPLEMENTARY DIAGNOSTIC PROCEDURES

## 5.1 INTRODUCTION

## 5.2 ISOLATION OF INFECTIVE LARVAE FROM HERBAGE

- **5.2.1 Principle**
- **5.2.2 Application**
- **5.2.3 Equipment**
- **5.2.4 Procedure**

## 5.3 PACKED CELL VOLUME DETERMINATION (PCV, HAEMATOCRIT)

- **5.3.1 Principle**
- **5.3.2 Application**
- **5.3.3 Equipment**
- **5.3.4 Procedure**
5 SUPPLEMENTARY DIAGNOSTIC PROCEDURES

5.1 INTRODUCTION

This chapter considers two supplementary diagnostic procedures. The first is the isolation and identification of infective larvae from the herbage. This procedure allows the estimation of larval availability on the pasture and can be used to define larval seasonality and distribution. The second is the estimation of anaemia in clinically or sub-clinically affected animals by the determination of packed cell volume (PCV) in a blood sample.

5.2 ISOLATING INFECTIVE LARVAE FROM HERBAGE

5.2.1 Principle

This is a qualitative and semi-quantitative procedure for isolating, counting and identifying L₃ from representative samples of herbage taken from defined grazing areas.

5.2.2 Application

This is a useful procedure for determining seasonal variations in L₃ availability on the pasture. However, the number of larvae on the herbage is influenced by a variety of factors, and may vary considerably even when repeated samplings are done at the same time of day and the same site; herbage larval counts should therefore be interpreted with extreme caution.

5.2.3 Equipment

- Scissors and collecting bags
- Small bucket (e.g., 7 litre), calibrated at one-half litre gradations
- Gauze bag, made from terylene netting or cheesecloth, large enough to fit over the rim of the bucket
- Large plastic or glass filter funnel (20-cm diameter), fitted with a length of flexible, transparent tubing carrying 2 screw clamps arranged so that about 15 ml can be trapped between the two clamps
- Domestic detergent
- Test tube, holding at least 15 ml
- Dissecting microscope or microscrope
- Balance
5.2.4 Procedure

(a) Collect small samples of grass from a large number of sites randomly scattered throughout the area to be sampled, using a "W" or "N" collecting route. Avoid areas of heavy faecal contamination and avoid collecting soil.

(b) Grass can be collected by hand. Where grasses are coarse, scissors may be used. The grass collections of approximately 300-600 g should then be placed in a plastic bag.
(c) Place the collected grass sample inside a gauze bag and immerse the bag in water in a bucket but keep the bag clear of the bottom of the bucket.
(d) In the first 3–4 hours, remove, drain and replace the bag in the water several times to agitate the sample. Leave the bag in the water at room temperature overnight.

(e) The next morning, remove the bag and run fresh tap water over it and into the bucket. Leave the contents of the bucket to sediment for about 1 hour.
(f) The bag of grass should be dried (sun/oven/incubator) and weighed when completely dry.

(g) Carefully decant or syphon off the supernatant, leaving about 1 litre containing all the sediment.
(h) Suspend the sediment and pour it into the large funnel in its stand with the bottom clamp fastened. Wash out the bucket into the funnel, but discard any heavy debris that sediments rapidly.

(i) Leave the funnel to stand for another hour.

(j) Close the top clamp and collect the trapped sediment with about 15 ml of fluid in a test tube. Procedures (h) and (i) may need to be repeated when large volumes of sediment have to be processed, or when only a small funnel is available. Leave to cool at 4°C for at least 1 hour.
(k) Siphon off the supernatant to leave 3–5 ml, which is stained with 3–5 drops of iodine for at least 1 hour. Counter stain with 3 drops of sodium thiosulphate.
(l) Count and identify the parasitic larvae seen. There will usually be many more non-parasitic nematodes and larvae present than parasitic larvae and the count may therefore be rather laborious.

(m) Parasitic larvae all have sheaths discernible at the tail end and tend to retain the brown colouration of the iodine for 30–40 minutes.

Larval identification keys can be found in Tables 3.3 and 3.4.

