The epidemiology, diagnosis and control of helminth parasites of ruminants

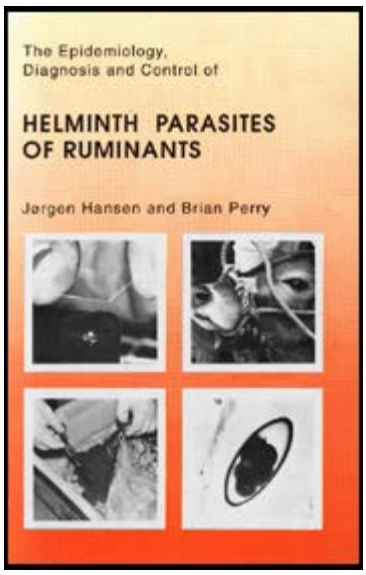


Table of Contents

A Handbook

Jørgen Hansen, DVM, PhD
Animal Production and Health Division Food and Agriculture Organization Rome, Italy

Brian Perry, BVM&S, DTVM, MSc, DVM&S, MRCVS
International Laboratory for Research on Animal Diseases Nairobi, Kenya

Published by the International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya

Cover Design by Dynamic Advertising Ltd.

Printed by the International Livestock Centre for Africa Addis Ababa, Ethiopia

ISBN 92-9055-703-1

Table of Contents

Foreword

Helminth parasites of ruminants are ubiquitous, with many tropical and sub-tropical environments of the world providing 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, infections with gastro-intestinal and other helminth parasites are among the most neglected areas of veterinary care in much of the developing world. The first edition of this handbook was written to help redress this imbalance.

The second edition of the handbook, in a simple style, again reviews the epidemiology of helminth parasites of ruminants, and presents procedures and techniques for their diagnosis, survey and control. It has been enlarged to encompass a broader range of helminth species in a wider geographical area. The book is designed for routine use in all types of animal health institutions, including universities, research institutes and field laboratories where diagnostic parasitology 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.

The first edition of this handbook was produced by ILRAD, in recognition of the need to address the problems of endemic helminth diseases in Africa in order to optimize the benefits from improved control of protozoan diseases, the main focus of ILRAD's research. In view of the value of effective helminth control in ruminant production systems throughout the world, FAO and ILCA have joined ILRAD in a partnership in publishing the second edition of this popular work.

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

Preface to the second edition

Helminth parasites are found in cattle, sheep and goats in all countries and regions of the world. Many of 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 the first edition of this handbook for several reasons. We have both lived and worked in various tropical and subtropical environments for several years, where we became conscious that helminth parasites play a controversial role. 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 in much of the world 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 helminth parasites are simple and require simple equipment. Much of this equipment is available in even the smallest of veterinary diagnostic laboratories. This handbook therefore presents procedures and techniques for the identification, diagnosis, survey and control of helminth parasites of ruminants that can be applied in a variety of circumstances and institutions, including universities, national laboratories and the more rudimentary veterinary outposts. The epidemiology of helminth 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 in the world.

In the first edition, we focused on gastro-intestinal helminths in Africa. In this second edition, while maintaining the simple style, we have expanded the scope of the handbook to include helminth parasites of other body systems, and have widened the geographical range beyond Africa to include other areas of the world.

We are grateful to the many people who helped us translate our ideas into reality. This work was initiated when we were Associate Professors of Parasitology (JH) and Epidemiology (BP) at the Virginia-Maryland Regional College of Veterinary Medicine, Virginia, USA. 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 of laboratory materials and to Mr. Jerry Baber, Mr. Dave Elsworth and Mr. Don Massie for taking the photographs. We thank Ms. Janet Schultz for original manuscript preparation, and Ms. Lucy Kirori for preparing the manuscript of the second edition. Camera ready copy of the manuscript was prepared by Mr. Peter Werehire, to whom we are extremely grateful. We also thank the following people for reviewing and commenting on earlier drafts of the original

handbook: Professor James Armour, Dean of the Faculty of Veterinary Medicine, University of Glasgow, Scotland; Professor Peter Nansen, Royal Veterinary and Agricultural University, Copenhagen; Dr. K. Pfister, Faculty of Veterinary Medicine, University of Berne; and Dr. P.J. Waller, Director of the McMaster Laboratory, University of Sidney, New South Wales. Dr. L. Baker of the International Livestock Centre for Africa (ILCA) provided valuable comments on sections of the second edition. We are extremely grateful to Dr. M. Smalley and Mr. P. Neate of ILCA for arranging for the printing of the second edition to be undertaken by ILCA in Addis Ababa. Finally, we thank Merck Agvet Division, Rahway, New Jersey, USA, for contributing to the costs of producing this handbook.

JWH
BDP
Nairobi, November, 1993

---

1. Initial surveys for determining the parasite species present

1.1 Introduction

As a first step in the investigation of helminth infections of ruminants, it is important to establish what parasite species are present in an area, country or region. 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.

While it may be relatively easy to identify some helminth parasites of ruminants simply on the basis of the tissues and organs in which they are located, it may be difficult to identify gastro-intestinal parasites. Many of the gastro-intestinal nematodes look alike but they can 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

Helminth parasites can be classified into four broad groups.

1.2.1 Nematodes

Haemonchus	*Bunostomum* (hookworms)
Ostertagia	*Strongyloides*
Trichostrongylus	*Oesophagostomum*
Mecistocirrus	*Chabertia ovina*
Cooperia	*Trichuris* (whipworms)
Nematodirus	*Dictyocaulus*
Protostrongylus	*Parafilaria*
Muellerius	*Onchocerca*

Toxocara	*Setaria*
Stephanofilaria	*Thelazia*

1.2.2 Cestodes

Monezia	*Cysticercus bovis*
Avitellina	*Cysticercus tenuicollis*
Thysaniezia	*Coenurus cerebralis*
Stilesia	Hydatid cysts

1.2.3 Trematodes

Fasciola	*Paramphistomum*
Dicrocoelium	*Schistosoma*

1.2.4 Protozoa

Coccidia* *(Eimeria)*

* Members of this family *Eimeriidae* are referred to here as Coccidia

As the protozoan parasites usually referred to as coccidia are commonly found in the intestines of ruminants, this manual will cover the main features of the epidemiology, diagnosis and control of these parasites.

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

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

1.3.1 Post-mortem examination

Generally the most useful data will be acquired from young animals and those that have not been recently dewormed. 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. This procedure should be performed towards the end of the rainy season.

Table 1.1 PARASITES LOCATED IN THE GASTRO-INTESTINAL TRACT AND THEIR EFFECT

Site	Host species	Parasites	Action
Rumen	Cattle	*Paramphistomum*	Mucosal damage
Abomasum	Cattle, sheep, goats	*Haemonchus*	Blood sucking
		Mecistocirrus	Blood sucking
		Ostertagia	Mucosal damage
		Trichostrongylus axei	Mucosal damage
Small intestine	Cattle, sheep, goats	*Trichostrongylus*	Mucosal damage
		Bunostomum	Blood sucking
		Cooperia	Mucosal damage
		Nematodirus	Mucosal damage
		Strongyloides	Mucosal damage
		Paramphistomum larva	Mucosal damage
		Coccidia	Mucosal damage
		Monezia	Minimal
Large intestine	Cattle, sheep, goats	*Trichuris*	Blood sucking
		Oesophagostomum	Mucosal damage, nodules
	Sheep	*Coccidia*	Mucosal damage
		Chabertia ovina	Minimal

The identification of parasite species is described in Chapter 4.

1.3.1.1 Gastro-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 which depends particularly on climate (especially rainfall), vegetation and livestock density.

1.3.1.2 Liver

The liver should be examined for migratory tracts (caused by immature *Fasciola* and *Cysticercus tenuicollis* larvae), swellings (hydatid cysts and *Schistosoma* nodules), enlarged bile ducts (adult *Fasciola)* and relatively large transparent cysts attached to the surface of the liver *(Cysticercus tenuicollis).* The presence of parasites associated with these lesions can be confirmed by incisions in the liver parenchyma. Following incision, *Dicrocoelium* and *Stilesia* may be observed in the bile ducts.

Table 1.2 PARASITES LOCATED IN THE LIVER AND THEIR EFFECT

Site	Host species	Parasites	Action
Parenchyma	Cattle, sheep, goats	Immature flukes *Fasciola hepatica, Fasciola gigantica*	Destruction of tissue, fibrosis
		Larval stages of *Cysticercus tenuicollis*	Fibrotic tracts, calcified nodules
		Eggs of schistosomes	Granulomas, destruction of tissue
		Hydatid cysts	Pressure atrophy
Bile ducts	Cattle, sheep, goats	Mature flukes *Fasciola hepatica, Fasciola gigantica*	Blood sucking, destruction of bile ducts, fibrosis
		Dicrocoelium	Minimal (fibrosis)
		Stilesia	Minimal
Liver capsule	Cattle, sheep, goats	*Cysticercus tenuicollis* cysts	Minimal

1.3.1.3 Lungs

Several species of lungworms may cause pathological changes and the post-mortem examination of the lungs may reveal signs of bronchitis, pneumonia, pleuritis, swellings and nodules. Adult *Dictyocaulus* species are found in the trachea and the main bronchi, *Protostrongylus* in the terminal bronchioles and *Muellerius* are usually embedded in grey nodules formed around the alveoli. The lungs are an organ in which hydatid cysts may be located and associated swellings can often be seen or palpated. Occasionally nodules containing liver flukes may be found in the lungs.

Table 1.3 PARASITES LOCATED IN THE LUNGS AND THEIR EFFECT

Site	Host species	Parasite	Action
Trachea	Cattle, sheep, goats	*Dictyocaulus*	Tracheitis,
Bronchi	Cattle, sheep, goats	*Dictyocaulus*	Bronchitis, pneumonia
Bronchioles	Sheep, goats	*Protostrongylus*	Pneumonia, pleuritis
Alveoli	Sheep, goats	*Muellerius*	Nodules
Lung tissue	Cattle, buffalo, sheep, goats	Hydatid cysts	Tissue atrophy

1.3.1.4 Other organs and tissues

The larvae of some cestode species may be found in muscle tissues of cattle *(Cysticercus bovis)* and sheep *(Cysticercus ovis).* The cysts are 6-9 mm in size and semi-transparent when young. As a result of the host's immune response they gradually degenerate, becoming caseous and eventually calcified. The predilection sites are the heart muscle, the tongue, the masseters and the diaphragm.

Some of the filarial worms are found in nodules located in ligaments and tendons *(Onchocerca* species), in muscles *(Onchocerca)* and in the skin or subcutaneous tissue *(Onchocerca, Parafilaria, Stephanofilaria).* Others are found living in the body cavities (*Setaria*).

Parasites of the conjunctival sac and/or lachrymal duct *(Thelazia)* may be very prevalent in some areas.

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 taken preferably 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 and sedimentation procedure for separating and concentrating parasite eggs (see section 3.3) and examined microscopically. In addition the sample should be subjected to a Baerman examination for isolation of lungworm larvae (see section 3.6). The eggs of some parasites are easy to differentiate. The following are examples of these parasites.

Table 1.4 PARASITES LOCATED IN VARIOUS ORGANS AND TISSUES AND THEIR EFFECT

Site	Host species	Parasite	Action
Muscle	Cattle, buffalo, sheep, goats	*Cysticercus* *Onchocerca*	Minimal Minimal
Ligaments, tendons	Cattle, buffalo	*Onchocerca*	Minimal
Skin and subcutaneous tissue	Cattle, buffalo	*Onchocerca,* *Stephanofilaria,* *Parafilaria*	Minimal Minimal Nodules, damage to carcass surface
Body cavities	Cattle, buffalo	*Setaria*	Minimal
Central nervous system	Cattle, buffalo sheep, goats	*Setaria* larvae	Neurological disturbance
	Sheep, (goats, cattle)	*Coenurus cerebralis*	Neurological disturbance
Circulatory system	Cattle, sheep, goats	Schistosomes	Minimal *
Eye	Cattle, sheep goats	*Thelazia*	Minimal

** Note: Most of the pathogenic effect of schistosomes is related to the migration of the eggs.*

Trichuris

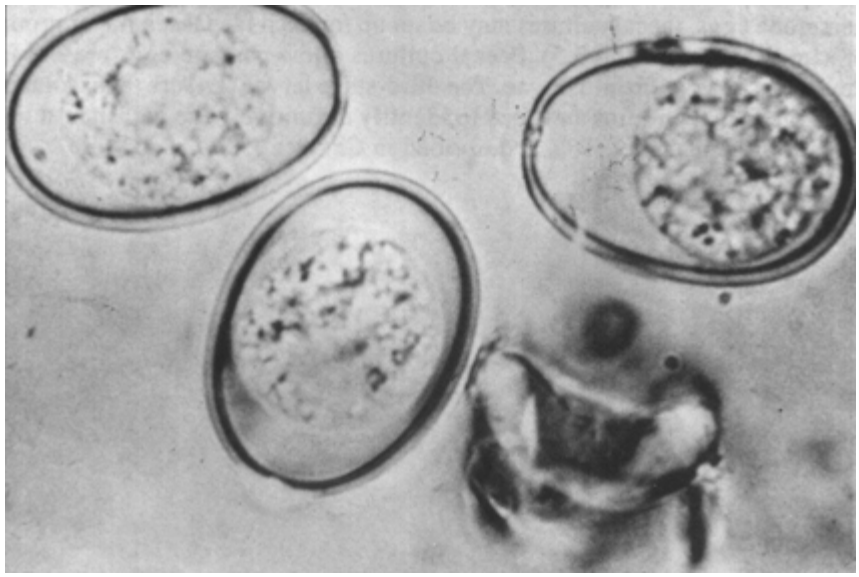
Strongyloides

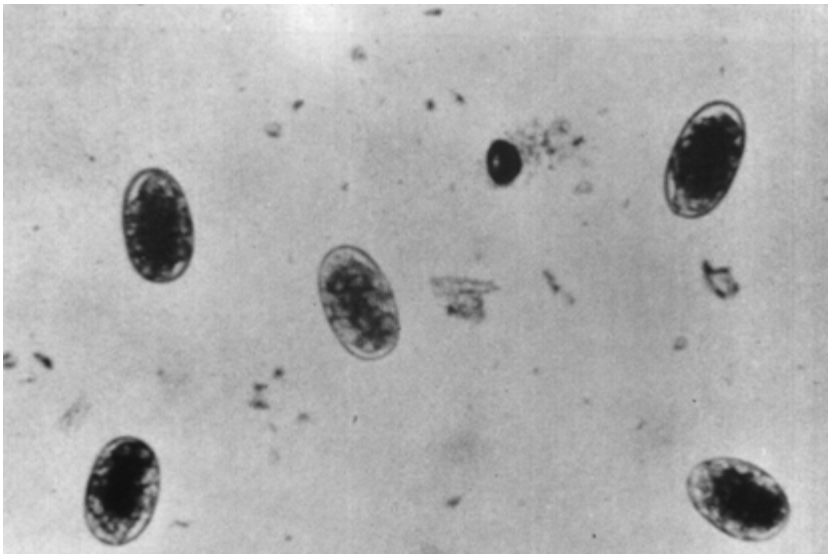
Nematodirus

Monezia

Paramphistomum

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 (L_3) 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.

---

2. The epidemiology of helminth parasites

2.1 Introduction

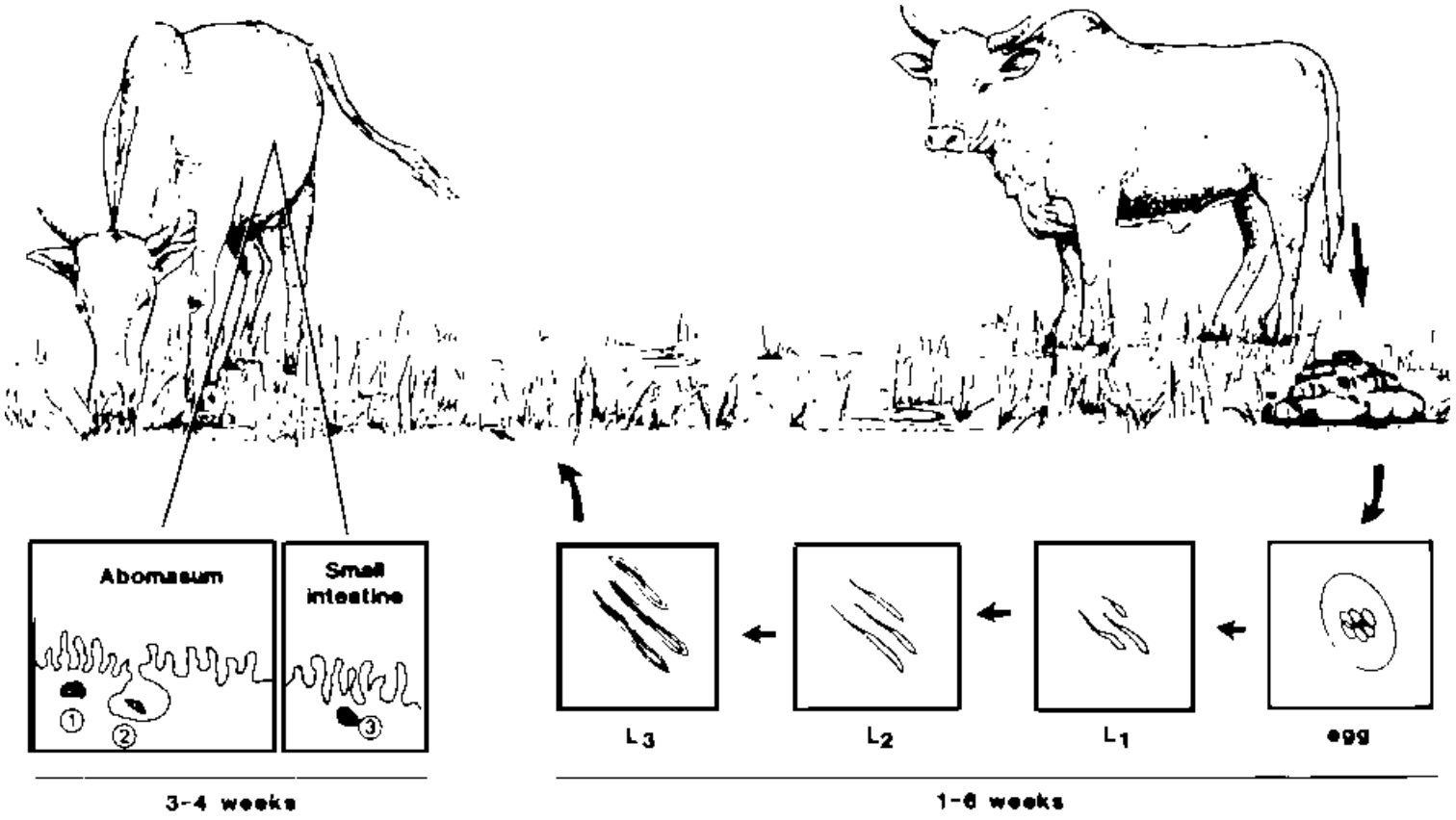
This section is devoted primarily to the epidemiology of the nematodes (roundworms), trematodes (flukes) and cestodes (tapeworms) of greatest economic importance.

2.2 Nematodes of the digestive tract

2.2.1 Life cycles

The most important and widely prevalent nematodes are the Trichostrongyle group *(Haemonchus, Ostertagia, Trichostrongylus, Mecistocirrus, Cooperia* and *Nematodirus), Oesophagostomum* and *Bunostomum.* 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 $L_1 \rightarrow L_4 \rightarrow L_5 \rightarrow$ Adult
(Haemonchus sp., T.axei)

② $L_3 \rightarrow L_4 \rightarrow L_5 \rightarrow$ Adult
(Ostertagia sp.)

③ $L_3 \rightarrow L_4 \rightarrow L_5 \rightarrow$ Adult
(T. colubriformis, Cooperia sp. Nematodirus)

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 (L_1) which in turn moult into second-stage larvae (L_2), shedding their protective cuticle in the process. The L_2 larvae moult into third-stage larvae (L_3), but retain the cuticle from the previous moult. This double-cuticled L_3 is the infective stage. 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 L_3. Most larvae are picked up during grazing and pass to the abomasum, or intestine, ex-sheathing the extra cuticle in the process. The L_3 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 L_3 moult to the fourth stage (L_4) and remain in the mucous membrane (or in the gastric glands) for about 10 to 14 days. They then emerge and moult into a young adult stage (L_5). Most Trichostrongyles 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 L_3 penetrate the lamina propria of the intestinal wall and the host response to the infection which surrounds the L_3 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 L_3 larvae of *Bunostomum* infect ruminants when they are ingested or penetrate the hosts skin. Following

skin penetration, the larvae are carried in the venous blood through the heart to the lungs, where 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 L_3 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

Nematode	Prepatent period
Haemonchus placei (cattle)	3-4 weeks
Haemonchus contortus (sheep)	2 weeks
Ostertagia (sheep and cattle)	3 weeks

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

Different species of nematodes have different egg-producing capacities as shown in Table 2.2. 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 gastro-intestinal parasites.

Table 2.2 DAILY EGG PRODUCTION PER FEMALE OF SOME GASTRO-INTESTINAL NEMATODES

Nematode	Daily egg production/female
Haemonchus	5000-15000
Ostertagia, Trichostrongylus	100-200
Cooperia	1000-3000
Nematodirus	50-100
Oesophagostomum, Chabertia	5000-10000

The number of eggs produced by an adult female nematode also depends on the level of immunity the host possesses to the intestinal parasites. 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 tropical and sub-tropical countries, temperatures are permanently favourable for larval development in the environment. Exceptions to this are the highland and mountainous regions throughout the world, and the winters of southern Africa and Latin America 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.

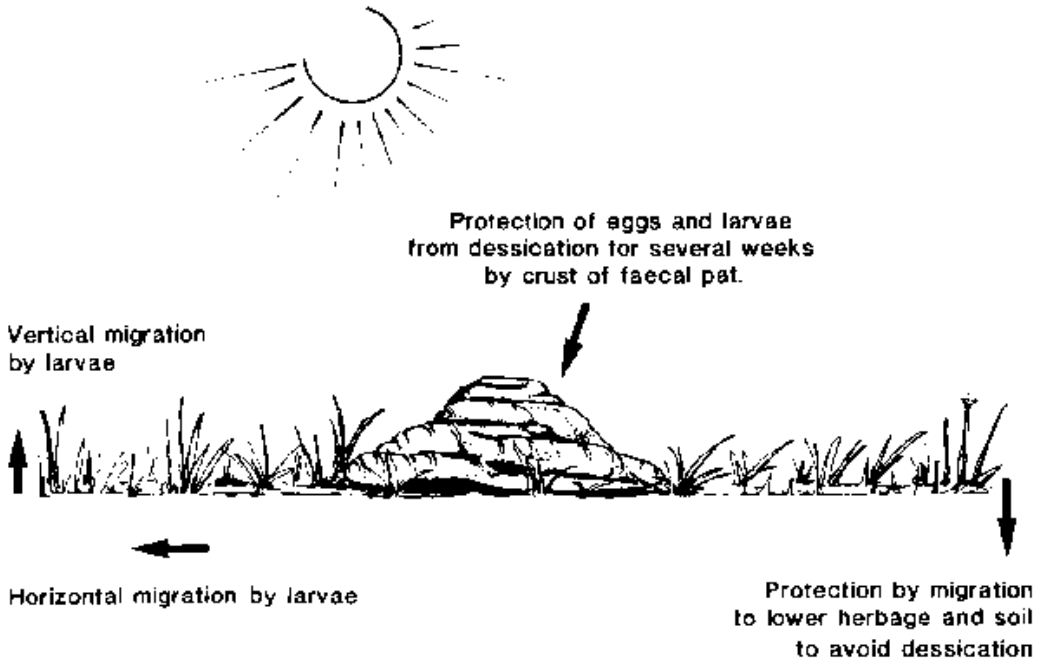
The ideal humidity for larval development in this microclimate is 100%; the minimum humidity required for development is about 85%.

The survival of larvae in the environment depends upon adequate moisture and shade. Desiccation from lack of rainfall kills eggs and larvae rapidly and is the most 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. Infective larvae may survive for up to 6 weeks or even longer in the manure pats, which act as a reservoir of infections during dry periods. The development of infective larvae ingested by an 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 (L_1, L_2, and L_3) it is the L_3 which has a protective sheath, that is the most resistant to variations in moisture, temperature and sunlight.

2.2.4 Dissemination of infective larvae

The parasite's eggs develop into third-stage, infective larvae L_3 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.

FIGURE 2.2 Survival and dissemination of larvae on pasture



The horizontal distance L_3 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 may 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 regions:

- humid tropical climate
- savannah-type tropical and sub-tropical climate with a long dry season
- arid tropical and sub-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. It is also the climate of much of southeastern Asia, central America and northern South America. This climate provides a more or less permanently favourable environment for the survival and development of parasitic larvae.