(n) Number of larvae per kg of dry herbage = count × 1,000/weight of dry herbage (in grams)

5.3 PACKED CELL VOLUME DETERMINATION (PCV, HAEMATOCRIT)

5.3.1 Principle

Infections with some parasite species, particularly Haemonchus but also Bunostomum and Trichuris, can cause anaemia. In acute haemonchosis, the pathogenic effect of the parasite is often present before eggs appear in the faeces. The PCV technique allows an estimation of the degree of anaemia present by measuring the volume occupied by the red blood cell in a sample of circulating blood.

5.3.2 Application

The PCV determination is a useful procedure to carry out on both individual animals and herds/flocks to assess the possible role of Haemonchus in a parasite problem. It is useful as an early aid to the diagnosis of haemonchosis. However, anaemia can occur as a result of other causes, in particular trypanosomiasis and some tick-borne diseases, so this test must be done in conjunction with both parasite egg counts and assays for circulating haemoparasites.

5.3.3 Equipment

- Needles and syringe (or Vacutainer needle and tubes containing anticoagulant)
- Micro-haematocrit tubes
- Micro-haematocrit centrifuge
- Micro-haematocrit reader
- Haematocrit tube sealant
5.3.4 Procedure

(a) Take a venous (jugular) blood sample into a test tube or Vacutainer containing anticoagulant (such as EDTA).

(b) Mix the blood sample well but gently for 2 minutes.

(c) Draw the well-mixed blood up a 75 × 1.5 mm capillary tube for 3/4 of its length.

(d) Seal one end with sealant.

(e) Place in the micro-haematocrit centrifuge, ensuring that the sealant is at the outer end.

(f) Close the centrifuge lid.

(g) Centrifuge the tubes at 12,000 rpm for 4 minutes.

(h) Place the tubes in the reader and note the reading.

(i) Express the reading as a percentage of packed red cells in the total volume of whole blood.
INVESTIGATING A POSSIBLE GASTRO-INTESTINAL PARASITE PROBLEM

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6 INVESTIGATING A POSSIBLE GASTRO-INTESTINAL PARASITE PROBLEM

6.1 INTRODUCTION

This chapter considers strategies for investigating a possible gastro-intestinal parasite problem in a herd or flock, and methods for applying the techniques described in previous chapters. This will be considered in three stages:

(a) Diagnosing a herd/flock problem

(b) Long-term monitoring of a herd/flock problem or of a control programme

(c) Plot experiments

The unit of investigation in the first two cases should be the herd or flock. Where groups of herds are communally grazed, the whole group should ideally be the unit of investigation. Gastro-intestinal parasite problems generally involve an entire herd or flock, and to be effective, diagnosis, treatment and control measures should be directed at the entire herd or flock.

The unit of investigation for plot experiments is a representative and convenient area of pasture in a similar environment to that in which animals are grazed.

6.2 DIAGNOSING A HERD/FLOCK PROBLEM

As many animals as possible, including those showing clinical signs, should be examined, and any abnormalities, such as diarrhoea and anaemia, recorded.

To understand the significance of the gastro-intestinal parasite problem in a particular group of animals, samples should be taken from live animals and dead (moribund or sacrificed) animals.

6.2.1 Sampling of Live Animals

(a) Which animals should be sampled?

If there are animals with obvious clinical disease suggestive of gastro-
intestinal parasitism, these should be sampled. However, it is also important to sample animals suspected of subclinical disease and those that appear healthy in order to fully understand what is occurring in a herd/flock.

(b) How many animals should be sampled?

There is no magic sampling number, but in general, the more animals sampled, the better the understanding of the problem and the greater the validity of the results. Table 6.1 should serve as a guide. It is based on both general principles and practical/logistical constraints.

Table 6.1 SUGGESTED SAMPLE SIZE FOR GIVEN TOTAL HERD/FLOCK NUMBERS

<table>
<thead>
<tr>
<th>Number in herd/flock</th>
<th>Number to sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>All</td>
</tr>
<tr>
<td>11-25</td>
<td>At least 10 animals</td>
</tr>
<tr>
<td>26-100</td>
<td>At least 20 animals</td>
</tr>
<tr>
<td>101-200</td>
<td>At least 30 animals</td>
</tr>
<tr>
<td>Over 200</td>
<td>At least 15 per cent</td>
</tr>
<tr>
<td>Over 500</td>
<td>At least 10 per cent</td>
</tr>
</tbody>
</table>

(c) What samples should be taken?

A faecal sample for a faecal egg count and/or a flotation procedure (see sections 3.3 and 3.4).

A blood sample in anticoagulant for a haematocrit (packed cell volume, PCV; see section 5.3).

6.2.2 Sampling from Dead (Moribund or Sacrificed) Animals

(a) Which animals should be sampled?

Any animal which dies of suspected gastro-intestinal parasitism. If a considerable number of the herd or flock are affected, one or two very sick or moribund animals should be sacrificed for examination.

(b) How many animals should be sampled?

As many as possible of those that die. However, economics and other factors will not normally allow more than one or two very sick or moribund animals to be sacrificed for examination.

(c) What samples should be taken?
A total worm count on the abomasum, small intestine and large intestine should be carried out (see section 4.3).

6.3 LONG-TERM MONITORING OF A HERD/FLOCK PROBLEM OR OF A CONTROL PROGRAMME

By sampling only the diseased animals as described for the diagnosis of a herd/flock problem, only a limited understanding of the epidemiology will be gained. If circumstances allow, a longer term monitoring of herds or flocks should be carried out.

Samples should be taken from live animals, dead (moribund or sacrificed) animals, pasture and tracer (sentinel) animals.

Climatic data should also be recorded.

6.3.1 Sampling of Live Animals

(a) Which animals should be sampled?

The animals to be sampled will normally be healthy animals identified at the beginning of the monitoring period. The same identified animals must be sampled at specified intervals (see below) for the period of monitoring.

(b) How many animals should be sampled?

Again, there is no magic sampling number. However, with the repeated sampling of the same animals, fewer numbers may be required than in the diagnosis of a herd/flock problem. The guidelines in Table 6.1 are recommended.

(c) What samples should be taken?

A faecal sample for a faecal egg count and a faecal culture. A blood sample in anticoagulant for a haematocrit (packed cell volume, PCV: see section 5.3).

(d) How often should samples be taken?

(i) Ideal

Under ideal circumstances, these procedures should be carried out every two weeks during the rainy season and every 4–6 weeks after one month into the dry season. Data should be collected over three calendar years.
(ii) **Acceptable**

If circumstances do not allow, it is acceptable to sample once a month during the rainy season, and once every 6-8 weeks after one month into the dry season. Data should be collected for a MINIMUM of one calendar year.

6.3.2 Sampling from Dead (Moribund or Sacrificed) Animals

(a) **Which animals should be sampled?**

Every opportunity should be taken of sampling animals that die for whatever cause. Sacrificing animals is not advocated for long-term monitoring programmes if tracer (sentinel) animals are used (see below).

(b) **What samples should be taken?**

Those animals that die should be subjected to a total parasite count on the abomasum, small intestine and large intestines (see section 4.3). Data on the quantitative seasonal availability of infective larvae on pasture can be gathered by pasture larval counts and/or the use of tracer animals.

6.3.3 Sampling of Pasture

(a) **From where should pasture samples be taken?**

During the dry season, pasture samples for larval counts should be taken regularly from the same grazing areas within "high risk" sites where larval survival is likely. These may include areas of impeded drainage and river beds. Additional data may be gathered from the following sites:

(i) Areas regularly grazed by the herd of flock

(ii) Areas frequently or periodically grazed by the herd or flock at high stocking density

During the rainy season, a representative sample from the largest possible area of grazing activity should be taken.

(b) **How often should samples be taken?**

Pasture sites should be sampled at the same intervals as animal sampling. Samples should be taken at the same time of day on each occasion.
(i) **Ideal**

Under ideal circumstances, this should be every two weeks during the rainy season, and every 4–6 weeks after one month into the dry season. Data should be collected over three calendar years.