The savannah type tropical, sub-tropical and temperate climate with a long dry season is found in much of eastern, central and southern Africa, much of South America, and areas of western, central and eastern Asia. 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 and sub-tropical climates characterize parts of western Asia, lowland Ethiopia, parts of Somalia and Sudan and much of northern Africa and 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 gastro-intestinal nematode infections

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

- The number of infective larvae (L_3) ingested by the host, which in turn is influenced by the climate, the amount of protection of larvae provided by vegetation, the livestock density 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 gastro-intestinal nematode infections

2.2.7.1 Effect of larval stages on the host

Considerable damage is caused by fourth-stage larvae (L_4) of abomasal parasites *(Haemonchus, Mecistocirrus, Ostertagia* and *T. axei).* The L_3 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 wall during the dry season until the next rainy season or until the animal experiences stress, such as that produced when the animal is calving/lambing or sick. The inhibition will then cease, and the L_4 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 emerging.

The L_4 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 any of the species *Haemonchus, Mecistocirrus, 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 feed intake
- diarrhoea
- mortality
- reduced carcass quality
- reduced wool production/quality

Severe blood and 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.

2.2.8 Toxocara vitulorum infections

Although *Toxocara vitulorum* is an intestinal nematode, the life cycle and the epidemiology of this parasite is markedly different from that of the Trichostrongyle group described on the previous pages.

Toxocara vitulorum is a large ascarid-type parasite (20-30 cm) which has a world-wide distribution. The prevalence is, however, much higher in the tropics and it causes severe problems in young calves (cattle, buffalo) in Southeast Asia and parts of Africa.

2.2.8.1 Life cycle

The life cycle is direct with possible prenatal infection and with neonatal infection through colostrum being the major route of infection for calves in Southeast Asia. The adult parasites which live in the small intestines are prolific egg producers and a very large number of eggs are produced every day. The thick-walled eggs are very resistant to adverse climatic and environmental conditions and may remain infective for a long period of time (several years).

Only if the infective eggs are ingested by young calves will the life cycle be completed. The *Toxocara* larvae penetrate the intestinal wall and migrate via the circulatory system to the liver and lungs where they enter the respiratory system. The larvae are coughed up and swallowed, returning to the small intestine where they mature and start egg production 3-5 weeks after infection.

If the infective eggs are ingested by older calves (more than 4 months of age) that possess immunity, the majority or all of the larvae that undergo somatic migration become arrested in organs and tissues. During pregnancy these larvae become reactivated and prenatal infection of the foetus is possible, but the majority of larvae are concentrated in the udder and new-born calves are usually infected through colostrum and milk. Following infection via this route, the larvae do not migrate in the hosts, but remain in the small intestine. This reduces the length of the prepatent period and eggs may be present in faeces 18-21 days after infection.

Whereas transmission through colostrum and milk is the major route of infection of calves in Southeast Asia, studies in the southern part of Africa have indicated that the ingestion of infective eggs from the environment is the most common route of infection there.

It is recommended that the local epidemiology of this parasite should be established for most efficient control.

2.2.8.2 Pathogenicity of Toxocara infections

Migrating larvae may cause damage to the liver and lungs. The presence of the adult parasites in the small intestine is often associated with diarrhoea and reduced weight gain. In untreated cases and heavy infections, the mortality rate may be up to 35-40 percent of infected animals, and it is believed to be the most serious disease of buffalo calves in Southeast Asia. The parasites are expelled by 5 months of age.

2.3 Nematodes of the lungs

2.3.1 Introduction

Lungworms are widely distributed throughout the world but are particularly common in countries with temperate climates, and in the highlands of tropical and sub-tropical countries. The species of importance in ruminants belongs to two different families; the Dictyocaulidae and the Metastrongylidae. The Dictyocaulidae include *Dictyocaulus viviparus* in cattle and buffaloes, and *Dictyocaulus filaria* in sheep and goats. These worms are 5-10 cm long and live in the trachea and bronchi. The Metastrongylidae are represented by at least three species in small ruminants. *Protostrongylus rufescens* a small worm (1.5-3.5 cm) found in the bronchioles, *Muellerius capillaris* (1.2-2.5 cm) which are located in the alveoli, and *Cystocaulus ocreatus* (2-5 cm) found in the terminal bronchioles.

An infection of the lower respiratory tract by any of these nematode species may result in bronchitis or pneumonia, or both.

2.3.2 Life cycles

The *Dictyocaulus* species have a direct life cycle and the behavior of the free-living stages is similar to that described for the trichostrongyles of the digestive tract (see section 2.2.1). The infective larvae are ingested by the final host during grazing and the larvae migrate from the intestine to the lungs via the lymphatic system and the pulmonary blood supply. They emerge from the pulmonary capillaries and enter the alveoli, migrating to the bronchi and trachea where they mature. The prepatent period is approximately 4 weeks for

D. viviparus and 5 weeks for *D. filaria.*

The *Metastrongylus* species have indirect life cycles that require a land snail as an intermediate host. The first stage larvae (L_1) which are passed in the faeces infect snails by penetrating the foot of the snail, or by being ingested. The development of infective larvae in the snails takes approximately 2 weeks. The final host is infected by accidentally ingesting snails with their food. The released larvae migrate from the intestine to the lungs via the lymphatic system, similar to the route of the *Dictyocaulus* species. The prepatent period is 6-7 weeks.

2.3.3 Development and survival of infective larvae

Eggs laid by the female worms hatch quickly and the L_1 larvae are coughed up, swallowed and appear in faeces.

For the *Dictyocaulus* species, the development into third stage larvae (L_3) takes a minimum of 5-7 days, but it may take longer depending on the ambient temperature and humidity. The *Dictyocaulus* larvae are generally more susceptible to adverse environmental conditions than are the larvae of the gastro-intestinal nematodes. Desiccation rapidly kills the larvae whereas moderate temperatures and high humidity will enhance their survival.

The L_1 *Metastrongylus* larvae are fairly resistant to drying and the stages in the snails are well protected during adverse conditions. The infective larvae probably survive in the snails for as long as the snails live and for up to a week after the death of the snail.

2.3.4 Pathogenic effect

The pathogenic effect of lungworms depends on their location within the respiratory tract, the number of infective larvae ingested and the immune status of the animal.

During the early stages of a *Dictyocaulus* infection (the prepatent phase) the small bronchioles are blocked by exudate, which obstructs the airways, and this may result in the collapse of the lung tissue distal to the blockage. The adult nematodes (the patent phase) in the bronchi cause a bronchitis. Emphysema, pulmonary oedema and secondary infections are common complications in severe cases. After 2-3 months all or most of the adult worms are expelled.

The pathogenic mechanisms of the other lungworm species *(Muellerius* and *Protostrongylus)* is similar but they rarely produce serious effects. This may in part be due to their more restricted localization in the lungs. The formation of granulomas seems to be the predominant reaction following the infection with *Muellerius* and they are often found subpleurally in the caudal lung lobes.

Following infection, most animals develop varying degrees of immunity, but in the absence of reinfection the immunity may decrease, rendering the animal susceptible again.

The clinical signs of lungworm infection may range from moderate coughing, exacerbated during stress, to severe persistent coughing with marked increase in respiratory rates accompanied by respiratory distress. Animals may lower their head and stretch it forward. Severe infections in cattle are often accompanied by production losses.

2.3.5 Factors influencing the epidemiology of lungworm infections

The transmission and maintenance of these infections from year to year is dependent on some infected animals harbouring small numbers of adult lungworms for several months and thus serving as carriers. The carrier animals continue to contaminate the pastures, and the infection cycle is maintained in the population at risk. As a result, the number of infective larvae on pasture may reach levels that cause outbreaks of clinical disease. Larvae of some lungworm species may become inhibited in the lung tissue during periods of adverse climatic conditions (such as a dry season) and then mature at the beginning of the rainy season.

2.4 Nematodes of other organs and tissues

2.4.1 Filarial nematodes

The economic importance of filarial nematodes varies according to the species. The distribution and impact of these parasites is dependent on the availability of the intermediate hosts (various insect species).

The filarial nematodes which may cause disease and loss of productivity in ruminants, belong to the genera *Stephanofilaria, Onchocerca, Parafilaria* and *Setaria.*

2.4.1.1 Life cycles

The life cycles of these parasites are similar to each other and are indirect, requiring insects as intermediate host for development and transmission.

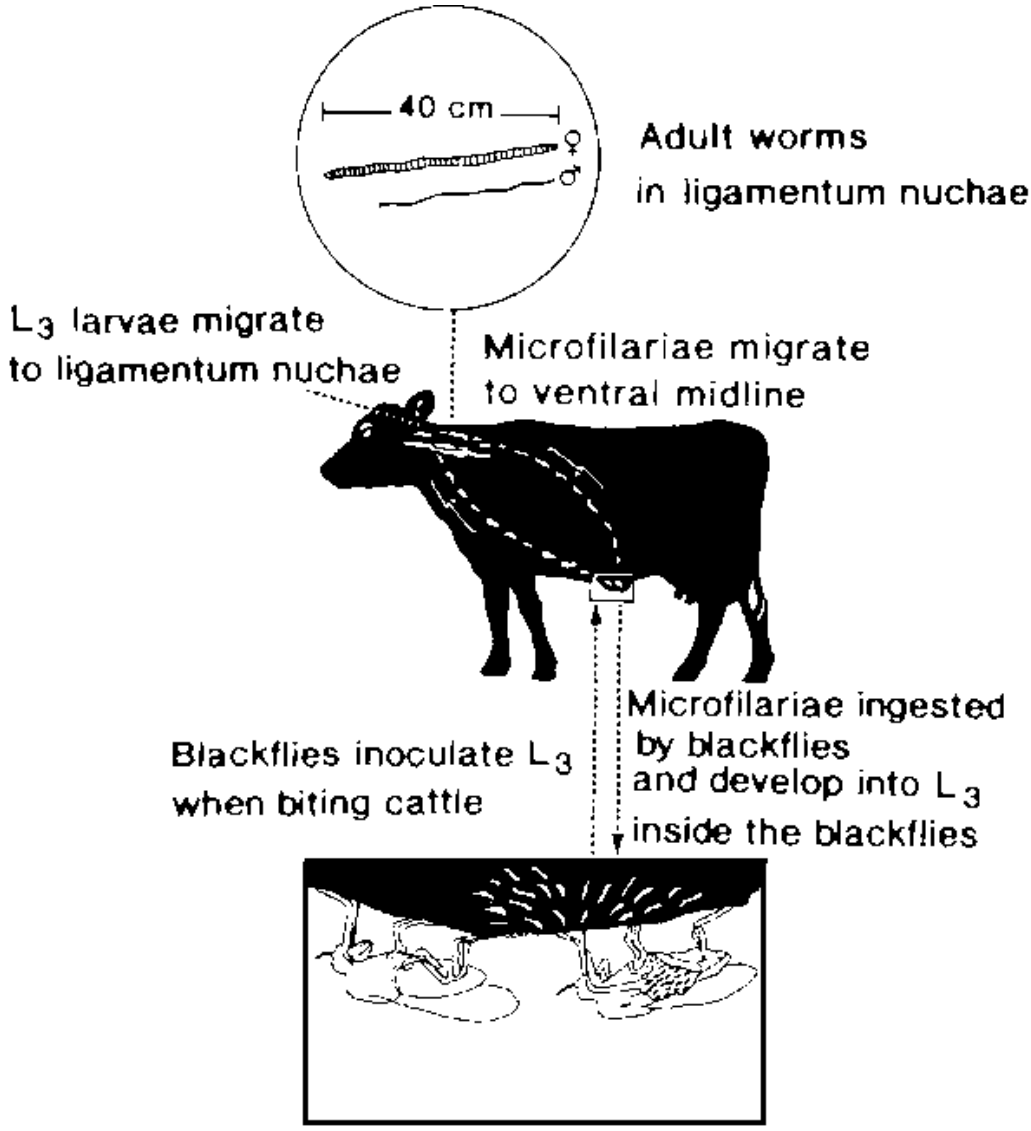
2.4.1.1.1 *Stephanofilaria*

The adult parasites live in the skin, where they cause an inflammation. Small papules develop and these coalesce to form larger crusty lesions. The female parasites produce microfilaria (first stage larvae) which remain with the adult parasites in the skin lesions. Several dipteran fly species act as intermediate hosts, with certain species predominating in a given geographical area. Adult flies ingest the microfilaria when they feed on the open lesions caused by the nematodes. Development into the third stage infective larvae occurs in the fly and takes from 10 to 25 days. The fly can then transmit *Stephanofilaria* to a new host.

2.4.1.1.2 *Onchocerca*

These parasites are found in the connective tissue of their hosts. Their presence often results in the formation of hard nodules in which the nematode is coiled up. The location of the nodules in the host varies according to the species of *Onchocerca* and may be found in intra-muscular and subcutaneous connective tissue *(Onchocerca dukei),* in ligaments *(Onchocerca gutturosa),* intradermally *(Onchocerca dermata* and *Onchocerca ochengi)* and in the aorta *(Onchocerca armillata).* The parasites produce microfilaria which circulate in the blood stream. The microfilaria are picked up by biting insects (midges, black flies and others) and develop into infective third stage larvae. These are transmitted when the insect feeds on a new host.

FIGURE 2.3 Life if cycle of *Onchocerca gutturosa*



2.4.1.1.3 *Parafilaria*

The adult parasites are located in subcutaneous nodules which are found primarily in the shoulder region and other dorsal areas of the body. When the female nematode invades the skin to lay eggs, it causes an inflammatory response and the nodules become enlarged. The penetration of the skin results in bleeding and these sites are known as bleeding points. Various species of dipteran flies become infected when they feed on the blood. The development into the infective third stage larvae takes *2-3* weeks in the fly. Transmission occurs when the flies feed on wounds. The maturation of the parasite in the final host may last 8-10 months.

2.4.1.1.4 *Setaria*

These worms are commonly found in the peritoneal cavity of ruminants. They are 6-15 cm long. The microfilaria produced by the female circulate in the blood and are ingested by biting insects. Several species of mosquitoes and flies serve as intermediate hosts.

2.4.1.2 Pathogenicity of filarial nematode infections

Although the prevalence of infections with these parasites may be high in some areas they usually only cause minor losses, with a few exceptions. In certain localized regions, *Parafilaria* species cause considerable losses at slaughter that result from the trimming of affected parts of the carcass. The major pathogenic effect of *Setaria* species occurs when the larvae accidentally migrate in the central nervous system of abnormal hosts, such as sheep and goats. The condition is known as endemic cerebrospinal nematodiasis and it is quite common in parts of Asia.

2.4.2 Nematodes of the eye

Thelazia species are common parasites of the conjunctival sac or lacrimal duct of cattle, buffaloes, sheep and goats. These worms are cosmopolitan in their distribution.

2.4.2.1 Life cycle

The life cycle is indirect and several dipteran flies act as intermediate hosts. The flies become infected when feeding on lacrimal secretions which contains eggs or larvae. Development of the infective stage in the fly takes from 2 to 4 weeks. When the flies feed in the eye region of a host, infective larvae may be released into the eye. The transmission is seasonal in some areas, following the seasonal variation in the abundance of fly populations.

2.4.2.2 Pathogenicity of eyeworms

These infections are common, but the majority of infections have no pathogenic effect on the host, apart from stimulating increased lacrimation from infected eyes. However, disease can occasionally occur in infected animals and an inflammatory reaction can be observed. In severe cases the cornea becomes cloudy, and the eye is swollen and covered with pus. Infections can be treated by injecting 2 ml of levamisole into the subconjunctival sac or by the use of eye ointments containing 4 % morantel tartrate or 1% levamisole.

2.5 Trematodes

2.5.1 Introduction

All the trematode species which are parasitic in livestock belong to the subclass Digenea. In general, these trematodes (known commonly as flukes) are dorso-ventrally flattened, some being leaf-shaped and some long and narrow; the gastro-intestinal flukes have thick fleshy bodies. The schistosomes, which also belong to this group, are elongated and almost roundworm-like in appearance. The flukes that parasitize livestock are hermaphrodites (except the schistosomes) but they have the ability to reproduce asexually and multiply in aquatic or amphibious snails, which they require as intermediate hosts in order to complete their life cycles. Most flukes are very discriminating in their choice of snail as intermediate host and the geographic distribution of trematode species is dependent on the distribution of suitable species of snails.

This section describes the ecology and epidemiology of the flukes of greatest economic importance.

2.5.2 Trematodes of the liver

Fasciola hepatica is leaf-shaped and may reach a size of 30 x 30 mm. It occurs in the bile ducts of a large number of ruminants, equines, pigs, rabbits and other animals and is cosmopolitan in its distribution. *Fasciola gigantica* resembles *F. hepatica* but is easily distinguished by its characteristic shape and larger size. It is common in Africa, the Indian sub-continent, Central and Southeast Asia, and other sub-tropical and tropical areas of the world. Mixed infections with the two flukes may occur.

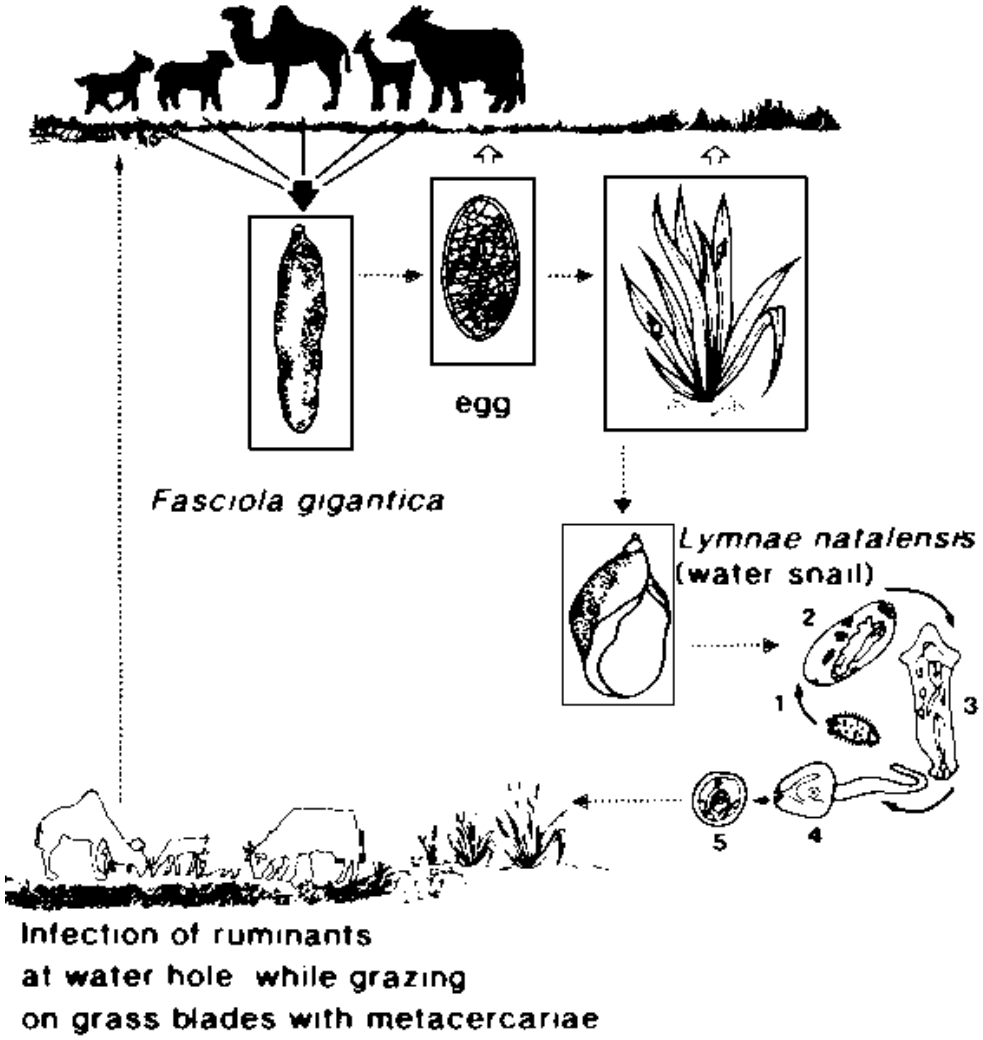
Dicrocoelium dendriticum is a small fluke, 6-10 mm long and 1-3 mm wide. It has an elongated lances-like body and inhabits the bile ducts of livestock and rodents. It occurs in Asia, North Africa, Europe, North America and rarely in South America.

2.5.2.1 Fasciola hepatica and Fasciola gigantica

2.5.2.1.1 Life cycles

Fasciola hepatica and *F. gigantica* have similar life cycles. The adult flukes inhabit the bile ducts of the final host (cattle, buffaloes, sheep, goats). The hermaphroditic parasite produces eggs which are expelled with the bile into the intestine and shed in the faeces. The eggs embryonate and hatch in water or wet pastures, releasing a free-swimming miracidium. The ciliated miracidia actively seek and penetrate suitable intermediate hosts and undergo several stages of development by asexual multiplication. Five to seven weeks after infection of the snail the tadpole-like motile cercariae emerge from the snail and swim until they make contact with herbage. They then encyst on blades of grass close to streams or in low-lying damp pasture areas. Infection of the final host occurs by ingestion of herbage contaminated with the encysted metacercariae. After ingestion, the young flukes are released from the cysts in the small intestine. They penetrate the intestinal wall and migrate through the abdominal cavity and the liver capsule into the liver parenchyma. Following the penetration of the capsule the immature flukes migrate through the liver tissues for about 6-8 weeks and then enter the bile ducts where they mature and commence egg production.

FIGURE 2.4 Life cycle of Fasciola gigantica



2.5.2.1.2 Life cycle of the intermediate snail host

The important *Lymnaea* species of snails involved in the transmission of fascioliasis vary in their geographical distribution in the world. The habitat requirements of the intermediate hosts of the two most important liver flukes differs slightly. The intermediate hosts for *F. hepatica* are amphibious snails that live close to the edge of slow moving or stagnant water whereas those transmitting *F. gigantica* live in deeper water and are close to being true aquatic snails in their behaviour. They can, however, adapt to an amphibious existence in adverse conditions. The optimum temperature range for development of the snail is 15-26 °C, when rapid production of snail egg masses occurs. These eggs hatch within two weeks and the

resulting snails mature a month later. Thus one snail can produce several thousand descendants within a period of 10-12 weeks. No development and no reproductive activity takes place at temperatures below 10 °C, but snails may survive adverse conditions for months buried in the mud.

2.5.2.1.3 Egg production

The prepatent period for *F. hepatica* is about 8-12 weeks and 10-14 weeks for *F. gigantica.*

The egg-producing capacity of the liver flukes is very high and each fluke may produce 5,000-20,000 eggs per day throughout its life. The factors which influence the egg production of *Fasciola* species are not well understood, but include the level of nutrition of the parasites in the bile ducts, and this can be influenced by crowding of flukes and reduced blood intake when bile ducts calcify in cattle. The eggs are carried through the bile to the gall-bladder, which may serve as a reservoir of eggs for a considerable time. Eggs are expelled from the gall-bladder when it contracts during digestion, and large numbers of eggs are released during these contractions. This means that the number of liver fluke eggs in a faecal sample is not an accurate indication of the number of parasites in the liver, nor of the amount of damage being done to the host.

The factors which influence the number of parasite eggs in faeces are similar to those listed for nematodes (see section 2.2.2).

2.5.2.1.4 Development and survival of eggs and miracidia

Light is essential for the embryonation and hatching of eggs and this process does not take place while the eggs are in the faecal mass. For development to start the eggs need to be washed out of the manure by rain or surrounding water. Eggs are rapidly killed by desiccation, but they may survive for months in moist faeces; manure pats may thus act as a reservoir of the infection for a considerable length of time. The embryonation of eggs also depends on suitable temperatures and the availability of oxygen. In most tropical and sub-tropical countries, temperatures are permanently favourable for the embryonation and hatching of the eggs and larvae. Exceptions to this are some highland and mountainous areas.

The time taken for development to be completed is temperature-dependent; this is about 6 weeks at 15 °C and 10 days at 22 °C. The ideal temperature for development is between 20 and 25 °C. At temperatures above 25 °C (up to approximately 35 °C) development takes place at an ever increasing speed but the mortality rate of embryos will increase at higher temperatures. No development takes place below 10 °C.

The miracidia do not survive for more than approximately 24 hours following their release from the egg and their survival depends on locating a snail within this period. The swimming pattern of the miracidia is guided by numerous factors including phototaxis and chemotaxis. When a snail is located the miracidia penetrate the snail.

2.5.2.1.5 Development and survival of larvae in the intermediate host

Following penetration of the snail, the miracidia transform into sporocysts; through several stages of development and asexual multiplication, one miracidium may produce 600-4,000 cercariae. The development in the snail is temperature-dependent and at optimum temperatures cercariae may be shed from the snail as early as 5 weeks after infection. At lower temperatures the development rate slows down and at temperatures below 10 °C no development takes place. The larval stages may survive in the snails for several months and this is often the mechanism for persistence of the infection from season to season.