(ii) **Acceptable**

If circumstances do not allow, it is acceptable to sample once a month during the rainy season, and once every 6–8 weeks after one month into the dry season. Data should be collected for a MINIMUM of one calendar year. During the dry season and in arid regions with limited grass cover, herbage larval counts may be of limited value. Under these circumstances, the use of tracer animals (see below) is recommended.

6.3.4 Sampling of Tracer (Sentinel) Animals

Tracer animals are intended to provide an indicator of the availability of infective larvae on the pasture. Tracers should be parasite-free and non-immune animals. These can be produced by:

(a) Rearing parasite-free animals from birth in animal houses. This is the best method, but it requires good animal housing facilities.

(b) Using young (6–12 month old) animals which have been treated twice at 14-day intervals with a larvicidal anthelmintic before starting as tracers. This is the most satisfactory method of producing tracer animals where animal housing facilities are not available.

Tracer animals should be introduced to a herd or flock at intervals of 4 weeks over a minimum period of one year. The group of tracers introduced each month to join the herd or flock should not be treated with an anthelmintic and should be removed after one month. The tracer animals should then be held in a house or pen with no access to pasture, fresh or cut, for a period of at least 21 days, following which they should be slaughtered. (The larvae remaining in the abomasal and intestinal mucous membrane after this period will be considered inhibited larvae.) Slaughtered tracer animals should then be subjected to a total parasite count for the abomasum (including larval stages) and small and large intestines (see section 4.3). Normally it is sufficient to introduce two tracer animals to a herd or flock each month, but the accuracy of the data will be improved by introducing more than this.
6.4 PLOT EXPERIMENTS

Useful epidemiological information can be obtained from studying the seasonal dissemination and survival of L₃ on herbage surrounding faecal pats containing a known number of nematode eggs per gram of faeces which are deposited on pasture at regular intervals throughout a minimum period of one year.

6.4.1 Procedure

(a) Identify a parasite-free grass covered area (600-900 m²). Construct fencing to prevent grazing if necessary. If such an area is not available, consider preparing one by planting/sowing similar grass species as are found in grazing areas. If neither alternative is possible, monitor herbage larval counts in the study plot prior to and during the study to establish the background contamination of L₃.

(b) Identify a source of infected animals for continuous supply of faecal material containing nematode eggs. If not available, calves can be infected for the supply of faeces.

(c) Collect as much faeces as possible (10 kg) from the source animals. Mix the faecal material well. If it is too dry, add water.

(d) Determine the approximate parasite egg count by running five McMaster tests. Calculate the mean count in e.p.g.

(e) Prepare 4–6 plots of 9 m² each. Ensure that no traffic of personnel occurs directly from one plot to another.

(f) Deposit a faecal pat of 1 kg in the centre of each plot.

(g) Repeat procedures (c)–(f) every 4–6 weeks for a minimum of one year.

(h) From each plot with faecal pats of the same age, collect a small amount of herbage by hand from the grass growing near the faecal pat. Combine these samples and place in a plastic bag/container. Repeat this procedure, collecting grass 40–50 cm from the faecal pats. Grass sampling should be carried out at two-weekly intervals for a period of one year.

(i) Samples from plots containing faecal pats of different age must be kept separate.
(j) Process the herbage samples for isolation of $L_3$ (see section 5.2).

6.4.2 Monitoring the Climate in Plot Experiments

The two parameters important for monitoring the climate are:

- ambient temperature
- rainfall

These should be monitored daily, if possible, for the entire period of the sampling/study.
TREATMENT AND CONTROL STRATEGIES

7.1 PRINCIPLES OF CONTROL

7.1.1 Parasite Species Present
7.1.2 Herd Structure and Grazing Management
7.1.3 Availability and Abundance of Infective Larvae on Pasture
7.1.4 Type of Climate
7.1.4.1 Control in savannah-type climates
7.1.4.2 Control in arid climates
7.1.3.3 Control in humid climates

7.2 CHOICE OF ANTHelmINTIC

7.2.1 Anthelmintics for the Treatment of Gastro-Intestinal Nematodes, Lungworms, Tapeworms and Flukes
7.2.2 Chemotherapeutics for the Treatment and Control of Coccidiosis of Cattle, Sheep and Goats
7 TREATMENT AND CONTROL STRATEGIES

7.1 PRINCIPLES OF CONTROL

The principle of a parasite control strategy is to keep the challenge to young livestock by the pathogenic trichostrongyle 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 favourable 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 assuring an adequate plane of nutrition. Control programmes should reduce the effect of parasites to sub-economic levels.