2.5.2.1.6 Development and survival of the metacercariae

Survival of the motile cercariae emerging from the snail depends on their ability to locate a suitable object on which to encyst within 1 hour. When contact is made with blades of grass the cercariae lose their tails and encyst, becoming metacercariae. These are the infective stages to the final host. The metacercariae are relatively resistant to unfavourable climatic conditions and may survive for as long as a year under conditions of high humidity and moderate temperatures. Many die during prolonged periods of drought, freezing or very high temperatures.

2.5.2.1.7 Infection of the final host

Infection of the final host occurs by ingestion of encysted metacercariae on herbage, or less commonly by ingestion of suspended metacercariae in drinking water. Once ingested, the young flukes encyst in the small intestine, penetrate the gut wall and traverse the abdominal cavity to reach the liver capsule and the liver tissue. The immature flukes migrate in the liver parenchyma for 6-8 weeks before entering a bile duct where

they mature and commence egg production.

2.5.2.1.8 Pathogenicity of liver fluke infections

Fascioliasis in ruminants ranges in severity from a devastating highly fatal disease in sheep to an asymptomatic infection in cattle. The severity of pathological manifestations usually depends on the number of metacercariae ingested over a period of time and the relative susceptibility of the animal. In sheep, acute fascioliasis occurs seasonally and is manifest by anaemia and sudden death. Deaths can occur within 6 weeks of infection. Cases of chronic fascioliasis occur in all seasons and the clinical signs may include anaemia, reduced weight gain, decreased milk production, unthriftiness, submandibular oedema and possibly death in sheep. In contrast, even heavily infected cattle may show no obvious clinical signs, but some production losses may be evident.

Under natural conditions there is little evidence of any acquired immunity to fascioliasis in sheep and the effect of additional infections is additive as far as pathogenicity is concerned. This is evident at post-mortem examination where a succession of developmental stages may be found. Cattle seem to be less susceptible than sheep, and following the first infection immunity develops, reducing the migration of immature flukes in subsequent infections. The development of fibrosis of the liver, the calcification of the bile ducts and acquired immunity may be responsible for the elimination of the infection which occurs in some animals.

2.5.2.1.9 Effect of immature flukes on the host

The migration through the intestinal wall appears to be non-damaging to the host but the penetration of the liver capsule by a large number of young flukes results in an inflammatory response of the capsule (peri-hepatitis). The subsequent simultaneous migration of many immature flukes through the liver parenchyma causes severe destruction of liver tissue, especially during the last 2-3 weeks before they enter the bile ducts. This may result in bleeding into the abdominal cavity which can be severe enough to cause sudden death of the animal (acute fascioliasis). With smaller numbers of migrating immature flukes, the liver damage may be considerably less and clinical signs may even be absent. During the reparatory phase following fluke migration, the liver tissue may show varying degrees of fibrosis.

2.5.2.1.10 Effect of adult flukes on the host

After 6-8 weeks of migration in the liver tissue the young flukes enter the bile ducts. Their blood sucking activities irritate the lining of the ducts, resulting in an inflammatory response and the associated blood loss results in anaemia. Considerable thickening of the bile duct walls occurs with the result that these protrude markedly from the surface of the liver. In cattle the ducts often become calcified giving rise to the name "pipe-stem liver". The inflammatory effect is not limited to the bile ducts. Irritation of the ducts and obstruction of the bile flow may cause severe fibrosis of the liver.

2.5.2.1.11 Seasonal transmission and epidemiological patterns

Moisture is the critical factor determining the presence and extent of snail habitats, which serve as transmission foci for liver flukes. Temperature is an important factor affecting the rate of development of snails and of the stages of the parasite outside the final host. The interaction between moisture and temperature determines the survival and reproduction rate of the snails and the parasites.

The liver flukes have a versatile survival strategy; certain stages of the parasites and their intermediate hosts have a relatively well-developed ability to persist through adverse weather conditions such as drought and freezing. Thus persistence of infection from one season to the next may occur by several mechanisms: as adult flukes in mammalian hosts, as eggs on pasture, as larvae developing in snails and as metacercariae encysted on herbage.

2.5.2.2 Dicrocoelium dendriticum

2.5.2.2.1 Life cycle

The life cycle is unusual for flukes in that this parasite does not require an aquatic environment at any stage in the development. The adult flukes live in the bile ducts and eggs are passed in the faeces. The eggs are small, operculated, dark brown and typically flattened on one side. Two intermediate hosts, a terrestrial snail and an ant, are required for the completion of the cycle. The eggs are ingested by the first intermediate host, the snail, when it feeds on manure. A miracidium hatches out which undergoes asexual multiplication and further development in the snail. Cercariae are released from the snail and different species of ants eat these. Metacercariae are formed in the ant and the final host is infected by accidentally ingesting the infected ants. After excysting in the gut of the final host young immature flukes enter the liver via the main

bile ducts and mature in the smaller bile ducts.

2.5.2.2.2 Development and survival of larvae in the intermediate hosts

Miracidia hatch out in the snail after it has ingested embryonated eggs. The miracidia migrate in the snail and transform into sporocysts, the first stage of asexual multiplication. Numerous cercariae develop from a single miracidium. The cercariae emerge from the snails clumped together in masses called "slime-balls", in which several hundred cercariae are held together by a sticky material which adheres to the vegetation. The slime-balls are ingested by ants, and metacercariae develop in the abdominal cavity of the ants. Some of these may enter the central nervous system of the ant resulting in behavioural changes. These changes render the infected ants more susceptible to ingestion by grazing animals. The rate of development in the snail is usually slow, and lasts *3* months or more.

2.5.2.2.3 Pathogenicity of *Dicrocoeleum dendriticum*

The young flukes migrate directly up the biliary duct system of the liver. There is no penetration of the gut wall or the liver capsule as in *Fasciola* and no migration in the liver tissue. The effect on the liver depends on the number of flukes and the duration of the infection. Massive long-lasting infections may cause hypertrophy of the bile ducts and progressive fibrosis of the liver. Clinical signs and loss of production are rare in cattle; however, older sheep may suffer reduced milk production and loss of weight. Sheep production can be unprofitable in areas with a high prevalence of the infection.

2.5.2.2.4 Factors affecting the epidemiological pattern

The eggs of *D. dendriticum* are relatively resistant and may survive for months in soil and faeces. In addition the intermediate hosts are not restricted to aquatic environments and both snails and ants may be widely distributed over pastures. The infection is often maintained in wildlife, which may serve as a source of infection to livestock.

2.5.3 Gastro-intestinal trematodes

A large number of gastro-intestinal trematode species (paramphistomes) have been described. They are usually thick, short (4-12 mm), fleshy, maggot-like worms. They may infect all ruminants but young calves and lambs are the most susceptible. The infections are very common in Africa, Asia, Oceania, Eastern Europe, Russia and some of the Mediterranean countries. Not all the species are pathogenic, but clinical outbreaks of paramphistomiasis have been caused by *Paramphistomum microbothrium* (Africa), *Cotylophoron cotylophorum (Asia), P. ichikawar, C. calicophorum* (Australasia) and *P. cervi* (Europe).

2.5.3.1 Life cycles

Paramphistomes require an aquatic snail as an intermediate host and the pre-parasitic stages of the life cycle (miracidia and stages in the snail) are very similar to those of *F. hepatica* and *F. gigantica* described earlier.

The metacercariae encysted on the herbage are ingested and young flukes are released in the duodenum of the final host. They attach to - or invade - the duodenal mucosa, usually in the proximal 3 m of the gut. The immature flukes re-emerge/detach from the mucosa 10-30 days after initial infection and migrate towards the rumen and reticulum, where they attach to the mucosa and mature into egg-producing adult parasites. The pre-patent period is reported to vary from approximately 56 days in cattle to around 70 days in sheep and goats. The parasites appear as reddish/pink clusters between the papillae of the rumen and reticulum. Adult paramphistomes may survive for many years in the rumen of the host.

2.5.3.2 Pathogenicity of paramphistomes

2.5.3.2.1 Effect of larval stages on the host

The size of the paramphistome burden is the most important factor determining the degree of small intestinal damage and the possible clinical effects. The immature flukes may be responsible for severe damage while embedding in and penetrating the mucosa. This causes bleeding and necrosis in the gut wall. If present in

sufficient numbers the damage may be responsible for clinical signs. Affected animals are listless, with reduced appetite and increased thirst. A profuse foetid diarrhoea develops which may be accompanied by anaemia, oedema and emaciation. Severe cases lead to death in a few days, especially in young calves and lambs.

2.5.3.2.2 Effect of adult flukes on the host

Little if any pathogenic effect is associated with the presence of the adult flukes in the rumen and/or reticulum, even though large numbers may be present. Localized destruction of rumen papillae may be seen, but this appears to have no measurable effect on the host.

Two species of the genus *Explanatum (Gigantocotyle)* develop to maturity in the bile ducts and cause severe fibrosis.

2.5.3.3 Factors affecting the epidemiological pattern

The infection of cattle, sheep and goats with paramphistomes is very common. These parasites may survive for years, so there is a virtually constant source of infection for successive generations of snails. The intermediate hosts (snails of the genus *Planorbidae* and for some species *Lymnaeidae)* are extremely adaptable and prolific breeders, which ensures a widespread availability of the snails within infested areas. Massive asexual multiplication of the parasites in infected snails and the survival of snails for several months may result in the shedding of large numbers of cercariae. Infected snails may also survive in mud for months.

Clinical outbreaks of paramphistomiasis are usually confined to the drier months. During this period, the snail population becomes concentrated around natural sources of water and as these areas may provide the only dry season grazing, animals may become heavily infected. Older animals, especially cattle, seem to acquire immunity to the infection.

2.5.4 Pancreatic trematodes

Several *Eurytrema* species have been found in the pancreatic ducts of sheep, goats, cattle and buffaloes in eastern Asia and Brazil. Their length varies from 8-16 mm.

2.5.4.1 Life cycles

The pancreatic flukes have two intermediate hosts. The eggs are passed in the faeces and ingested by different species of land snails. A miracidium is released and asexual multiplication and development takes place in the snails. Cercariae are released onto herbage where they are ingested by second intermediate hosts, grasshoppers and crickets. The final hosts become infected accidentally by ingesting the infected insects. Immature flukes migrate from the small intestine to the pancreas via the pancreatic ducts. The prepatent period is 80-100 days.

2.5.4.2 Pathogenic effect

The presence of flukes in the pancreatic ducts cause an inflammation and destruction of the ducts. The severity varies according to the number of parasites. In cases of massive infestations, severe fibrosis of the pancreas occurs resulting in atrophy of the organ. Reduced weight gain or weight loss are usually the only clinical signs observed.

2.5.5 Schistosomes (blood trematodes)

Schistosomes are elongate, sexually differentiated (an exception among the trematodes) flukes which live in

the circulatory system of their hosts. The flattened male carries the female in a special ventral groove. The males are 4-22 mm and the females from 12-28 mm in length. Several different species exist. These include *Schistosoma bovis* in central, eastern and west Africa, the Mediterranean area and the Middle East; *S. mattheei* in central, southern and eastern Africa; *S. intercalatum* in central Africa; and *S. japonicum* in the Far East (a species infecting humans but which may also cause schistosomiasis in ruminants and other host species). *S. nasalis* is found in the veins of the nasal mucosa of livestock in the Indian subcontinent. Infection rates of 40-50% have been reported in buffaloes and cattle. It will be described in section 2.5.5.4.

2.5.5.1 Life cycle

Like many of the other trematodes, Schistosomes require an aquatic or amphibious snail as an intermediate host in order to complete their life cycle. The adult parasites live in the mesenteric veins of the final host. During the period of egg-laying, the female parasite enters the small vessels of the gut wall. The eggs, which have a sharp spine, penetrate the wall, enter the intestinal lumen and are passed out in the faeces. Different snail species act as intermediate hosts. The development in the snail is similar to that of other trematodes. The infective forms released from the snails are free-swimming, fork-tailed cercariae. Infection of the final host takes place when the animal is drinking from a contaminated water source. Infection occurs either via skin penetration by the parasite, or by penetration of the digestive tract after ingestion of cercariae with the water. The immature flukes migrate through the lungs and the liver to the mesenteric veins, where they mature.

2.5.5.2 Pathogenic effect

The effects of schistosome infections of livestock are not easily recognized and the non-specific clinical signs are often overlooked by farmers. Infections may, however, result in severe clinical signs. The infections are often manifest by acute intestinal signs, 7-9 weeks after infection (the time when the females produce large numbers of eggs which penetrate the gut wall). The mucosa of the intestine is severely damaged and the animal develops profuse, sometimes bloody diarrhoea, dehydration and loss of appetite. Anaemia and general loss of condition may also be seen. The intensity of the pathogenic effects depends on the duration of the infection and the number of Schistosomes present. Signs associated with chronic hepatic disease may develop when eggs are washed back to the liver by the portal circulation during their penetration of the gut wall. The eggs become lodged in the liver and an intense immunological response results, followed by the formation of a granuloma. A large proportion of the liver may be destroyed and the liver function severely disturbed.

2.5.5.3 Factors affecting the epidemiological pattern

Schistosomiasis is closely associated with large permanent water bodies such as ponds, lakes and marshy pastures. A key determinant in the epidemiology of this infection is the relative abundance of the intermediate hosts and their ability to develop and survive in the environment. Contamination of water with schistosome eggs results when animal defaecate in the water while drinking or if manure is used for feeding fish in ponds. As sheep and goats are reluctant to enter water, cattle are largely responsible for the transmission of the important schistosome species. Cattle become infected through skin penetration and the oral route, whereas sheep and goats generally become infected by drinking contaminated water. The type of watering facilities used by domestic stock is therefore a crucial factor in the maintenance and transmission of the infection.

2.5.5.4 Nasal schistosomes

The adult parasites live in the blood vessels of the nasal mucosa. They are present in abscesses and granulomas that are full of eggs; these pass out in the nasal secretions and exudate. The development of the free-living stages, the stages in the snail and the transmission to the final host are similar to the processes in other schistosomes. Infected animals show varying degrees of sneezing, snoring and discharge from the nostrils.

2.6 Cestodes

2.6.1 Introduction

Cestodes in ruminants can conveniently be classified into two distinct groups; one in which ruminants act as the final host (the intestinal and hepatic cestodes) and one in which cattle, buffaloes sheep and goats act as the intermediate hosts for the larval stages *(Cysticercus, Coenurus* and hydatid cysts) of various tapeworm species. In the latter group, the adult parasites live in the small intestines of domesticated and wild carnivores *(Taenia ovis, T. hydatigena, T. multiceps, Echinococcus granulosus)* and man (*T. saginata).*

2.6.2 Cestodes with ruminants as the final hosts

2.6.2.1 Intestinal tapeworms

This group comprises species of the genera *Moniezia* (cosmopolitan), *Thysaniezia* (Africa) and *Avetellina* (Africa, Asia).

2.6.2.1.1 Life cycles

The life cycles of these tapeworms are indirect and herbage mites of the family *Oribatidae* act as intermediate hosts. The mites, which are soil-inhabiting, surface during the night and early morning to feed on manure. During their feeding they accidentally ingest eggs of the intestinal tapeworms present in the manure, and the larval stage called a cysticercoid develops in the mites. Ruminants become infected by ingesting herbage containing mites carrying the infective stage of the parasite.

2.6.2.1.2 Pathogenesis of intestinal tapeworms

Lambs, kids and calves under six months old are commonly infected. Light to moderate infections are considered to be non-pathogenic. Heavy infections have been reported to cause poor growth and diarrhoea in lambs. Whether the intestinal cestodes are directly responsible for production losses is still a controversial issue and the pathogenicity of these parasites has not yet been established conclusively.

2.6.2.2 Hepatic tapeworms

Stilesia hepatica (Africa) occurs in the bile ducts of ruminants and is very common in certain parts of Africa. It is believed that certain antelope species act as a reservoir of this infection.

2.6.2.2.1 Life cycle

The life cycle is similar to that described for the intestinal tapeworms; ruminants become infected by ingesting infected herbage mites. The parasite occurs in animals of all ages.

2.6.2.2.2 Pathogenicity of *Stilesia hepatica*

This parasite is considered to be non-pathogenic, and no clinical signs are associated even with heavy infections. Affected livers may show signs of mild cirrhosis with some thickening of the bile ducts. The economic importance of this infection results from the condemnation of affected livers at meat inspection.

2.6.3 Cestodes with ruminants as the intermediate hosts

2.6.3.1 Muscular cysticercosis

2.6.3.1.1 Bovine cysticercosis

This is caused by the presence of the vesicular larvae *Cysticercus bovis* in the striated muscles of cattle. Bovine cysticercosis has a cosmopolitan distribution being particularly common in certain parts of Africa.

2.6.3.1.1.1 Life cycle

Cysticercus bovis is the larval stage of *T. saginata,* a tapeworm of man. Tapeworm segments containing thousands of eggs are passed in faeces or shed from the intestine of a parasitized individual. If the eggs are ingested by a receptive intermediate host, the embryos migrate through the blood stream and become disseminated throughout the body. Usually only the embryos which reach the striated muscle tissues will develop further, but viable cysts have been identified in other organs and tissues. Development takes 3-5 months and the majority of cysts remain viable (and thus infective) for 1-2 years. Man becomes infected by ingesting live cysts in raw or undercooked meat. Following infection of man an adult tapeworm develops in the intestine within 3 months.

2.6.3.1.1.2 Infection of animals

Cattle become infected when they ingest the eggs of *T. saginata.* Poor standards of personal hygiene of infected human populations is responsible for the spread of cysticercosis. In some societies such as nomadic pastoral people there is a high risk of animals becoming exposed to infected faeces. Abnormal eating habits of cattle due to certain mineral deficiencies (pica) may result in cravings that increase the exposure through the ingestion of faeces. The survival of the eggs is strongly influenced by climatic conditions. Under wet and moist conditions, eggs may survive for months, exposing animals to a source of infection for a prolonged period of time. Eggs are very susceptible to dry conditions and are rapidly destroyed during the dry season.

2.6.3.1.1.3 Pathogenicity of bovine cysticercosis

The cysticercus appears as a small (6-10 mm) oval vesicle which is at first semitransparent. These lesions are known as beef measles. The majority of cysts will gradually undergo degenerative changes resulting in loss of transparency, and this is followed by caseation and calcification.

Not all striated muscles are infected to the same degree. It appears that the parasite has preferred muscle sites for development (predilection sites) and these are usually the myocardium, the tongue, the masseter and the shoulder muscles.

The development and presence of cysts in bovines is generally not clinically apparent. The importance of this parasite is its public health significance and the resultant losses encountered during meat inspection when infected carcasses are condemned.

2.6.3.1.2 Ovine cysticercosis

This is caused by the presence of the vesicular larvae *Cysticercus ovis* found in the striated muscles of sheep and goats Ovine cysticercosis is common in many countries of the world.

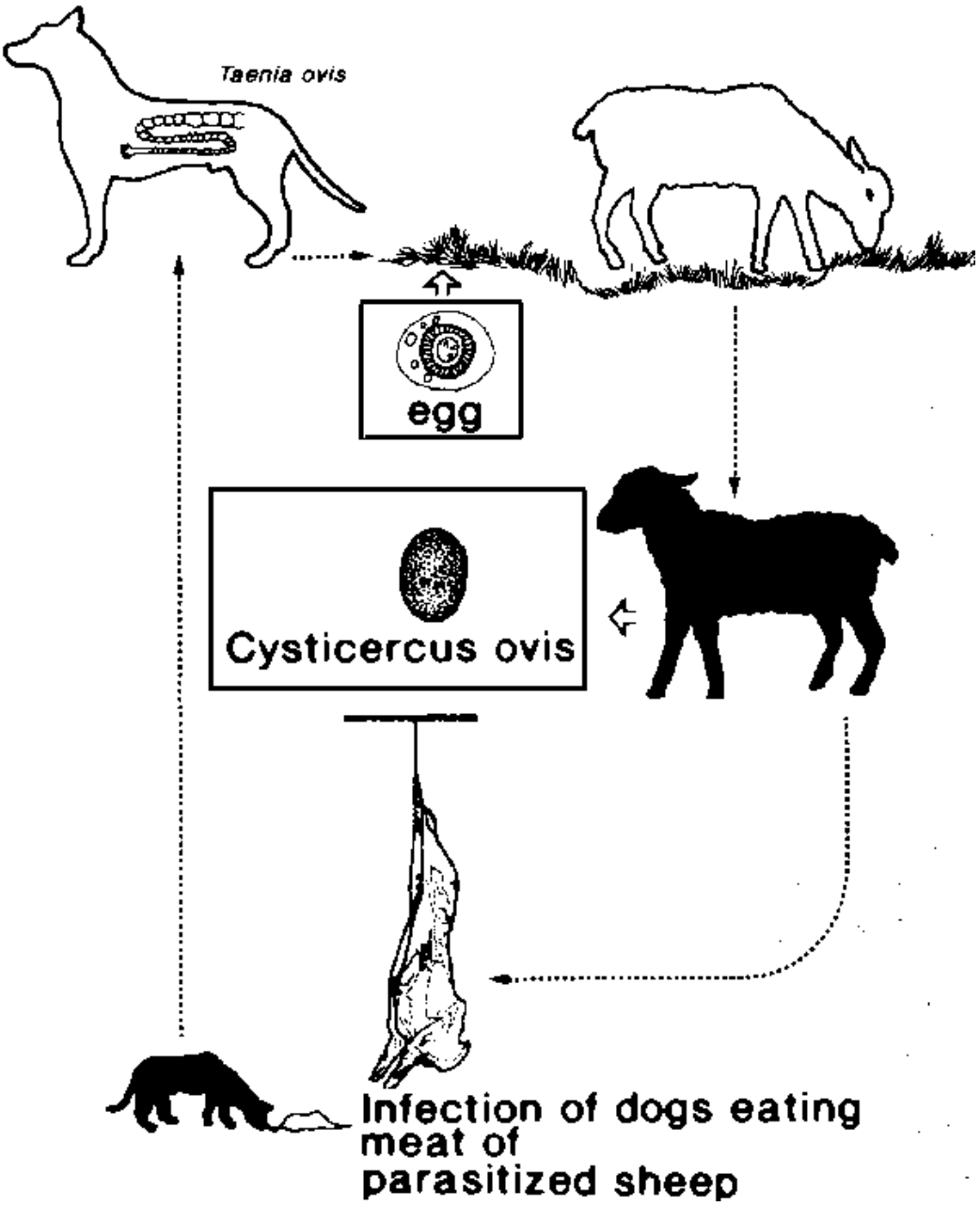
2.6.3.1.2.1 Life cycle and pathogenicity

Cysticercus ovis is the larval stage of *T. ovis,* a tapeworm of dogs and other carnivores. The development cycle is similar to that of *C. bovis.* Although the cysts are found in the muscles of sheep, this parasite is not considered of public health importance because man cannot become infected with *T. ovis.* The most common sites of infection are the heart and the diaphragm, but other muscle groups may also be affected. The detection of the cysts usually results in condemnation of the meat for aesthetic reasons. Some reports indicate that massive infestations can kill animals.

2.6.3.2 Abdominal cysticercosis

Abdominal cysticercosis of ruminants is caused by *C. tenuicollis,* the larval stage of *T. hydatigena,* a dog tapeworm. This parasite is cosmopolitan in its distribution.

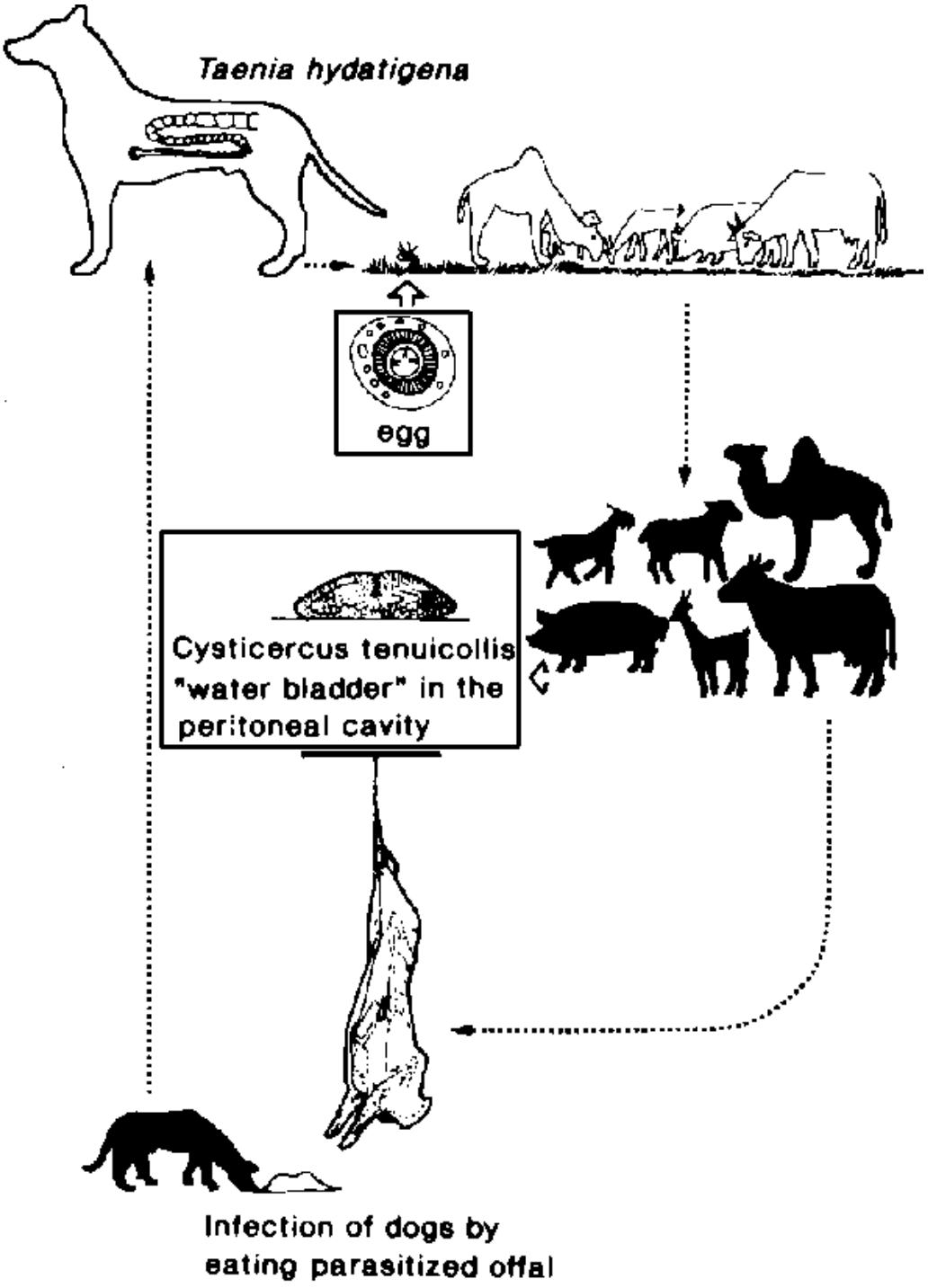
FIGURE 2.5 Life cycle of Cysticercus ovis



2.6.3.2.1 Life cycle

The adult tapeworms live in the small intestines of dogs and other carnivores and segments containing numerous eggs are passed in the faeces. After disintegration of the segments, eggs can be disseminated by wind and by insects contaminating the pasture. Ruminants then become infected by ingesting eggs. The embryos penetrate the wall of the digestive tract and migrate to the liver, where they migrate through the liver surface to enter the abdominal cavity. The fully developed cyst is a large (5 cm or more in diameter), soft, semi-transparent bladder within which the invaginated head of the tapeworm is clearly visible. The final host becomes infected by ingesting the-cysts.

FIGURE 2.6 Life cycle of Cysticercus tenuicollis



2.6.3.2.2 Pathogenicity of abdominal cysticercosis

The developed *C. tenuicollis* has no pathogenic effect while situated in the abdominal cavity. When many embryos migrate simultaneously through the liver clinical signs may be seen. The migration may cause severe destruction of liver tissue and the pathology seen in the liver may be similar to that observed in liver fluke infections.

2.6.3.3 Coenurosis of the brain

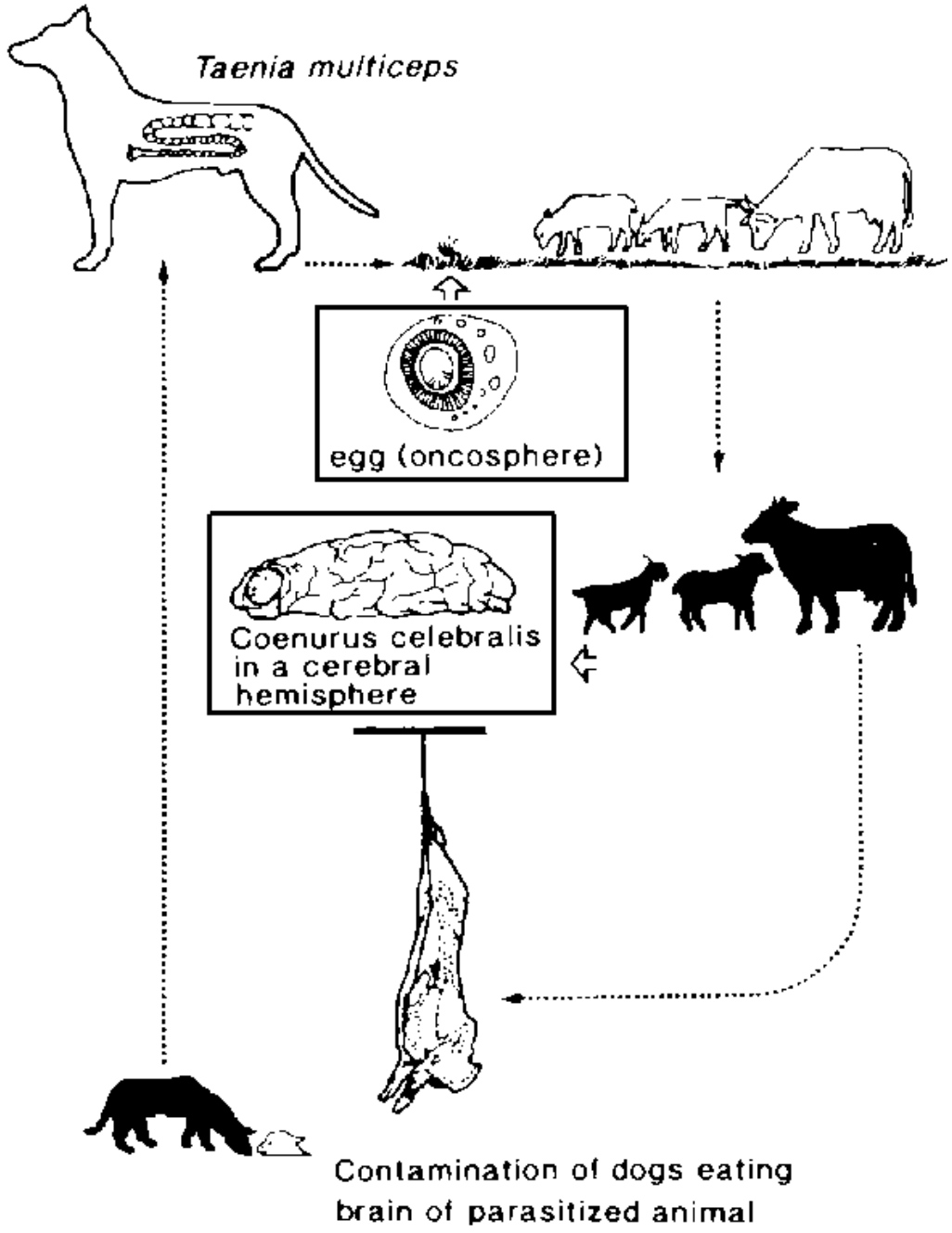
Cysticercus cerebralis is the cystic larval stage of *T. multiceps,* a tapeworm of dogs and other wild carnivores. The coenurus develops in the brain of sheep. This parasite is cosmopolitan in its distribution but not very prevalent.

2.6.3.3.1 Life cycle

Egg-filled segments of *T. multiceps* are passed in the faeces of dogs. When sheep ingest the eggs, the embryos migrate via the circulatory system to the brain and spinal cord where the cystic stage, the

coenurus, develops. The cyst takes 6-8 months to develop and at maturity may be up to 5 cm in diameter. The cyst is characterized by numerous invaginated tapeworm heads. The final host becomes infected by ingesting the cyst.

FIGURE 2.7 Life cycle of Coenurus cerebralis



2.6.3.3.2 Pathogenicity of *Coenurus cerebralis*

The pathogenic effect is that of a space-occupying lession and the resulting pressure applied to the brain by the cyst during its development. The clinical signs depend on the size and site of the Coenurus in the brain. These include uncoordinated movements of the legs and abnormal positioning of the head. Affected animals may become blind in one or both eyes and indifferent to food and water. This can result in emaciation and eventual death.

2.6.3.4 Hydatidosis

Hydatidosis or larval echinococcosis is the cystic stage of *Echinococcus granulosus,* a very small tapeworm of dogs and other canids. This parasite has a cosmopolitan distribution and is very common in parts of Africa, Latin America and some countries of Southeast Asia.

2.6.3.4.1 Life cycle

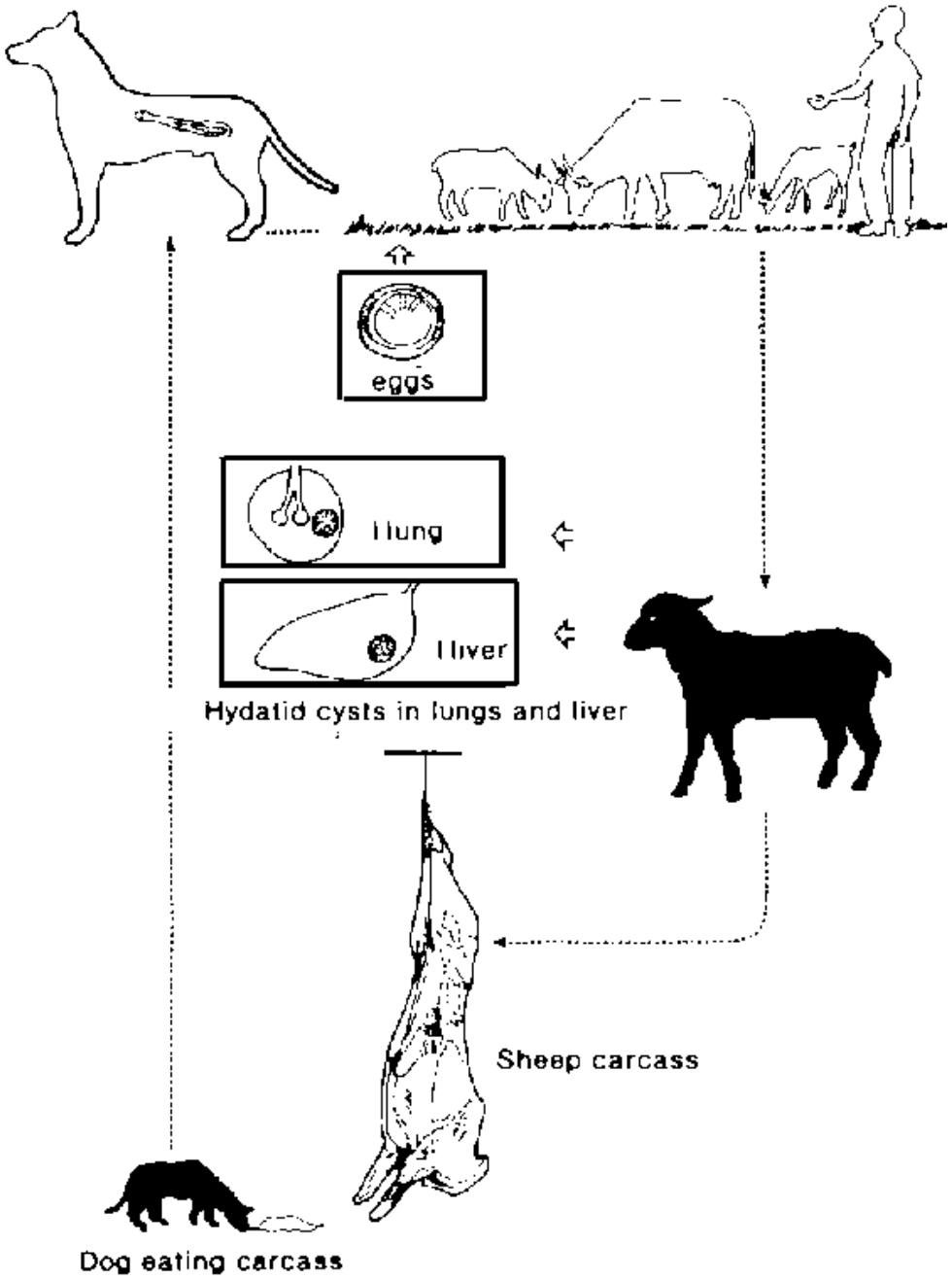
There are several types of life cycle involving different mammalian species. One cycle involves domesticated ruminants and dogs; another cycle involves wildlife species, for example the warthog-lion cycle in Africa. Other cycles involve domesticated animals and wildlife, such as the dromedary camel-jackal cycle in some regions of sub-Saharan Africa.

The gravid segments of the *E. granulosus* tapeworm are excreted in the faeces of dogs and the eggs released from the segments are very resistant to adverse climatic conditions. They may be carried by wind in dust or be mechanically transported by flies. Following ingestion of the eggs by the intermediate hosts, which include man, domesticated animals and numerous wild mammals, the embryos emerge and migrate to the blood stream through which they are carried to various organs and tissues in which the hydatid cysts develop. Hydatid cysts are most commonly found in the liver and the lungs. The embryos grow slowly into large fluid filled cysts, 5-10 cm or more in diameter. Hydatid cysts are lined with a thin layer of germinal epithelium. After 5 months this germinal layer is capable of producing tapeworm scolices, which can be found individually in the fluid of the cyst as "hydatid sand". It also produces brood capsules which consist of several scolices held together by a thin membrane. Part of the germinal epithelium may occasionally form daughter cysts with a germinal layer of their own. In some cysts the germinal layer does not produce infective protoscolices and brood capsules; these cysts remain sterile. The final host acquires the infection by eating viscera containing fertile hydatid cysts. Cysts maintain their infectivity for several weeks after the death of the intermediate host and carrion feeders are therefore considered important in disseminating this infection.

2.6.3.4.2 Pathogenicity of hydatid cysts

The effects of hydatid cysts depend on the organs in which they are situated and the number of cysts present. It is generally considered that hydatid infections of ruminants are not clinically important and disease due to the presence of hydatid cysts in ruminants is rare. Occasionally numerous large cysts may cause respiratory problems when they are situated in the lungs; digestive disturbances and ascites may be seen associated with heavy infections of the liver. The major significance of *Echinococcus granulosus* is the risk of human infection.

FIGURE 2.8 Life cycle of Echinococcus granulosus



2.7 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.

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 cattle. An exception to this may occur in a primary infection in young grazing animals during their first exposure. There are some indications that the correlation is stronger in sheep and goats with mixed infections.

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% formal in 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.) When samples are received in the laboratory they should

immediately be stored in the refrigerator (4 °C) until they are processed. Samples can be kept in the refrigerator for up to *3* weeks without significant changes in the egg counts and the morphology of eggs. SAMPLES SHOULD NEVER BE KEPT IN THE FREEZER.

3.3 Qualitative techniques for separating and concentrating eggs/larvae

---

A number of different methods are available for separating, concentrating and demonstrating eggs, oocysts and larvae in faecal samples. Three methods are described:

- Simple test tube flotation
- Simple flotation
- Sedimentation technique (for trematode eggs)

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.

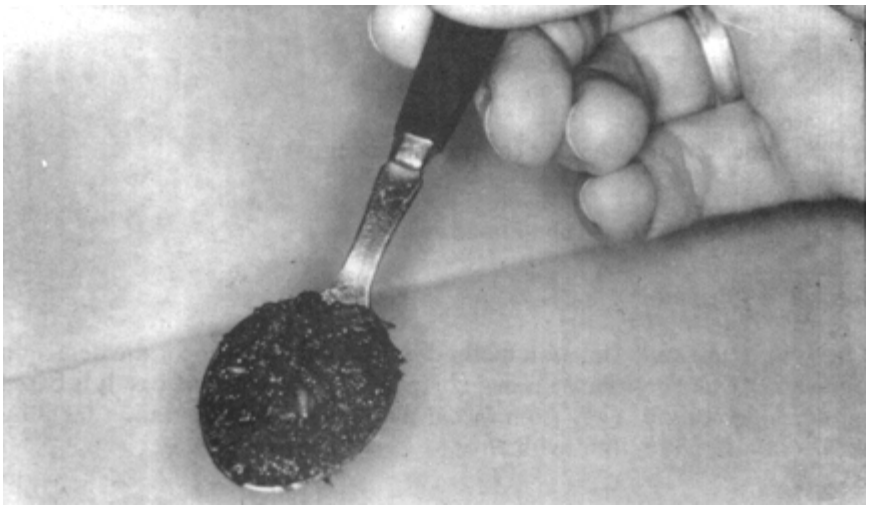
3.3.1.3 Equipment

- 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 or teaspoon

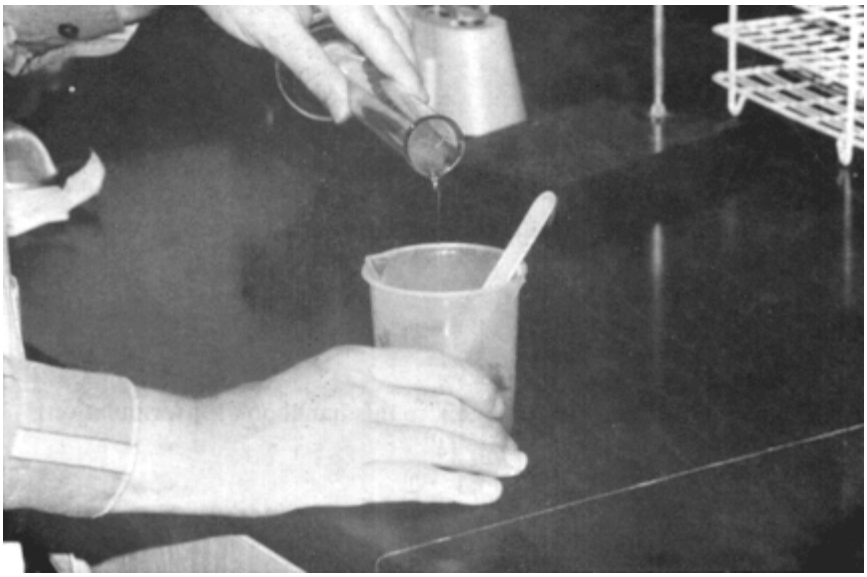
- Flotation fluid (see the Appendix to this handbook for formulation)

3.3.1.4 Procedure

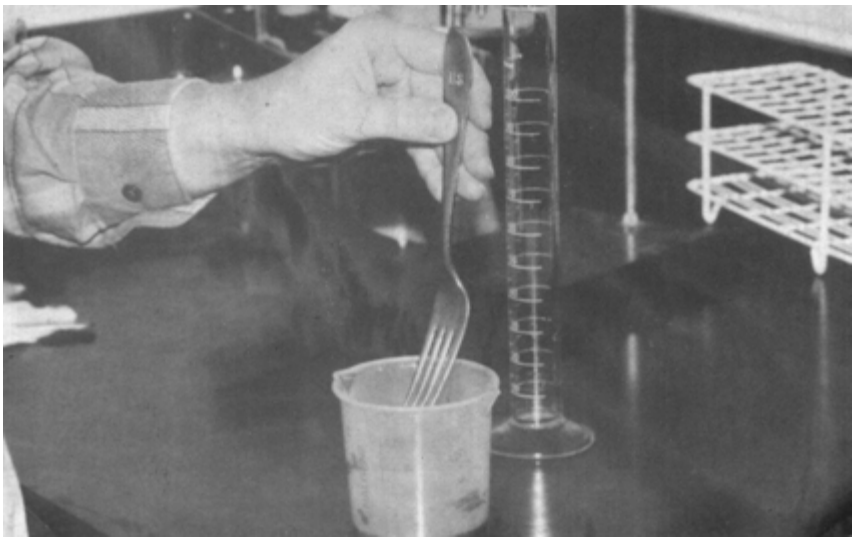
(a) Put approximately 3 g of faeces (weigh or measure with a precalibrated teaspoon) into Container 1.



(b) Pour 50 ml flotation fluid into Container 1.

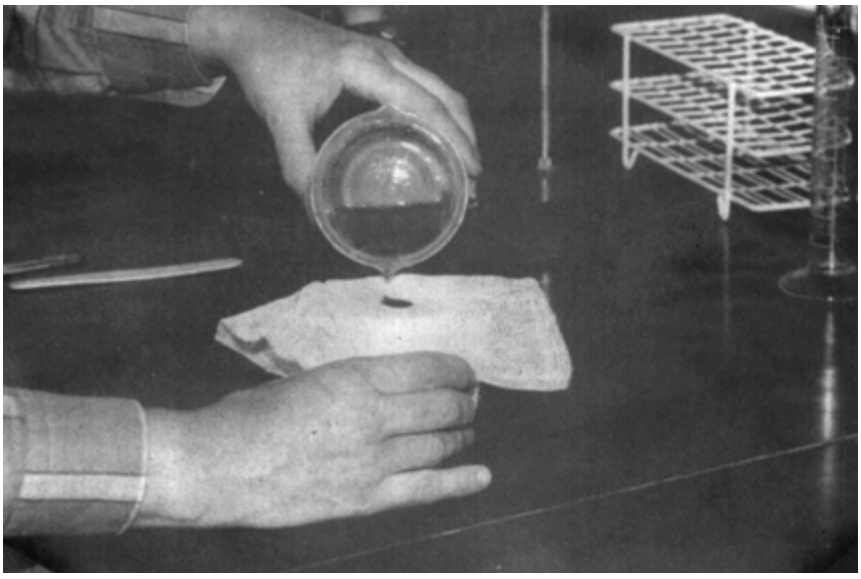


(c) Mix (stir) faeces and flotation fluid thoroughly with a stirring device (tongue blade, fork).

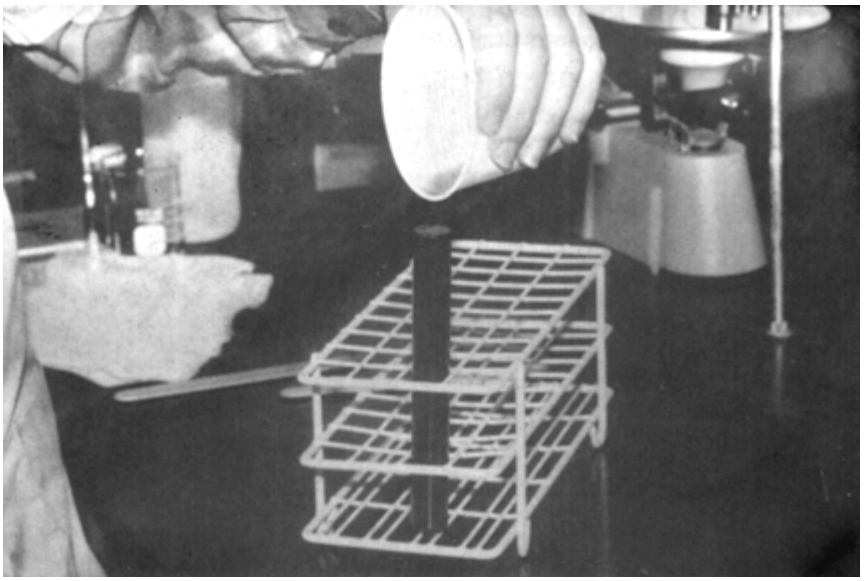


(d) Pour the resulting faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.

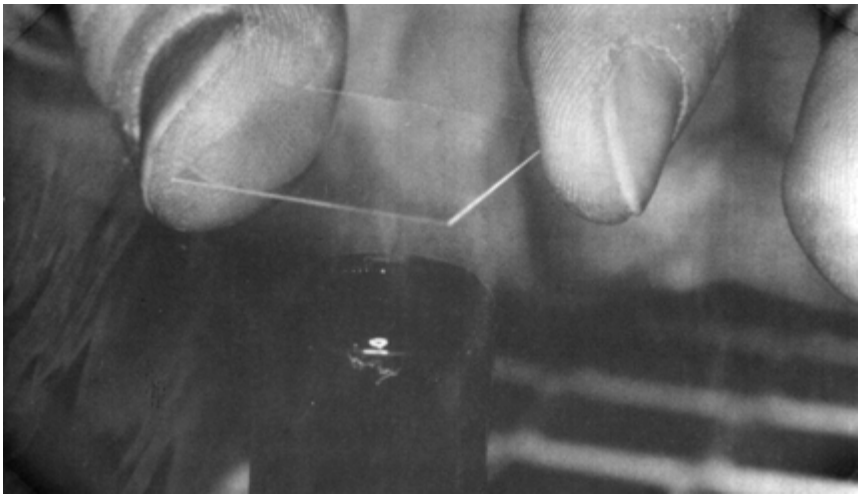
(e) Pour the faecal suspension into a test tube from Container 2.