The development of such programmes 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.

7.1.1 Parasite Species Present

Most infections are mixed infections and involve several species of gastro-intestinal parasites. The pathogenicity is usually high when *Haemonchus*, *Trichostrongylus* and *Oesophagostomum* are present. The presence of *Haemonchus*, especially in sheep, requires immediate control measures to prevent severe weight losses and mortality. *Ostertagia* may be a severe problem in certain areas, especially at higher altitude. According to the season, the ingested infective larvae of *Haemonchus*, *Trichostrongylus* and *Ostertagia* may become inhibited in the mucosa of the gastro-intestinal tract. This usually happens prior to the dry season. Development continues at the beginning of the following rainy season. *Cooperia* and *Trichuris ovis* are in most cases relatively non-pathogenic. Faecal egg counts should always be obtained on a herd or flock basis.
7.1.2 Herd Structure and Grazing Management

The data required include the number of animals and the broad age structure of the herd/flock, the time of calving/lambing in relation to the rainy season(s), grazing management (communal, confined, transhumant) and stocking rates.

7.1.3 Availability and Abundance of Infective Larvae on Pasture

If herbage larval counts or tracer animal studies have been performed at regular intervals and grass samples picked at the same time of the day on each sampling occasion, a pattern of seasonal availability of L₃ can be established for any particular pasture. Similarly, trends in relative abundance of L₃ by season and by location can be established. On the basis of these results, the timing and frequency of anthelmintic treatments can be proposed.

7.1.4 Type of Climate

Strategic control programmes are based on the seasonality of development and survival of L₃ on the pasture. This is strongly dependent upon climate. Climatic data from each study site or from local meteorological stations are therefore required.

7.1.4.1 Control in savannah-type climates

The ideal approach in savannah-type climates is an integration of

- adjusting stocking rate
- optimum use of safe pastures
- strategic use of anthelmintics

Ungrazed pastures are parasitologically safe at the end of the dry season. Other types of safe pasture are those previously grazed by other species and those used for hay production. 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. If separation of the age groups is not possible, all animals should be treated at the start of the rains and placed on safe pastures. If no safe pasture is available, two to three consecutive treatments at three-week intervals, beginning at the onset of the rains, should prevent livestock from acquiring excessive parasite burdens.

NOTE: In sheep, the intervals between treatments should be 2 weeks to prevent haemonchosis.

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Older animals may experience an increase in their parasite burdens at the beginning of the rainy season. This "rains rise" is due to further development of previously (end of last rainy season) inhibited larvae in the gastrointestinal mucosa. If not treated, this "rains rise" results in a heavy contamination of the pasture with eggs, and subsequently L3, which when ingested by young, susceptible animals may result in severe clinical disease.

7.1.4.2 Control in arid climates

Infection with gastro-intestinal parasites in arid regions is often limited to local areas with surface water or irrigation. Infection over a wider area may occur during the brief intermittent periods of rainfall. Haemonchosis is the main hazard at such times. With the infrequent and/or low level of infection which may occur under these climatic conditions, animals are usually highly susceptible, and disease is often severe. The timing and frequency of anthelmintic treatments under such climatic conditions will vary greatly from place to place. As such, they should be based on results of prior epidemiological studies described earlier.

7.1.4.3 Control in humid climates

Humid climates are permanently favourable for the development of infective larvae. In these climates it is important to ascertain levels of parasitism and the epidemiology of the species present in order to determine satisfactorily the frequency and timing of strategic anthelmintic dosing.

The adjustment of stocking rate is very important as a controlling factor in humid climates. If a high stocking rate is maintained, regularly repeated treatments with anthelmintics may be essential. However, regularly repeated treatments throughout the year may not be economically feasible, and a strategic anthelmintic regimen should be devised based on optimal cost/benefit assessments.

7.2 CHOICE OF ANTHELMINTICS

Most modern anthelmintics are effective against both adult and larval gastrointestinal parasites. Only a limited number, however, are effective against the inhibited larval stages.

7.2.1 Anthelmintics for the Treatment of Gastro-Intestinal Nematodes, Lungworms, Tapeworms and Flukes

Table 7.1 below shows anthelmintics for treating gastro-intestinal nematodes, lungworms and tapeworms, their route of administration, dose rate and spectrum of activity.
<table>
<thead>
<tr>
<th>Generic name</th>
<th>Route of administration*</th>
<th>Dose rate (mg/kg)</th>
<th>Spectrum of activity**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benzimidazoles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albendazole</td>
<td>O</td>
<td>5-7.5</td>
<td>GI, L, T</td>
</tr>
<tr>
<td>Cambendazole</td>
<td>O</td>
<td>20-25</td>
<td>GI, L, T</td>
</tr>
<tr>
<td>Fexantel</td>
<td>O</td>
<td>5-10</td>
<td>GI, L</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td>O</td>
<td>5-7.5</td>
<td>GI, L, T</td>
</tr>
<tr>
<td>Mebendazole</td>
<td>O</td>
<td>12.5</td>
<td>GI, L, T</td>
</tr>
<tr>
<td>Oxfendazole</td>
<td>O/IR</td>
<td>4.5-5</td>
<td>GI, L, T</td>
</tr>
<tr>
<td>Oxibendazole</td>
<td>O</td>
<td>10-15</td>
<td>GI</td>
</tr>
<tr>
<td>Parbendazole</td>
<td>O</td>
<td>20-30</td>
<td>GI</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>O</td>
<td>44-110</td>
<td>GI</td>
</tr>
<tr>
<td>Thiophanate</td>
<td>O</td>
<td>50-80</td>
<td>GI, L</td>
</tr>
<tr>
<td><strong>Imidazothiazoles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetramisole</td>
<td>O</td>
<td>15</td>
<td>GI, L</td>
</tr>
<tr>
<td>Levamisole hydrochloride</td>
<td>O/SC/SC</td>
<td>7.5</td>
<td>GI, L</td>
</tr>
<tr>
<td>Levamisole phosphate</td>
<td>O/SC</td>
<td>8-9</td>
<td>GI, L</td>
</tr>
<tr>
<td><strong>Organophosphates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coumaphos</td>
<td>O/F</td>
<td>8-15</td>
<td>GI</td>
</tr>
<tr>
<td>Halovon</td>
<td>O</td>
<td>40-50</td>
<td>GI</td>
</tr>
<tr>
<td>Naphtalophos</td>
<td>O</td>
<td>30</td>
<td>GI, T</td>
</tr>
<tr>
<td>Trichlorfon</td>
<td>IM/SC</td>
<td>10-15</td>
<td>GI</td>
</tr>
<tr>
<td><strong>Tetrahydropyrimidines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morantel</td>
<td>O</td>
<td>10</td>
<td>GI</td>
</tr>
<tr>
<td>Pyrantel tartrate</td>
<td>O</td>
<td>25</td>
<td>GI</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ivermectin</td>
<td>O/SC/SC</td>
<td>200 mcg/kg</td>
<td>GI, L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 mcg/kg</td>
<td></td>
</tr>
</tbody>
</table>

* 0: Oral  
  SC: Subcutaneous  
  SO: Spot-on  
  IM: Intramuscular  
  IR: Intraruminal  
  F: Feed

** GI: Gastro-intestinal nematodes  
  L: Lungworms  
  T: Tapeworms
Table 7.2 ANTHELMINTICS FOR THE TREATMENT OF INHIBITED LARVAE

<table>
<thead>
<tr>
<th>Cattle</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>Albendazole</td>
</tr>
<tr>
<td>Febantel</td>
<td>Febantel</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td>Fenbendazole</td>
</tr>
<tr>
<td>Oxfendazole</td>
<td>Oxfendazole</td>
</tr>
<tr>
<td>Thiophanate</td>
<td>Levamisole</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>Ivermectin</td>
</tr>
</tbody>
</table>