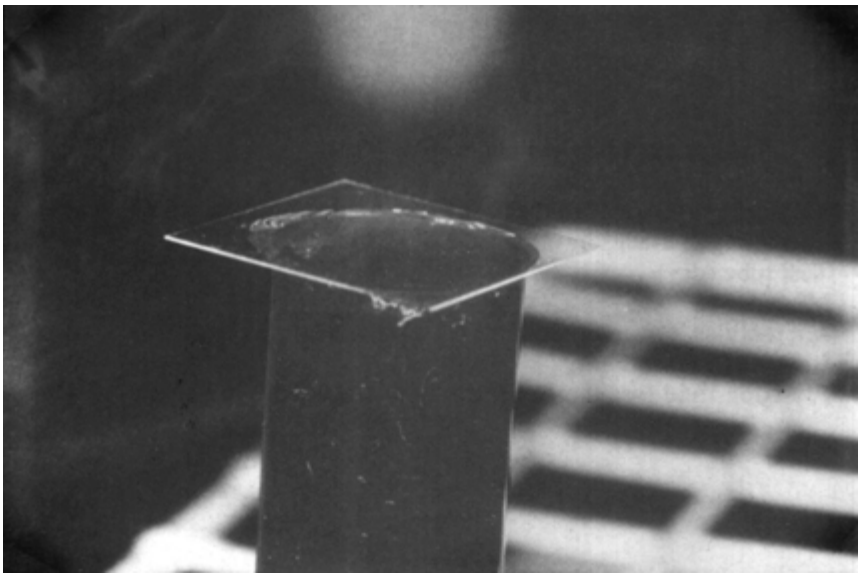
(f) Place the test tube in a test tube rack or stand.



(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.

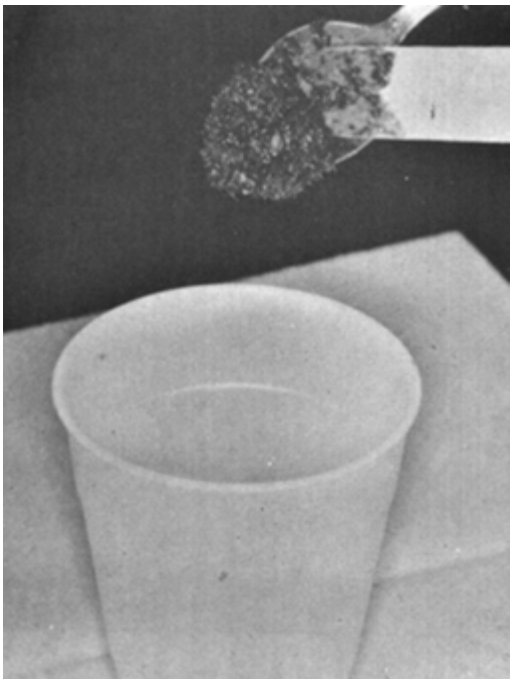
3.3.2.2 Application

This is another good technique for use in initial surveys. In addition, it can be used in conjunction with the McMaster technique (see section 3.4.1) to detect low numbers of eggs (when present below the McMaster sensitivity of 50 eggs per gram of faeces).

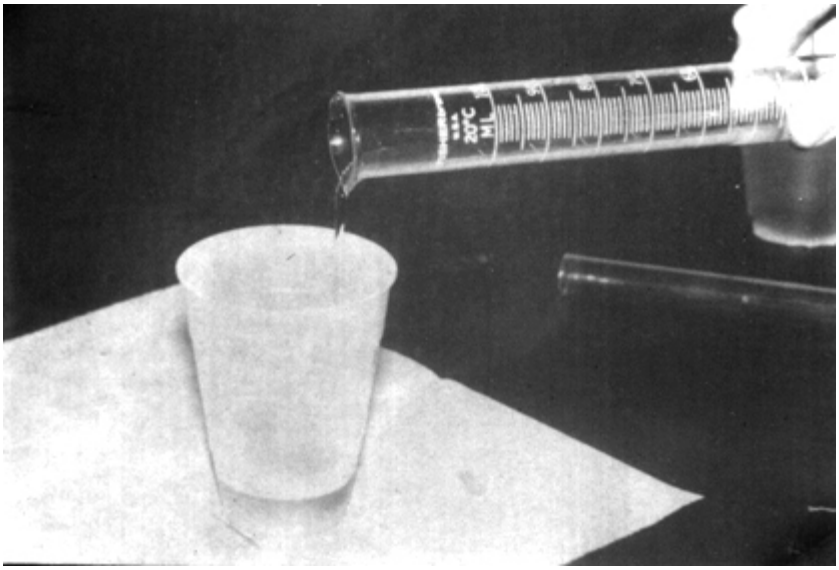
3.3.2.3 Equipment

- Two 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)

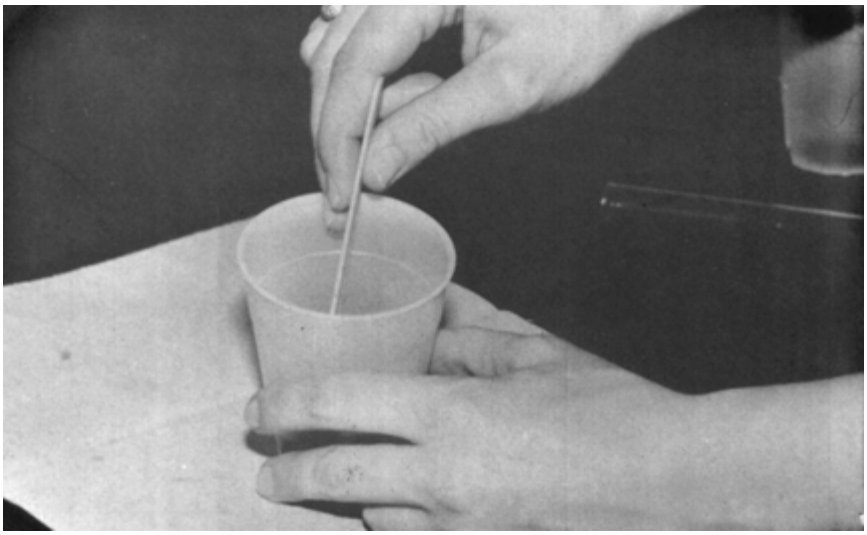
(a) Put approximately 3 g of faeces (weigh or measure the faeces with a precalibrated teaspoon) into Container 1.



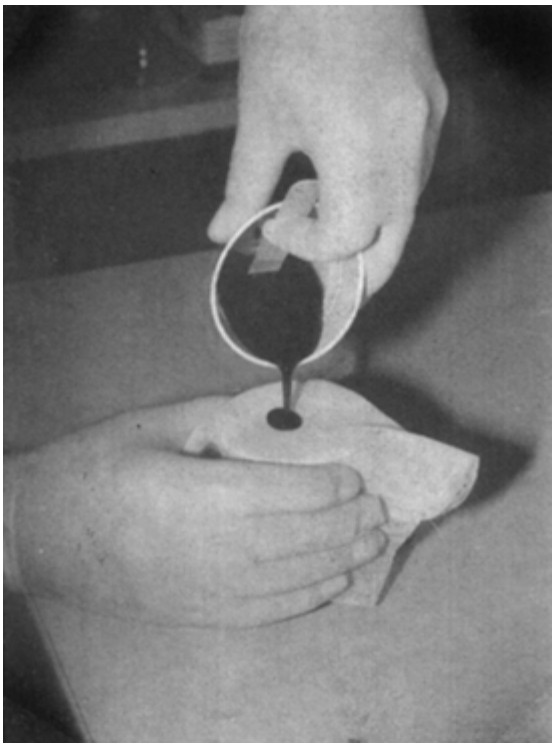
(b) Pour 50 ml of flotation fluid into Container 1.



(c) Mix (stir) the contents thoroughly with a stirring device (tongue blade, fork).

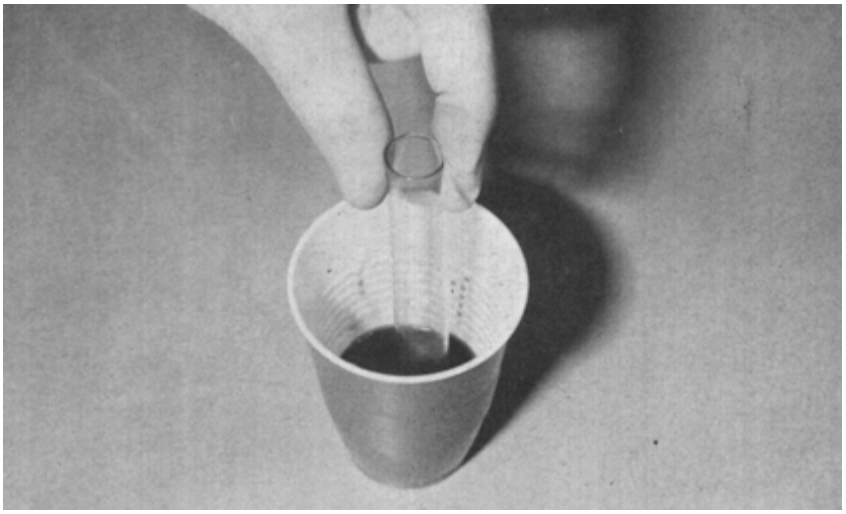


(d) Pour the resultant faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.

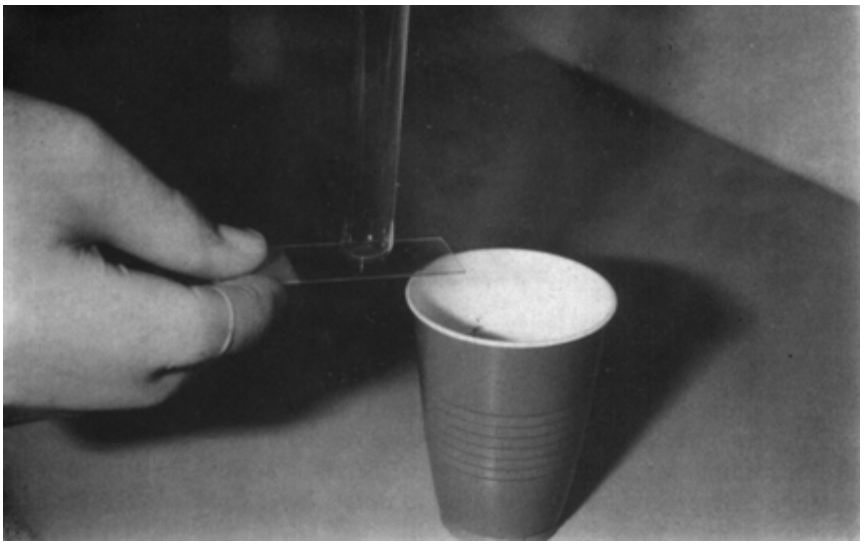


(e) Leave the container to stand for 10 minutes.

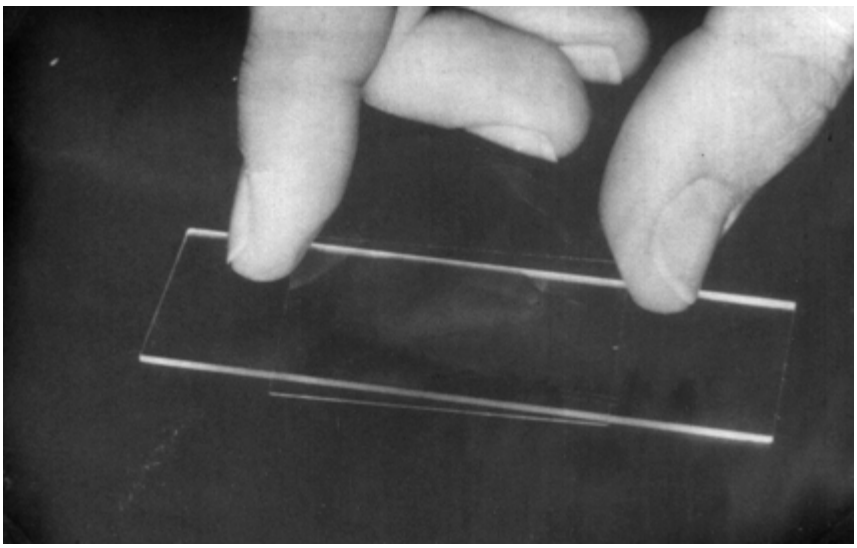
(f) Press a test tube to the bottom of the filtrate, lift it quickly and transfer a few drops adhering to the surface to a microslide.



(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 postmortem findings on other animals in the herd/flock area), and is not run routinely. The procedure can be used to detect liver fluke *(Fasciola)* and *Paramphistomum* eggs.

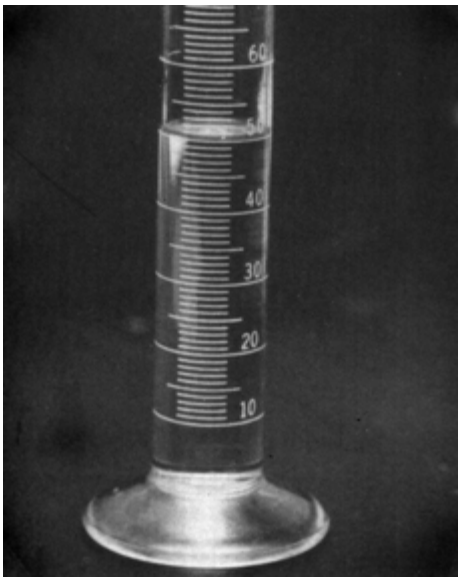
3.3.3.3 Equipment

- 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
- Balance or teaspoon
- Microscope

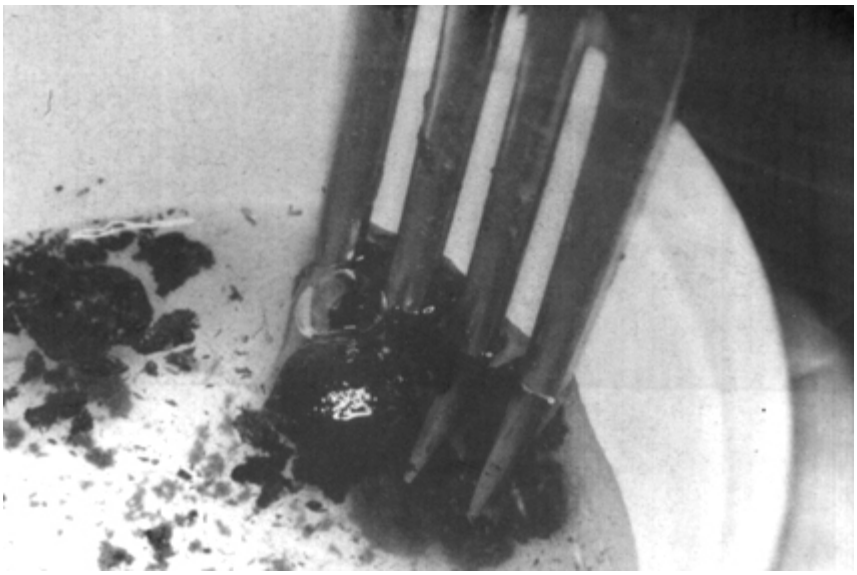
3.3.3.4 Procedure

(a) Weigh or measure approximately 3 g of faeces into Container 1.

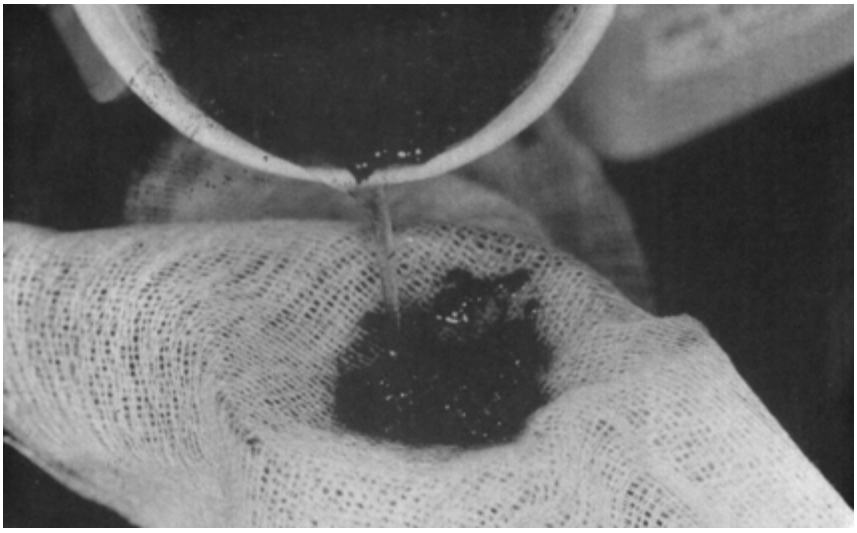
(b) Pour 40-50 ml of tap water into Container 1.



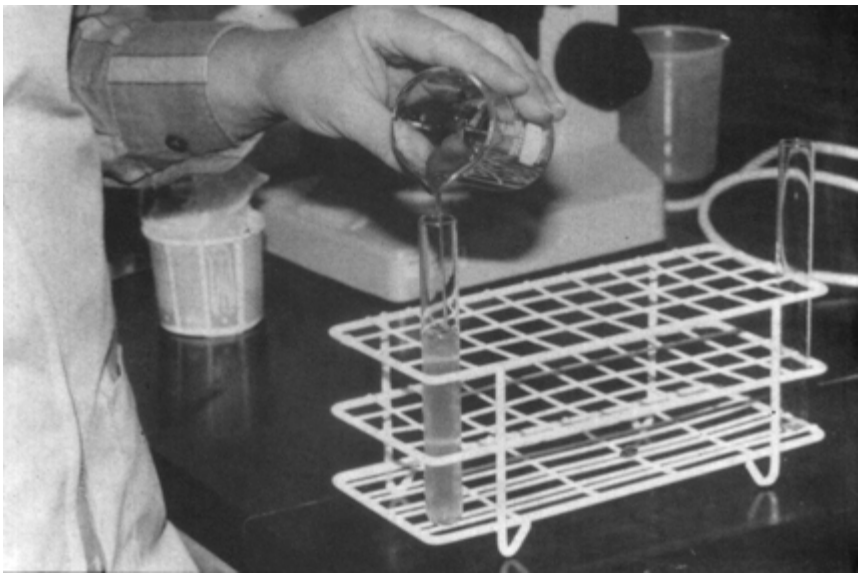
(c) Mix (stir) thoroughly with a stirring device (fork, tongue blade).



(d) Filter the faecal suspension through a tea strainer or double-layer of cheesecloth into Container 2.

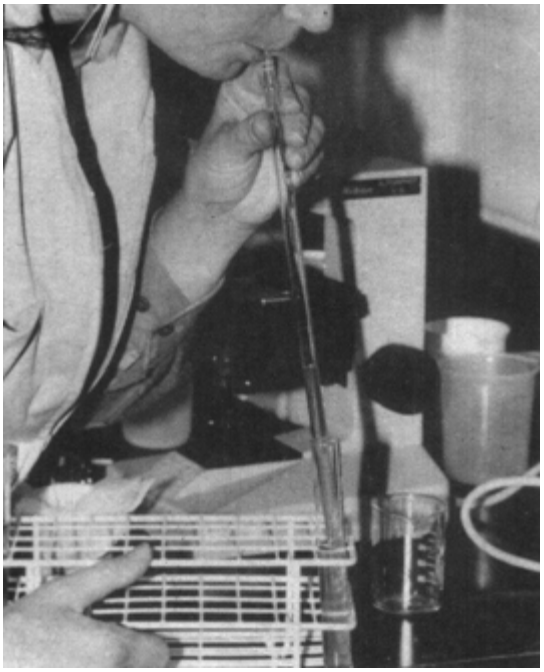


(e) Pour the filtered material into a test tube.

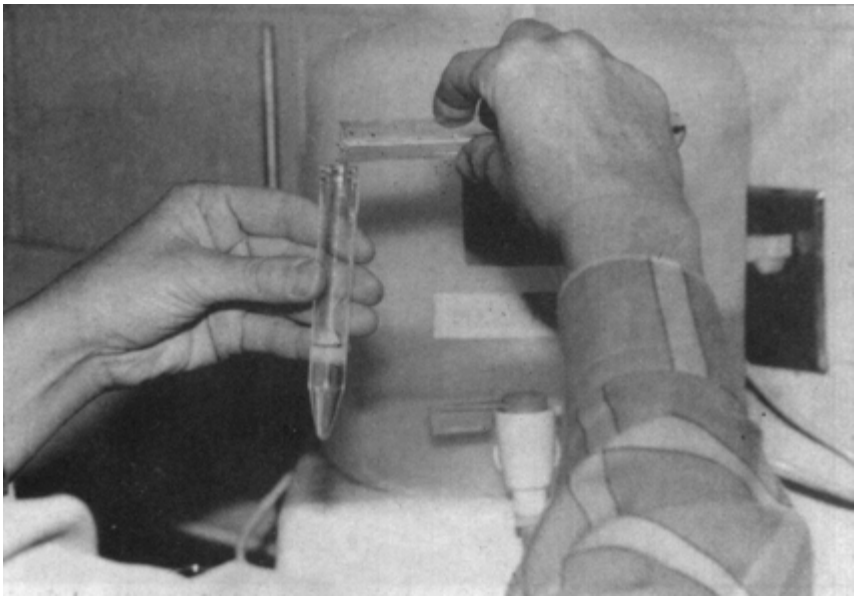


(f) Allow to sediment for 5 minutes.

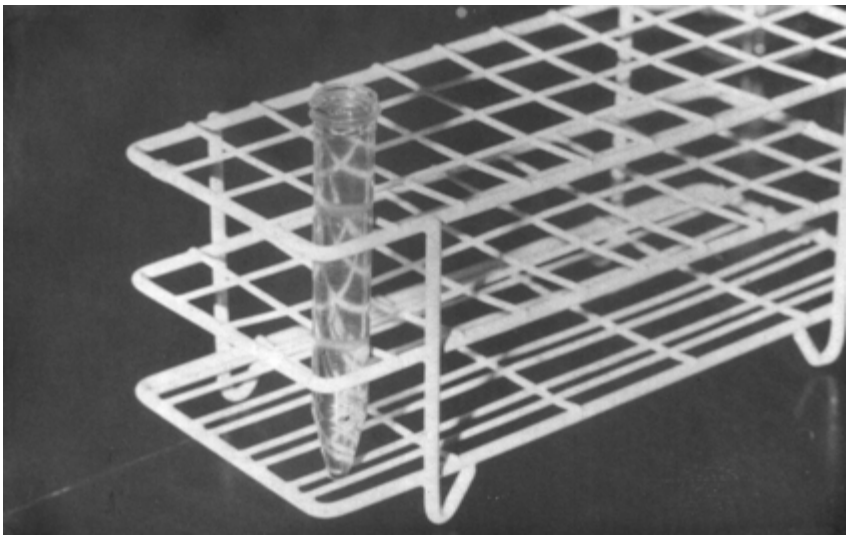
(g) Remove (pipette, decant) the supernatant very carefully.



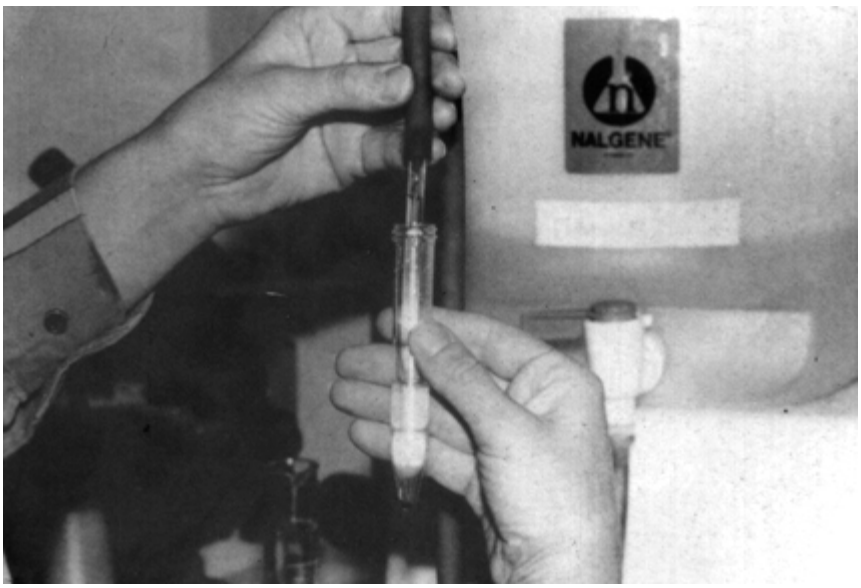
(h) Resuspend the sediment in 5 ml of water.



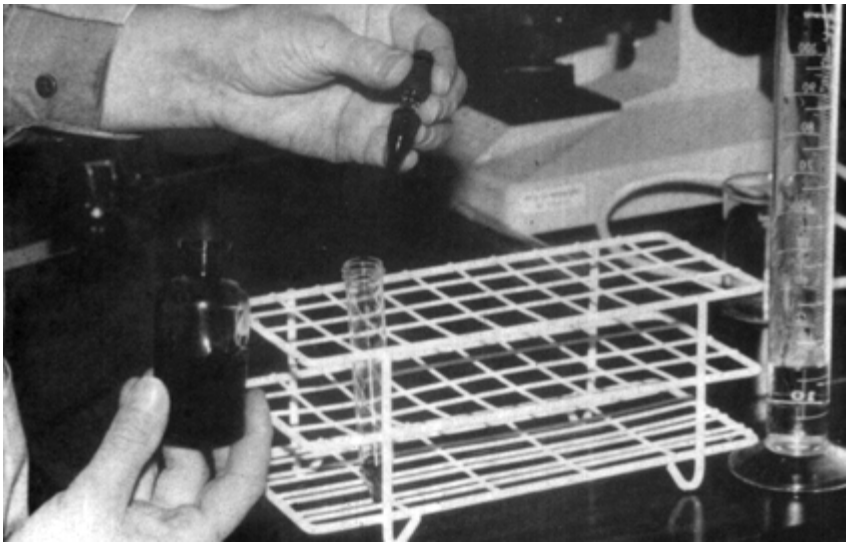
(i) Allow to sediment for 5 minutes.



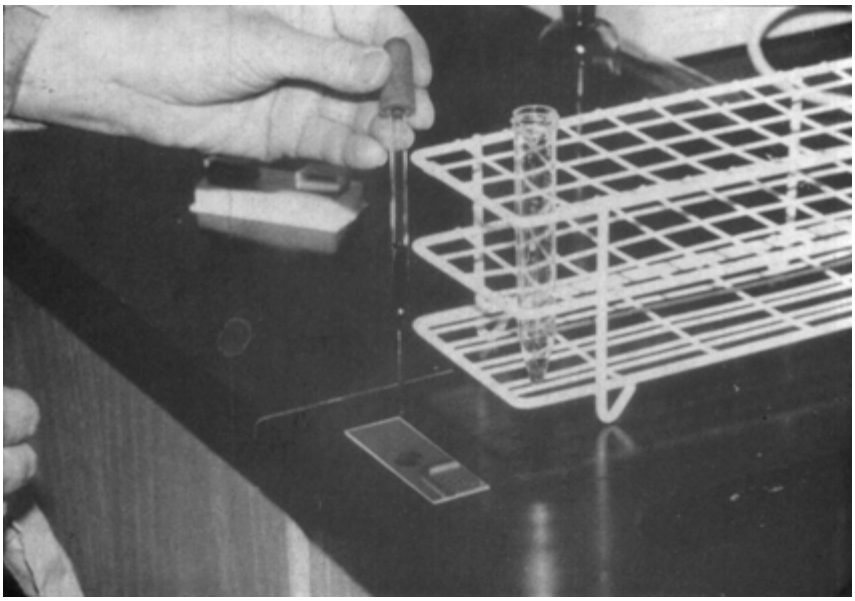
(j) Discard (pipette, decant) the supernatant very carefully.



(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

Magnification	Parasites
10 x 10	Nematode and cestode eggs
10 x 40	Coccidia oocysts
10 x 4	Trematode eggs

WARNING: In case of a time delay between processing the sample and reading the count, egg numbers may decline dramatically. Also, eggs may change their appearance, becoming crenated and "ghost-like". It is therefore advisable to prepare only a few samples at a time. These changes can be prevented by keeping prepared samples in the refrigerator after mixing. Using the salt-sugar solution as flotation fluid also reduces the morphological changes.

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 excretion.

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 are:*

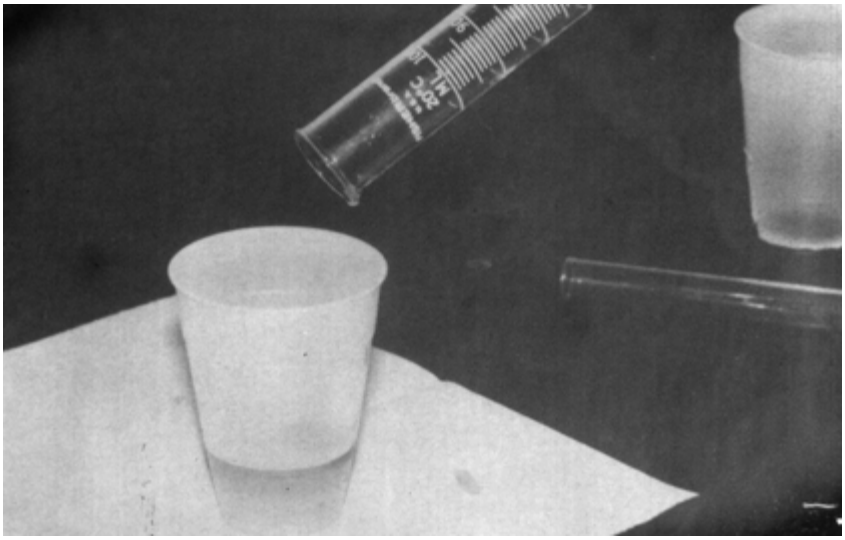
Philip Harris
618 Western Avenue
Park Royal
London W3 0TE
England
Tel: 081-992-5555

Fisons Scientific Equipment
Bishop Meadow Road
Loughborough
Leicestershire LE 11 ORS
England
Tel: 0509-231166

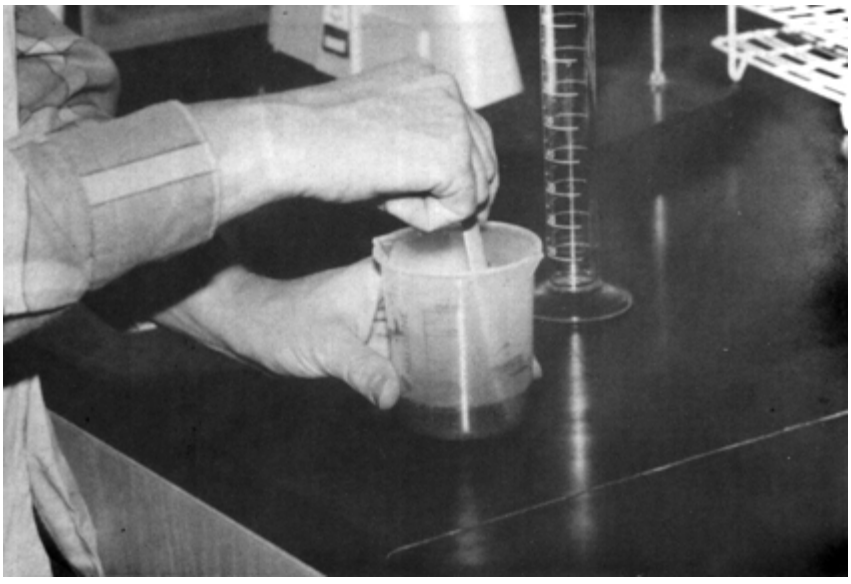
3.4.1.4 Procedure

(a) Weigh 4 g of faeces and place into Container 1.

(b) Add 56 ml of flotation fluid.

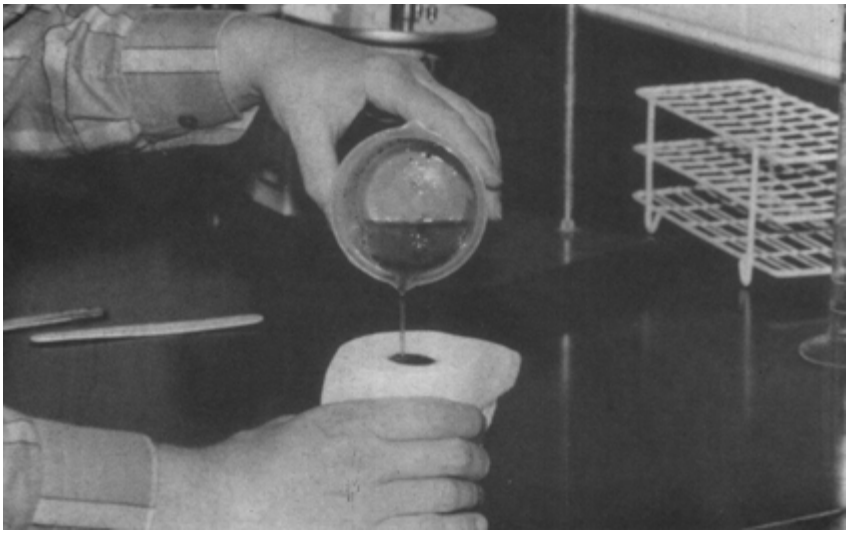


(c) Mix (stir) the contents thoroughly with a stirring device (fork, tongue blade).

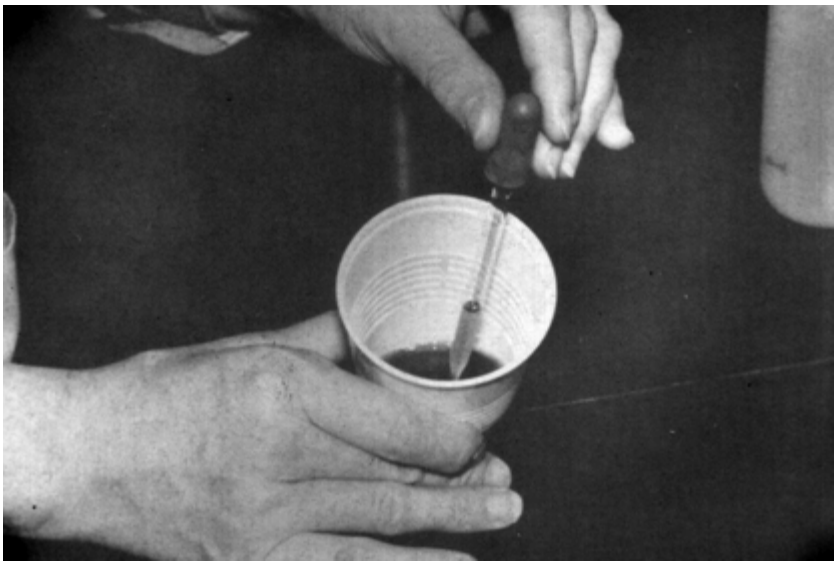


(d) Filter the faecal suspension through a tea strainer or a double-layer of cheesecloth

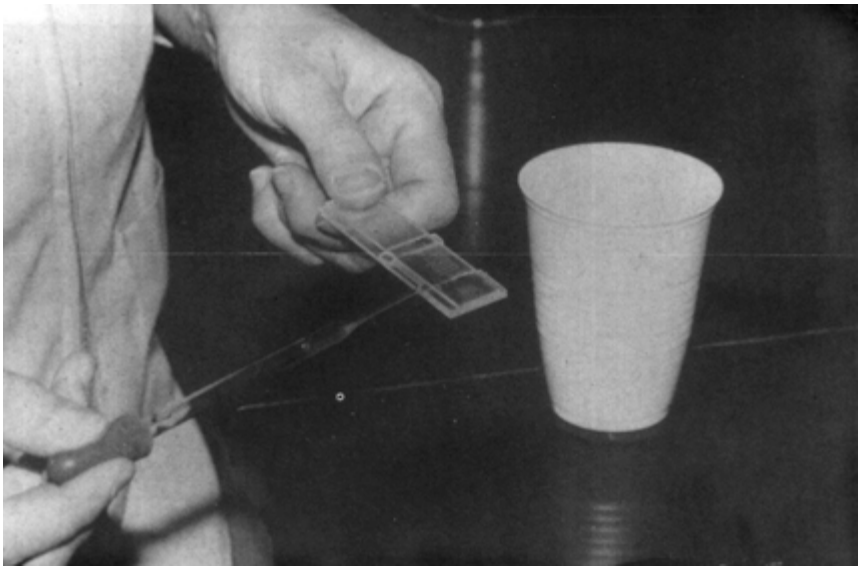
into Container 2.



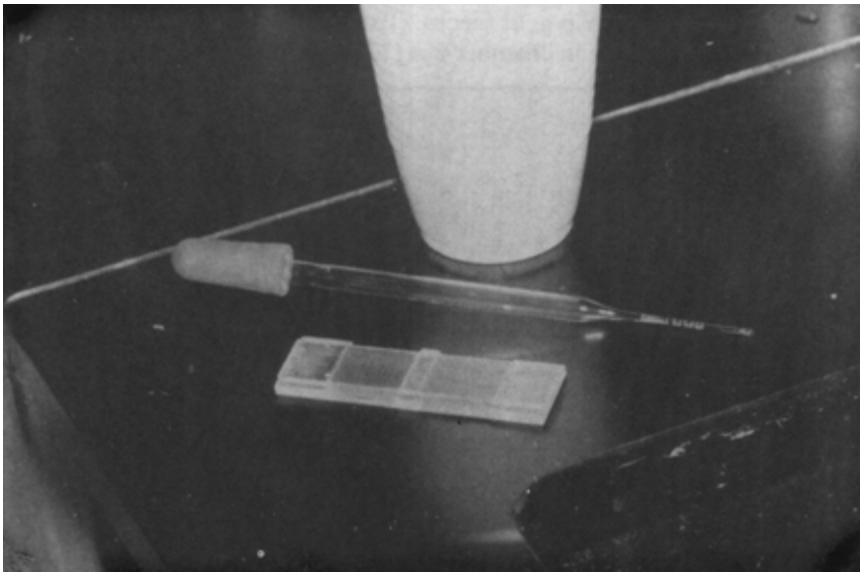
(e) While stirring the filtrate in Container 2, take a sub-sample with a Pasteur pipette.



(f) Fill both sides of the McMaster counting chamber with the sub-sample.



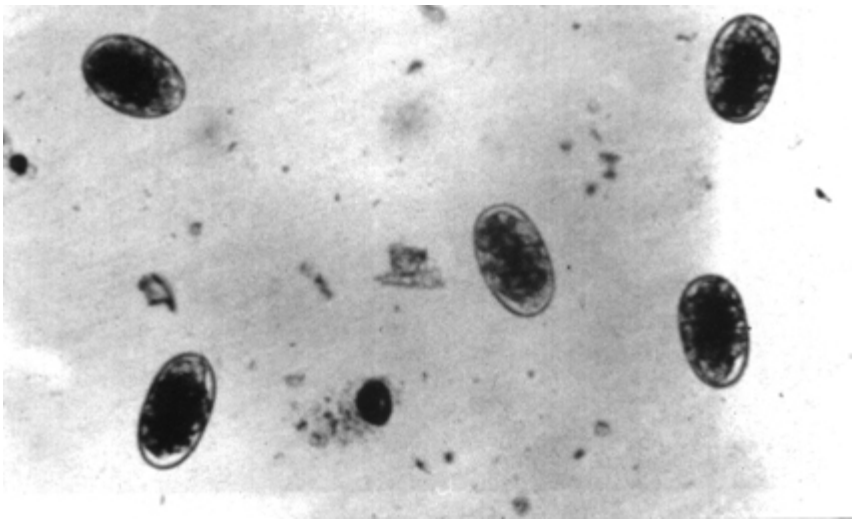
(g) Allow the counting chamber to stand for 5 minutes (this is important)



(h) Examine the sub-sample of the filtrate under a microscope at 10 x 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 (section 3.2.2), steps f, g and h.

WARNING: In case of a time delay between processing the sample and reading the count, egg numbers may decline dramatically. Also, eggs may change their appearance, becoming crenated and "ghost-like". It is therefore advisable to prepare only a few samples at a time. These changes can be prevented by keeping prepared samples in the refrigerator after mixing. Using the salt-sugar solution as flotation fluid also reduces the morphological changes.

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

Parasite	Degree of infection (eggs per gram of faeces)		
	Light	Moderate	Heavy
CATTLE			
Mixed infection	50–200	200–800	800+
Pure *Haemonchus* infection	200	200–600	600+
Pure Trichostrongylus infection	50–100	100–400	400+
Pure *Cooperia* infection	200–300	300–2500	2500+
SHEEP			
Mixed infection	50–800	800–1200	1200+
Mixed infection with *Haemonchus* absent	300–800	800–1000	1000+
Pure *Haemonchus*	100–2000	2000–7000	7000+
Pure *Trichostrongylus*	100–500	500–2000	2000+
Pure *Nematodirus*	50–100	100–600	600+
Pure *Oesophagostomum*	100–800	800–1600	1600+

If possible guidelines for the interpretation of faecal egg counts should be established for each area/country/region according to different climatic zones, as the composition and pathogenicity of parasite populations may differ from area to area.

3.5 Preparation of faecal cultures

3.5.1 Principle

Many nematode eggs are alike and species such as *Haemonchus, Mecistocirrus, Ostertagia, Trichstrongylus, 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 (L_3).

3.5.2 Application

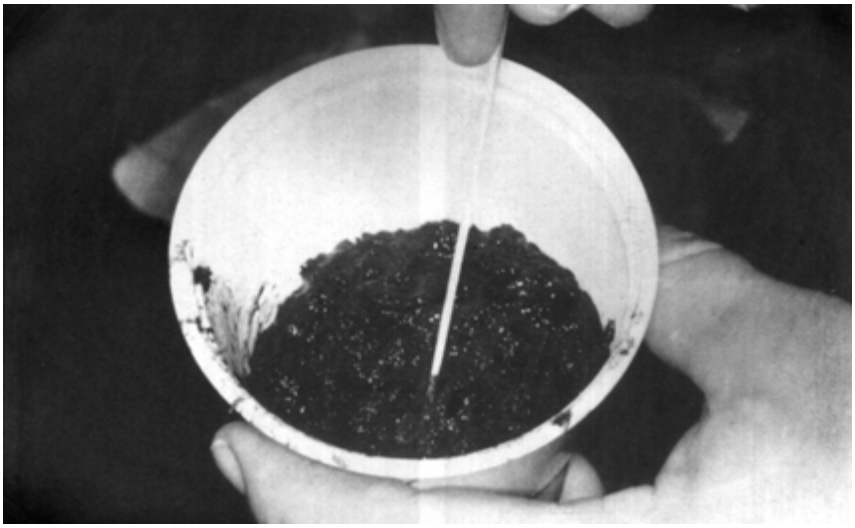
The identification of parasite species present is an important component of initial surveys and of the investigation of clinical disease caused by gastrointestinal nematodes.

3.5.3 Equipment

- Fork, spoon, tongue depressor, spatula
- Water
- Jars, containers
- Charcoal (dried, sterile bovine faeces may be used if charcoal is not available. This is prepared as follows. Faeces should be sterilized to remove any helminth eggs present, completely dried by heating to 70 °C and ground to a fine powder.)

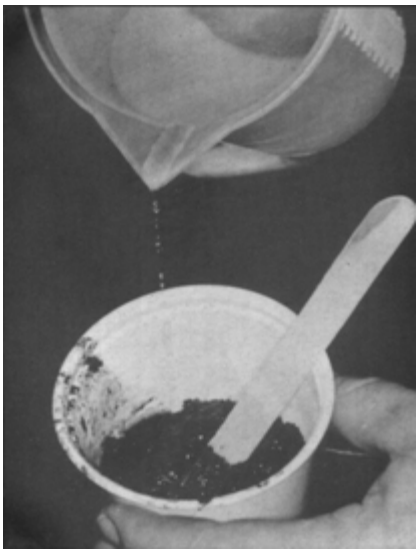
3.5.4 Procedure

(a) Break up collected faeces finely using a stirring device.

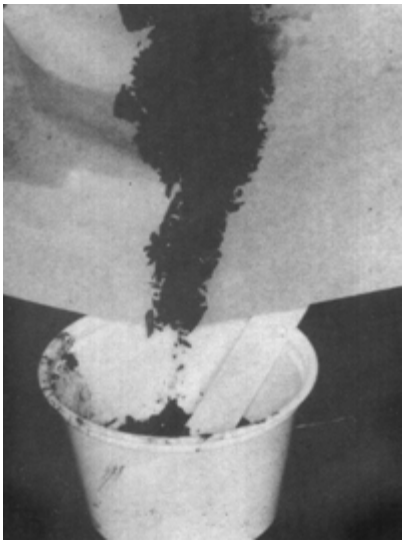


(b) Faeces should be moist and crumbly.

If faeces are too dry, add water.

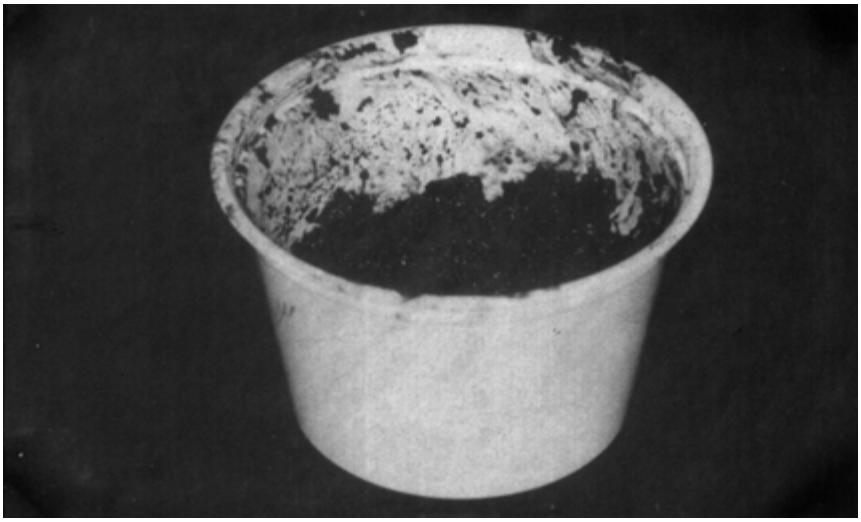


If faeces are too wet, add charcoal (or sterile bovine faeces) until the correct consistency is obtained.



(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.

(f) Add water to cultures regularly (every 1-2 days).

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

3.6 Isolation and identification of lungworm larvae and infective larvae harvested from faecal cultures (the Baermann technique)

3.6.1 Principle

The Baermann technique is used to isolate lungworm larvae from faecal samples and 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.

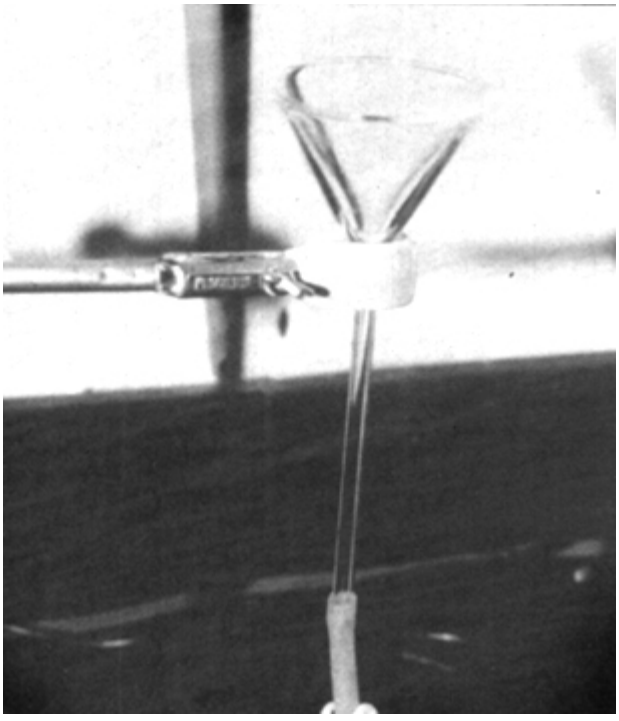
3.6.3 Equipment

- 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
- Small petri dish(es)

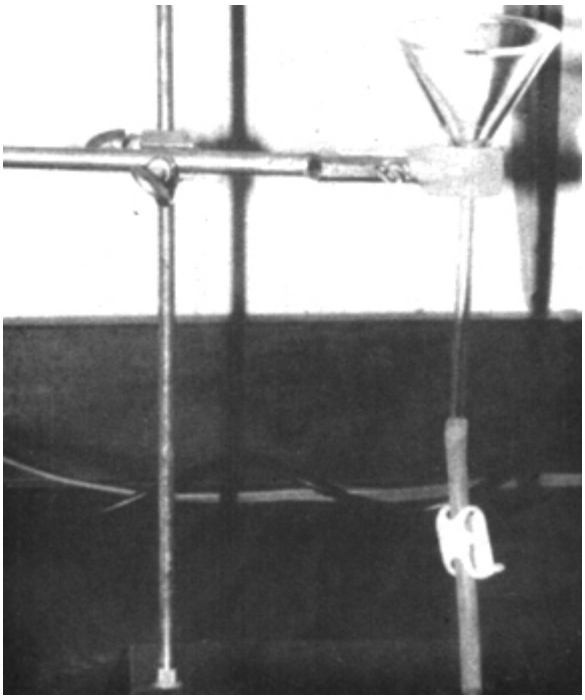
Equipment

3.6.4 Procedure

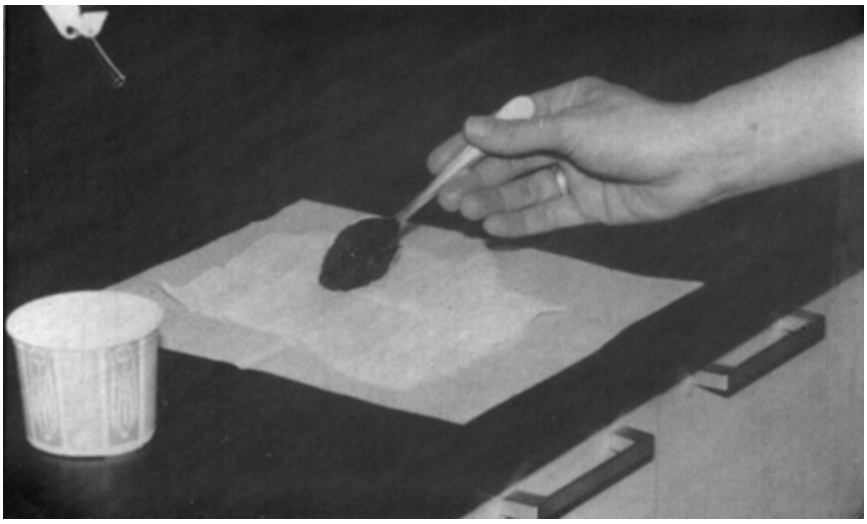
(a) Fit a short piece of tubing which is closed at one end with a clamp or spring clip, to the stem of a funnel of appropriate size.