Table 7.3 ANTHELMINTICS AND FORMULATIONS FOR STRATEGIC PROPHYLACTIC PROGRAMMES

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Formulation</th>
<th>Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>Slow release bolus, active 90–120 days</td>
<td>Cattle</td>
</tr>
<tr>
<td>Albendazole</td>
<td>Pulse release bolus</td>
<td>Sheep</td>
</tr>
<tr>
<td>Oxfendazole</td>
<td>Pulse release bolus</td>
<td>Cattle</td>
</tr>
<tr>
<td>Morantel tartrate</td>
<td>Slow release bolus, active 60 + days</td>
<td>Cattle</td>
</tr>
</tbody>
</table>

Table 7.4 ANTHELMINTICS FOR THE TREATMENT OF PARAMPHISTOMES

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Route of administration*</th>
<th>Cattle dose rate (mg/kg)</th>
<th>Sheep dose rate (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cioxanide</td>
<td>O</td>
<td>NA</td>
<td>20–40</td>
</tr>
<tr>
<td>Niclosamide</td>
<td>O</td>
<td>NA</td>
<td>90</td>
</tr>
<tr>
<td>Niclofolan</td>
<td>SC</td>
<td>3–4</td>
<td>4–8</td>
</tr>
<tr>
<td>Oxyclozanide</td>
<td>O</td>
<td>10–15</td>
<td>15–20</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>O</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Rafoxanide</td>
<td>O</td>
<td>7.5–10</td>
<td>7.5–10</td>
</tr>
</tbody>
</table>

* 0: Oral  
SC: Subcutaneous

The efficacy of these drugs varies considerably. In general, they are most efficient against the immature pathogenic stages in the duodenum and will in most cases reduce the numbers by 60–90%.

7.2.2 Chemotherapeutics for the Treatment and Control of Coccidiosis of Cattle, Sheep and Goats

Coccidiostats are most efficiently given as a preventive in feed or water. Drugs available include Decoquinate, Amprolecum, Monensin and Lasalocid.
APPENDIX

Formulations for flotation fluids and other reagents for use in diagnostic tests.

FLOTATION FLUIDS

The preparation of three different flotation fluids is described below. Any one of them can be used, depending on the availability of reagents. However, the salt/sugar solution (3) gives the best results due to its high specific gravity.

Good-quality inexpensive salt and/or sugar that give a clear solution should be used for the preparation of flotation fluids. For convenience, a stock supply can be prepared (preferably in a clear container so the amount of salt/sugar not in solution can be seen). The solution should be stirred thoroughly before use to ensure that it is saturated.

(1) Saturated salt solution

Sodium chloride (kitchen salt) 400 g
Water 1000 ml
Specific gravity: 1.200

(2) Saturated sugar solution

Sugar Q.S.
Water 1000 ml
Specific gravity: 1.120–1.200

Add sugar until saturation, indicated by the presence of sugar crystals at the bottom of the container after stirring for 15 minutes. Stir well before use.

(3) Salt/sugar solution

Sodium chloride (kitchen salt) 400 g
Water 1000 ml
Sugar 500 g
Specific gravity: 1.280

Dissolve the salt in water (saturated solution). Add the sugar to the saturated salt solution. Stir until the sugar is dissolved.
OTHER REAGENTS FOR USE IN DIAGNOSTIC TESTS

(1) Physiological saline solution (0.9%).

| Sodium chloride (kitchen salt) | 9 g |
| Distilled water                | 1000 ml |

Dissolve the salt in water.

(2) Aqueous iodine solution.

| Iodine re-sublimed crystals | 10 g |
| Potassium iodide            | 50 g |
| Water                       | 1000 ml |

Dissolve the potassium iodide in the water.

Then add and dissolve the iodine crystals.

(3) Formalin 3% solution.

| Commercial formalin (40% formaldehyde) | 3 parts |
| Water                                  | 97 parts |

N.B. The commercially available 40% formaldehyde solution is regarded as 100% formalin.

(4) Sodium thiosulphate.

| Sodium thiosulphate crystals | 124.1 g |
| Water                        | 1000 ml |

Dissolve the crystals in water.
BIBLIOGRAPHY