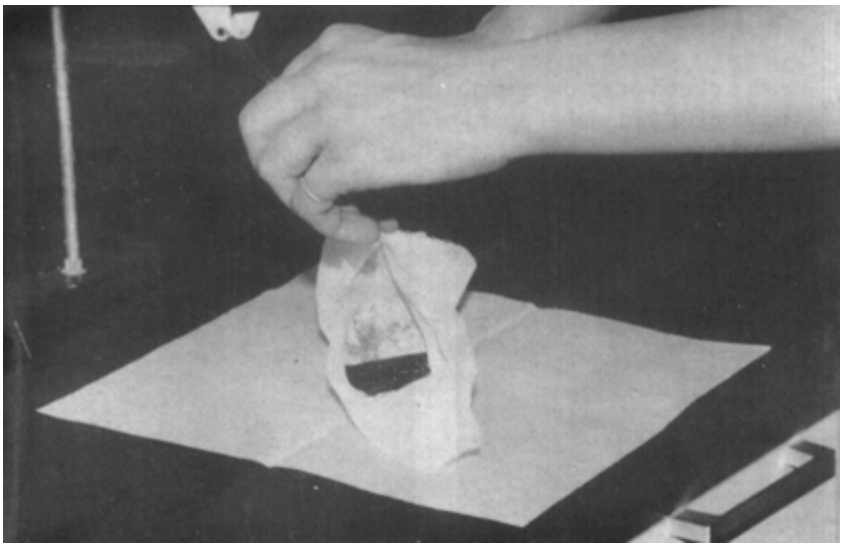
(b) Support the funnel by a stand.



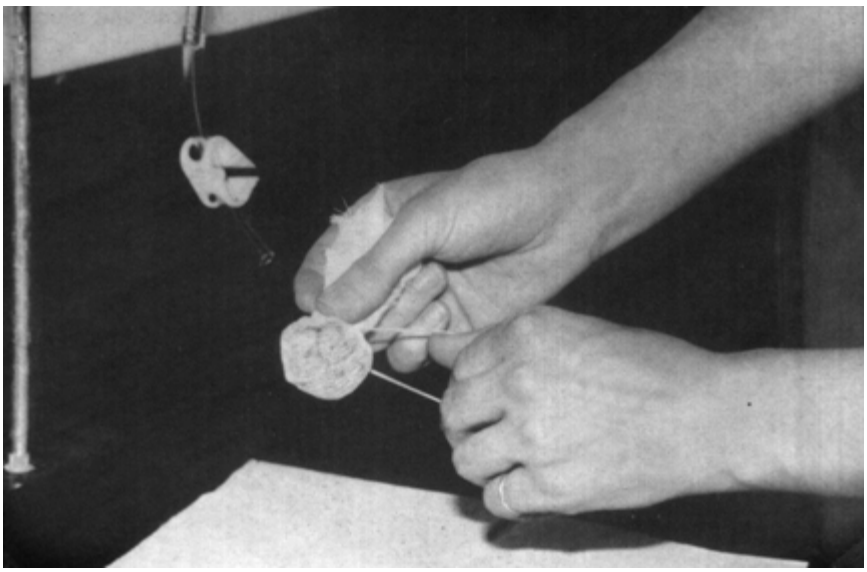
(c) Weigh or measure about 5-10 g of faecal culture/faeces and place it on a piece of double-layer cheesecloth.



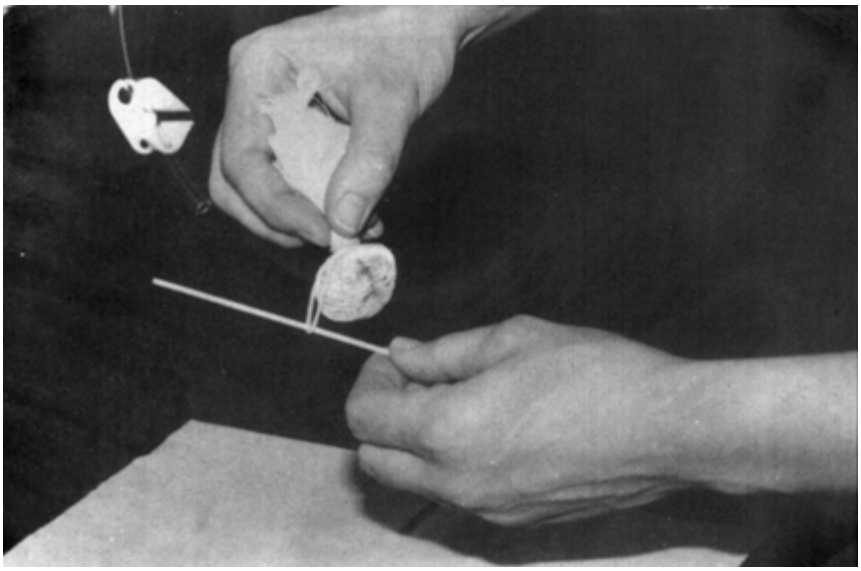
(d) Form the cheesecloth around the faeces as a "pouch".



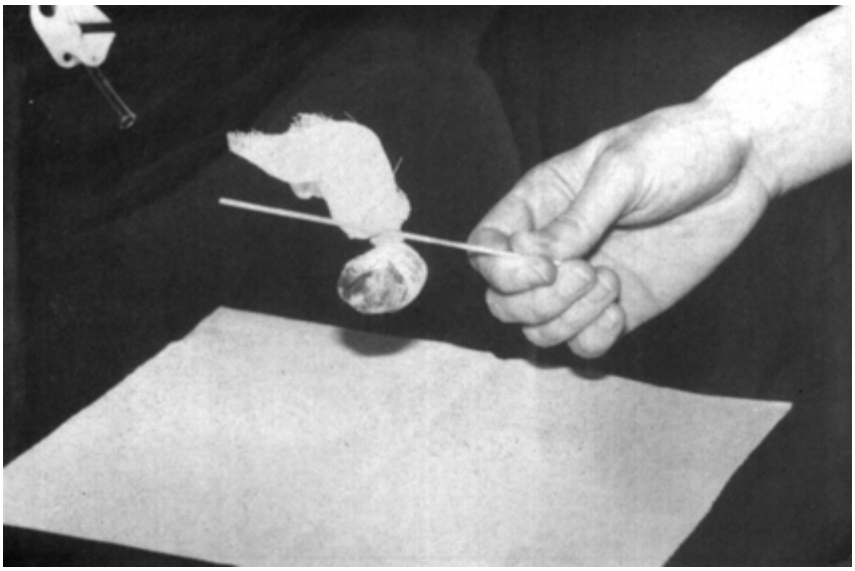
(e) Close the pouch with a rubber band.



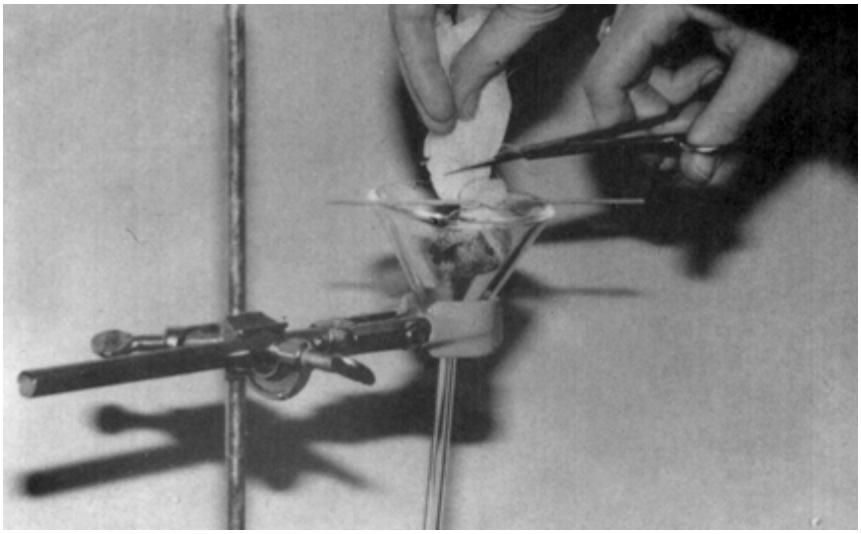
(f) Fix a supporting stick under the rubber band - Step 1



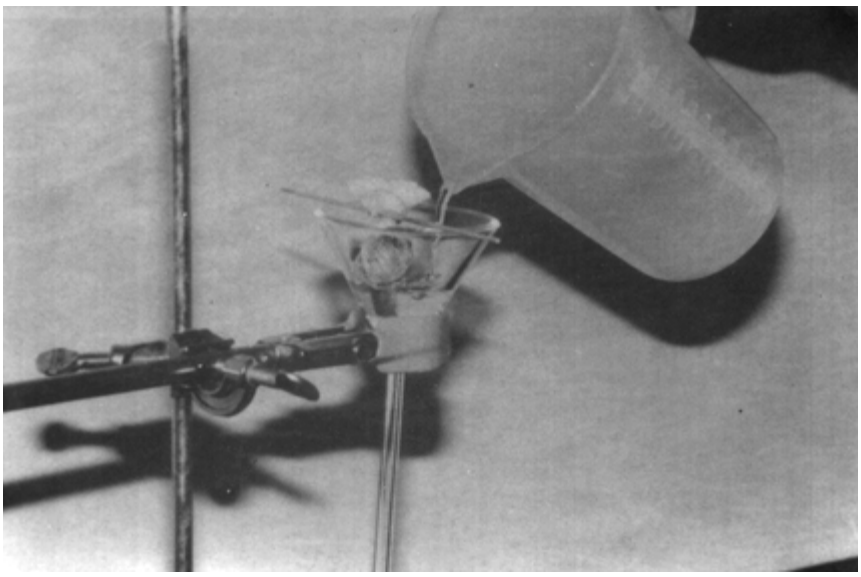
Fix a supporting stick under the rubber band - Step 2



(g) Place the pouch containing faecal culture material or faeces in the funnel. Trim the surplus cheesecloth off.

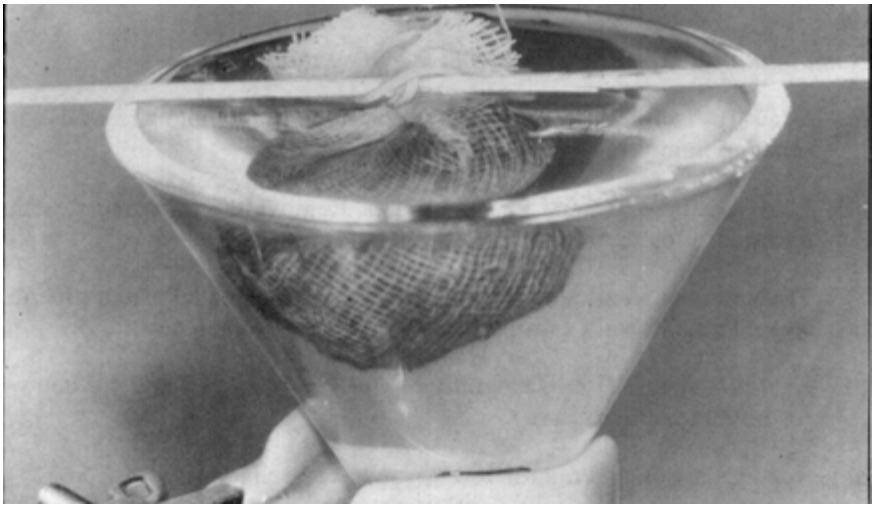


(h) Fill the funnel with lukewarm water, covering the faecal material.



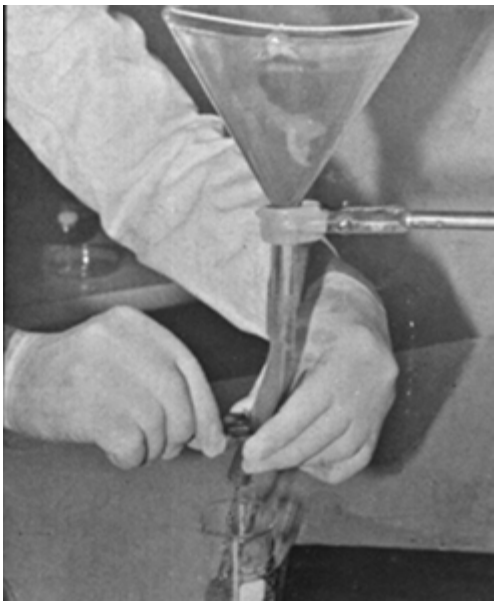
(i) Leave the apparatus in place for 24 hours, during which time larvae actively move

out of faeces and ultimately collect by gravitation in the stem of the funnel.

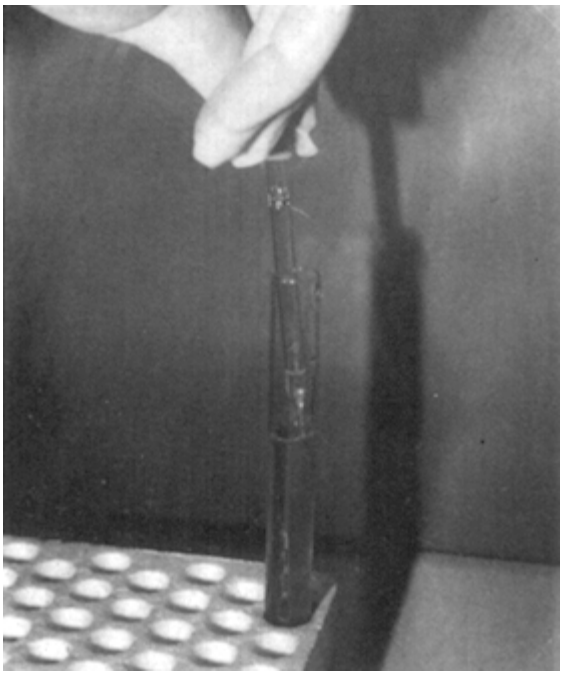


Examination for longhorns

(j) Draw a few ml of fluid from the stem of the funnel into a small petri dish.



(k) Examine under dissecting microscope for live lungworm larvae (L_1).



(l) For positive samples a transfer of larvae to a microslide for identification at 10 x 10 magnification may be required. It is important to differentiate between *Muellerius capillaris* and other species as the treatment is different.

Examination for infective larvae from faecal cultures

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

(n) Leave the tube to stand for 30 minutes. Remove the supernatant with a Pasteur pipette.

(o) 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.

(p) Examine under 10 x 10 magnification. (See Tables 3.3 and 3.4 below for larval identification).

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

(r) 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])

1.	Sheath absent, oesophagus more than $^1/_3$ the length of the body.	*Strongyloides*
	Sheath present, oesophagus short.	2
2.	Length, including sheath, less than 600 μm.	*Bunostomum*
	Length, including sheath, more than 600 μm.	3

3.	Tail of sheath less than 200 m.	4
	Tail of sheath more than 200 m.	5
4.	Two conspicuous oval bodies at anterior end of oesophagus.	6
	No such structures at anterior end of oesophagus.	7
5.	Length including sheath more than 1000 m; tail of sheath dorsal and ventral lobes with a rod-like process between.	*Nematodirus*
	Length including sheath less than 1000 m, tail of larva ending in a simple point.	*Oesophagostomum radiatum*
6.	Length, including sheath, usually more than 850 m; tail of sheath usually more than 150 m long, gradually to end bluntly.	*Cooperia oncophera*
	Length, including sheath, usually less than 850 m; tail of sheath tapering rapidly to a point or short fine filament less than 150 m long.	*Cooperia punctata C. pectinata*
7.	Tail of sheath short and conical, less than 110 m long.	*Trichostrongylus axei*
	Tail of sheath at least 126 m long.	8
8.	Tail of sheath ending bluntly.	*Ostertagia ostertagii*
	Tail of sheath ending in a fine whip-like filament.	*Haemonchus contortus*

Table 3.4 Provides a key to the infective nematode larvae of sheep and goats

Table 3.4 KEY TO INFECTIVE NEMATODE LARVAE OF SHEEP AND GOATS (Adapted from Dikmans and Andrews [1933])

Total length of larva (m)	Length, end of larva to end of sheath (m)	Species, with range of total length (m)	Other differential features
Short 500–700	No sheath 85–115	*Strongyloides* 570–700	Slender body with oesophagus, $^1/_3$ to ½ total length of larvae.
Short 500–700	Long 85–115	*Bunostomum* 510–670	Wide body with sudden tapering to long thin tail. "Band" constriction on oesophagus.
Medium 650–900	Short 20–40	*Trichostrongylus* 620–910	Short straight larva, conical tail sheath. Tubercles on tail of larva. Intestinal cells usually prominent.
Medium 650–900	Short 20–40	*Ostertagia* 790–910	Long, conical, "finger like" tail sheath.
Medium 650–900	Short 20–40	*Cooperia curtice* 710–850	Oval bodies at anterior end of larva. Tail of larva rounded.
Medium 650–900	Medium 30–60	*Haemonchus* 650–750	Tail sheath is usually "kinked". Pointed tail of larva.
Medium 650–900	Medium 30–60	Cooperia oncophera 800–920	Oval bodies anterior end of larva. Tail of larva rounded.
Long 900–1200	Long 60–80	*Chabertia* 710–790	Stout body with 24 to 32 rectangular intestinal cells.
Long 900–1200	Long 60–80	*Oesophagostomum* 770–920	Usually longer than *Chabertia.* Has 16 to 24 triangular intestinal cells.
Long 900–1200	Extremely long 250–290	*Nematodirus* 922–1180	Tail of larva is forked.

3.7 Diagnostic techniques for filarial nematodes

Diagnosis of filarial infections in living animals is done by isolating and identifying the microfilaria of these parasites.

As several of them have distinct predilection sites that have evolved in accordance with the feeding patterns of the insects which serve as their intermediate hosts, the majority of these infections can usually be diagnosed on the basis of clinical signs, site of lesions and type of tissue from which the microfilaria are isolated.

3.7.1 Stephanofilaria

The microfilaria (L_1 larvae) of this parasite are present in the skin lesions caused by the adult parasites. Skin scraping taken after the removal of the crust and exudate from the lesion may contain the microfilaria.

3.7.2 Onchocerca

Microfilaria of *Onchocerca* species are located in the subcutaneous tissue with the highest concentration found in the predilection sites. These sites vary according to the feeding habit of the intermediate hosts. For example, in Sudan the microfilaria are generally concentrated in the midline area of the hump and back of cattle; in Tanzania, microfilaria are found in the tissues of the neck and ears.

Infections can be diagnosed by demonstrating the microfilaria in a skin biopsy taken from the predilection site. The tissue sample is teased and placed in warm physiological saline solution for at least six hours. If present the larvae migrate out and can be found in the saline. If the sample is negative after six hours it should be left overnight to detect low levels of microfilaria in the skin.

3.7.3 Parafilaria

The microfilaria of *Parafilaria* are present in blood oozing from the lesions caused by the adult female penetrating the skin from the subcutaneous tissue. Blood from the "bleeding points" can be examined on a microslide. A drop of blood placed on the microslide should be covered by a cover slip and if present, microfilaria can be seen moving in the smear. Thin or thick blood smears taken from the bleeding points are also suitable for diagnosing *Parafilaria.*

3.7.4 Setaria

The microfilaria circulate in the blood and they can be detected by examining a blood sample as described for *Parafilaria.*

3.8 Identification and examination of snails

Aquatic lymnaid snails which act as intermediate hosts for *Fasciola hepatica* and *Fasciola*

gigantic can be identified fairly easily as compared to other freshwater snails. The opening of the lymnaid snails is on the right when the snail is held with the spires pointing away from the viewer. When the apex of the spire is facing the viewer the spires turn clockwise.

For closer identification of the species and examination of the snails for larval stages of the flukes, samples should be sent to the nearest appropriate laboratory.

---

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 postmortem 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 x 45 x 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 or nylon 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

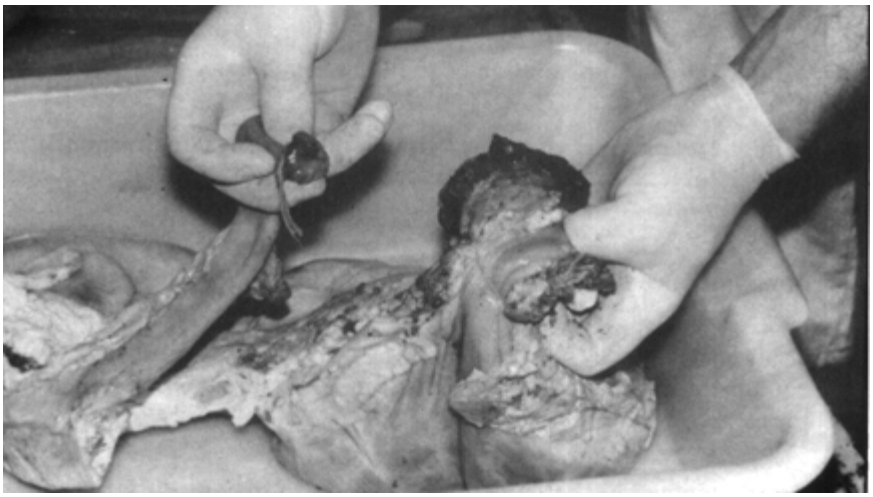
Counts of gastro-intestinal parasites are most conveniently done by examining the abomasum, small intestines and large intestines separately.

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.

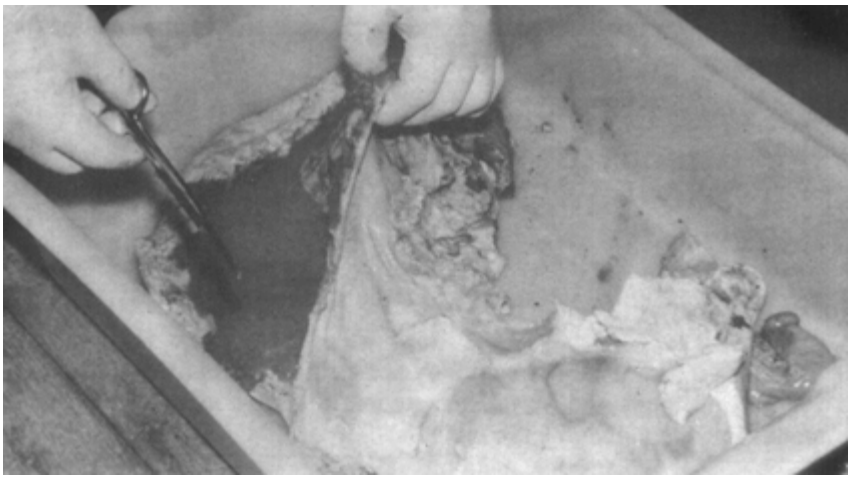
4.3.1 Differential parasite counts of the abomasum

4.3.1.1 Procedure

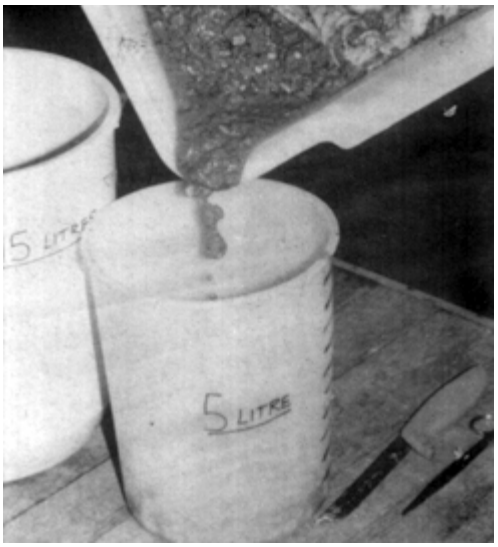
(a) During the post-mortem examination, ligate the abomasum with string and separate it from omasum and duodenum.

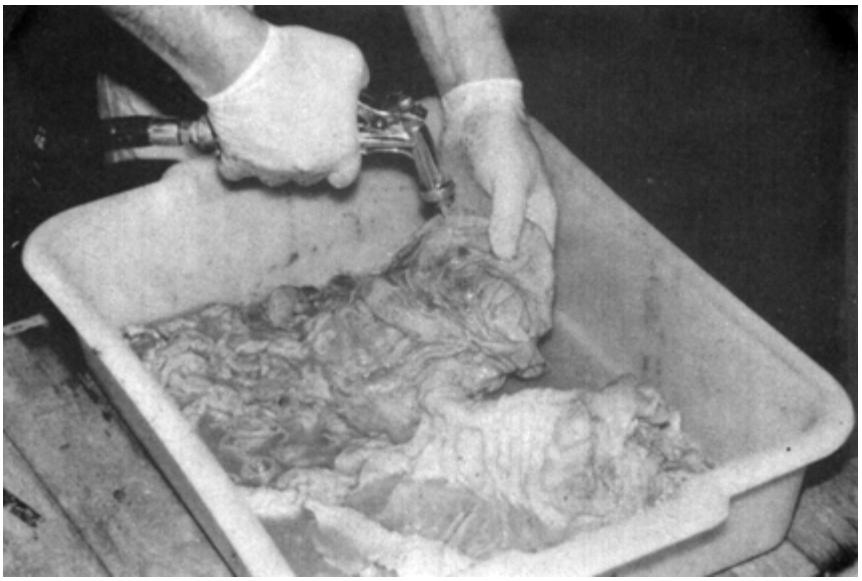


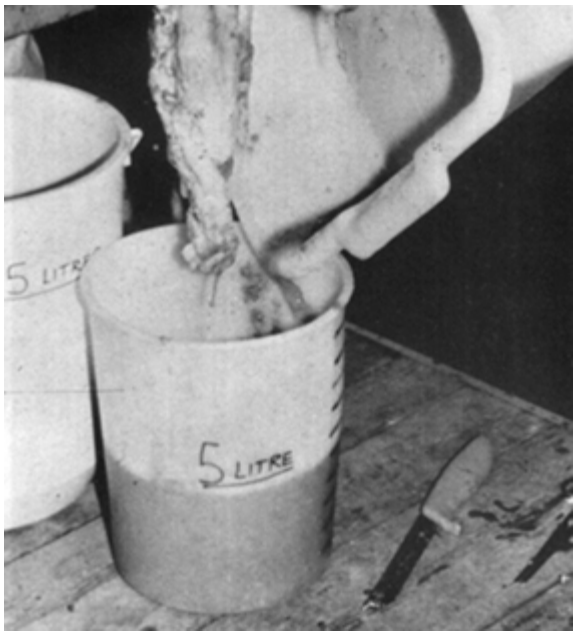
(b) Place the abomasum in a tray. Open the abomasum along the greater curvature so that its contents fall into the tray: empty the abomasal contents into the total content jar.



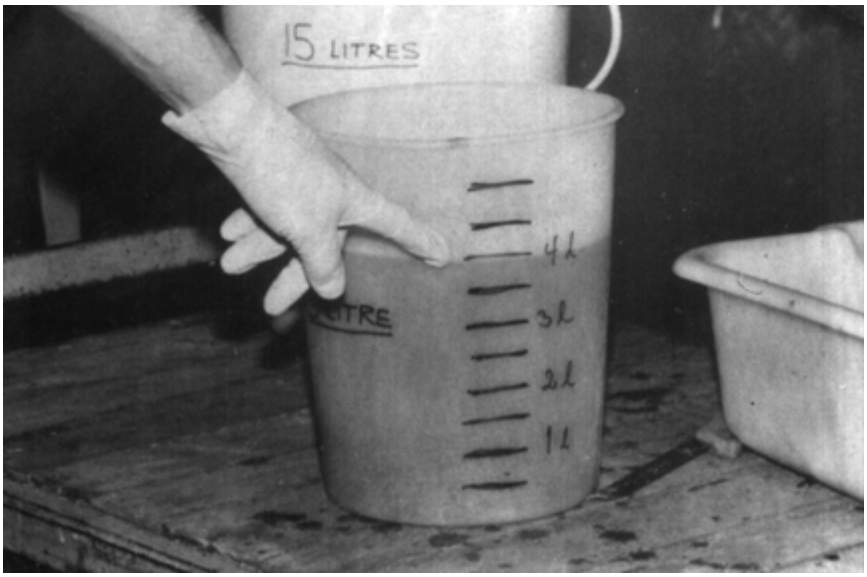
(c) Wash the empty abomasum thoroughly in the tray several times, paying particular attention to cleaning between the folds of the mucous membrane. Add the washings to the total contents jar.







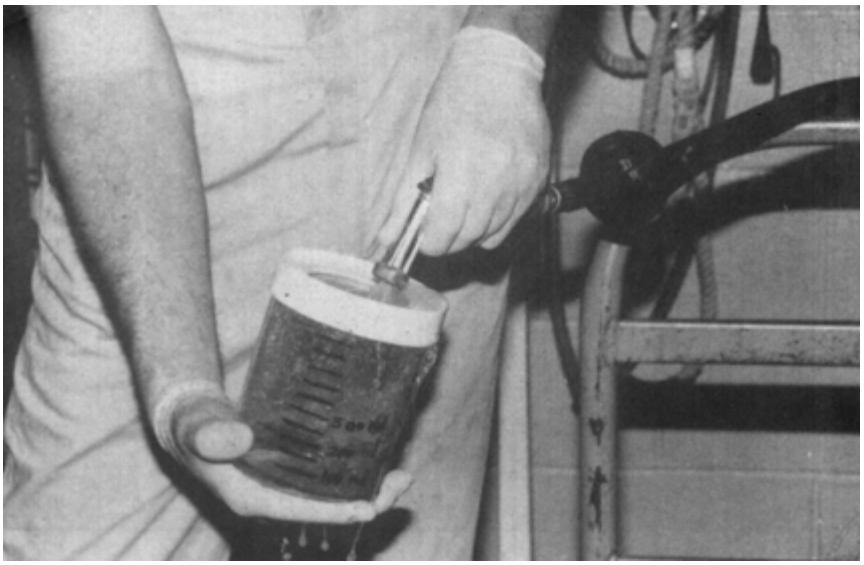
(d) For cattle, make the total volume of contents and washings in the total contents jar up to 4 litres with water. Occasionally it will be necessary to make the total volume up to 5 litres for cattle. For sheep and goats, make the volume up to 2 or 3 litres.



(e) Using the large ladle, stir vigorously until all food material, mucus and water are thoroughly mixed.

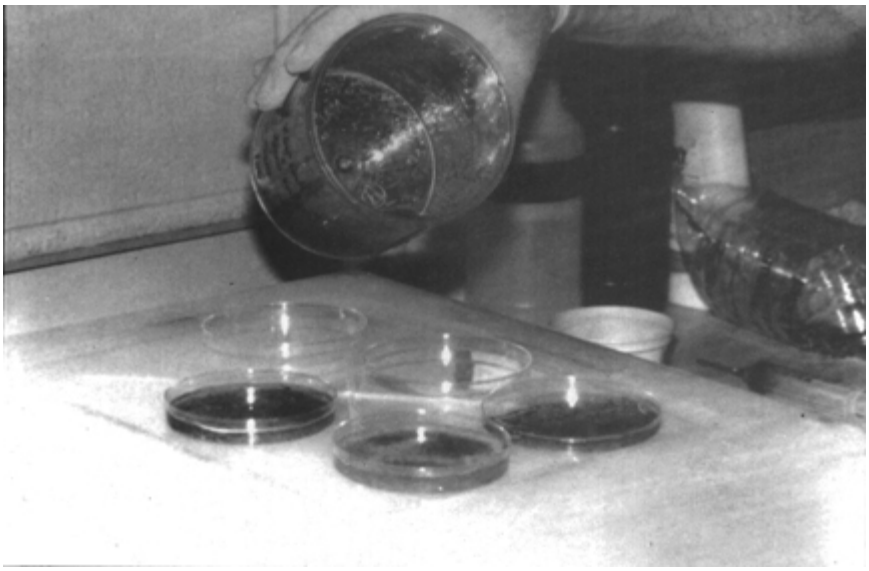
(f) Transfer a total of 200 ml of the contents to the wash jar in 5 steps of 40 ml per step, using the ladle container and stirring the mixture continuously.

(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 culturing matter is removed.

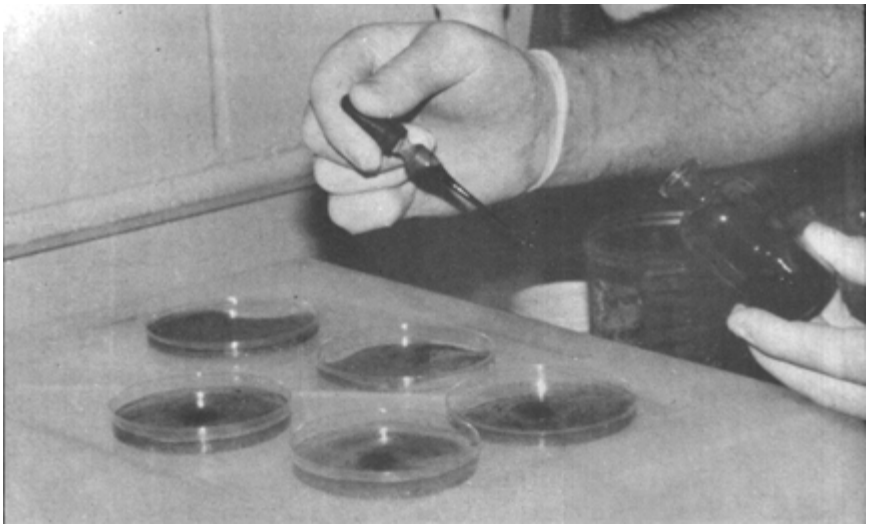


(h) Add water to make the volume in the wash jar up to 50 ml (for convenience).

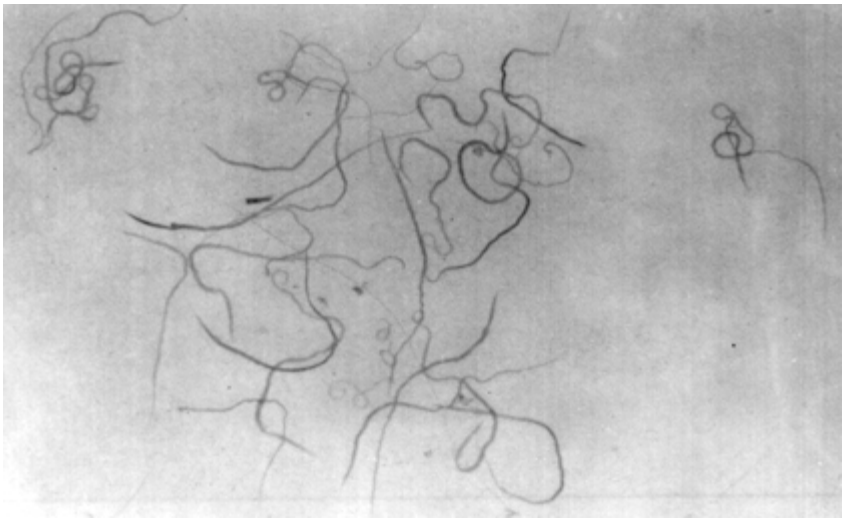
(i) Pour small volumes into petri dishes.



(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 35 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 mucous 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.

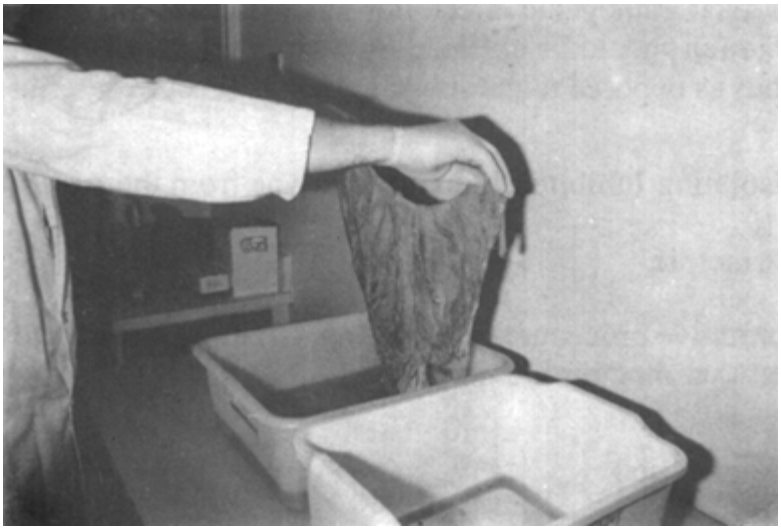
4.3.2.3 Equipment

- 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-m m mesh
- Baker
- Petri dishes
- Wash bottle

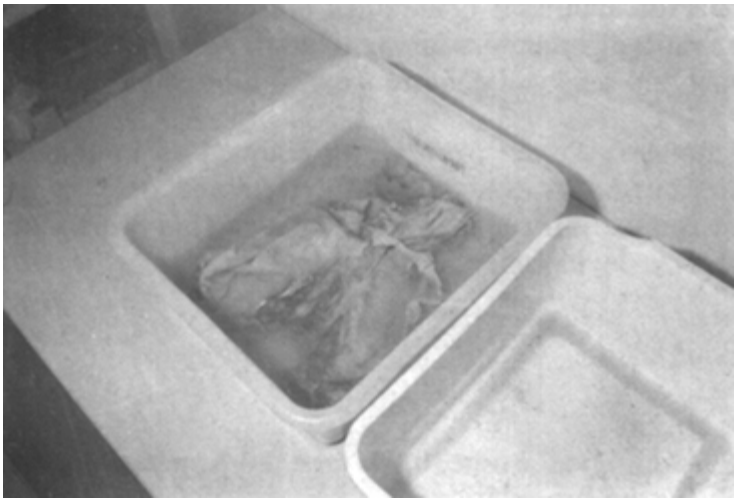
- Pasteur pipette
- Microslides/coverslips
- Microscope/dissecting microscope

4.3.2.4 Procedure

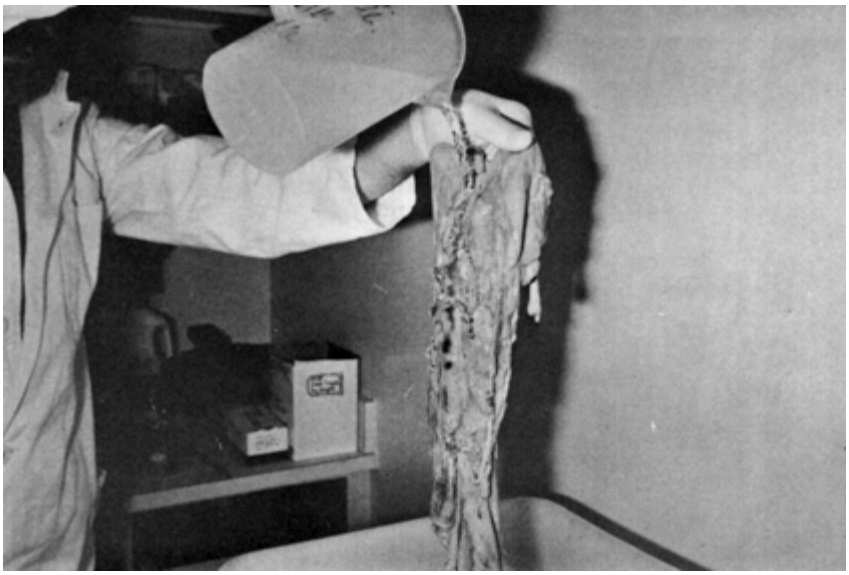
(a) Place the opened and washed abomasum with the mucous membrane face down in the tray/bucket containing lukewarm normal saline solution.



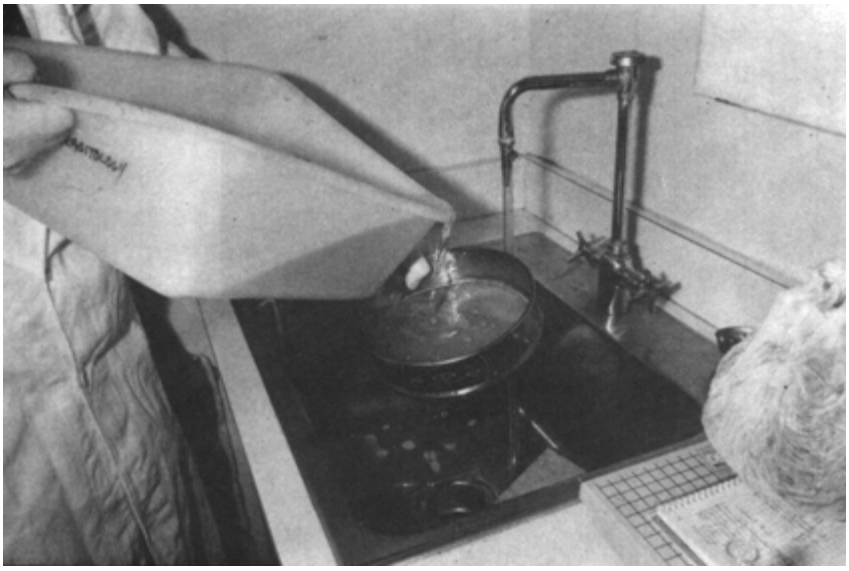
(b) Leave the abomasum to soak overnight.



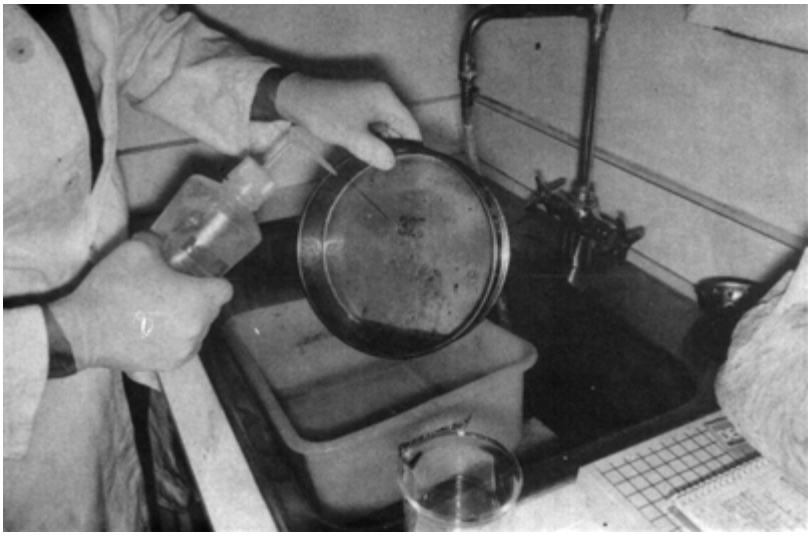
(c) Remove the abomasum, rinse well with saline solution and discard.



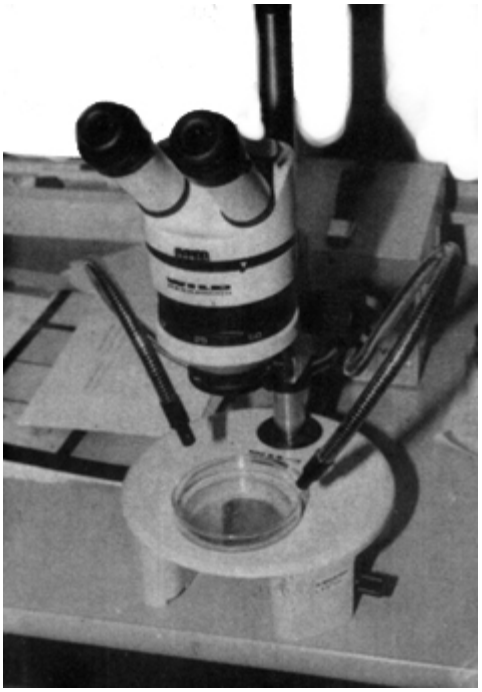
(d) Pour the saline solution left in the tray/bucket through the sieve/nylon net, which will retain the larvae.



(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.



(g) To identify the parasite species, transfer further sub-samples by Pasteur pipette to microslides for examination under the microscope.

(h) The total number of larvae is calculated as follows: number in 10 ml sub-sample x 20 = total abomasal larval count.

4.3.3 Differential parasite counts of the small intestines

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.

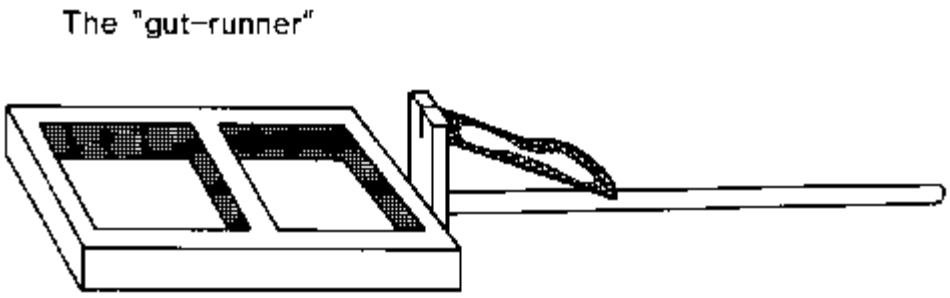
(c) Initially, the gut is washed by pouring water into one end of the gut and flushing it out into the total volume jar. For further washing and scraping, the intestine has to be opened.

(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, sub-sampling, 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 provides a guideline for interpreting adult nematode counts.

Table 4.1 A GUIDELINE TO THE INTERPRETATION OF ADULT NEMATODE COUNTS

Nematode species	Degree of infection (total number of parasites)		
	Light	Moderate	Heavy
CATTLE			
Abomasal parasites	1-5000	5000-10000	10000+
Small intestinal parasites	1-000	8000-20000	20000+
Haemonchus	1-400	400-1500	1500+
Trichostrongylus	1-10000	10000-25000	25000+
Cooperia	1-5000	5000-10000	10000+
SHEEP			
Haemonchus	1-500	500-1500	1500+
Trichostrongylus	1-1000	1000-10000	10000+
Nematodirus	1-2500	2500-8000	8000+
Oesophagostomum	1-50	50-150	150+

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 provide simple keys for identifying some common gastrointestinal 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

Organ location	Head region	Characteristics of Parasite		
		Anterior end	Mature size	Genus
Abomasum	No cephalic swelling	Cervical papilla present	Large	*Haemonchus*
			Medium	*Ostertagia*
		Prominent excretory pore	Small	*Trichostrongylus*
		Very long oesophagus	Medium	*Strongyloides*
Small intestines	Cephalic swelling with striations		Small, coiled	*Cooperia*
			Medium, tangled	*Nematodirus*
	Distinct buccal cavity with teeth	Head bent dorsally	Large, stout	*Bunostomum*
	Tapeworms Long, flat, segmented	Scolex with suckers	Large, 2 genital pores/seg.	*Moniezia*
			Small, indistinct segments	*Avitellina*
Caecum and large intestines	Small, indistinct	Very long thin neck	Large, whip-like	*Trichuris*
	Leaf crowns present	Cervical papillae level	Large, stout	*Oesophagostomum*

		with oesophagus		*columbianum*
		Cervical papillae behind oesophagus	Large, stout	*Oes. venulosum*

Further differential features are given in Tables 4.3-4.5.

Table 4.3 NEMATODES OF THE ABOMASUM

	Haemonchus	***Ostertagia***	***Trichostrongylus***
Mature size	Males 10 to 20 mm long. Females 18 to 30 mm.	Males 7 to 8 mm long. Females 9 to 12 mm.	Males 4 to 5 mm long. Females 5 to 7 mm.
	Large, easily seen, mostly in fundus region of abomasum	Mostly found at pyloric region of abomasum	Very small, difficult to see without washing or staining.
		Much the same thickness throughout length of worm.	Taper to very fine anterior end.
Head	Prominent large cervical papillae.	Small cervical papillae set more posteriorly.	No cervical papillae. Prominent excretory pore.
	Distance from anterior end about 3 times diameter between papillae.	Distance from anterior end about 5 times diameter between papillae.	
Female	Vulva covered by large vulval flap.	Small or no vulval flap.	Simple genital opening without vulval flap.
	Red and white spiral striping visible in fresh specimens resembling "Barber's pole".	Under high magnification tip of tail shows annular rings.	Cuticle striations are annular.
		Cuticle striations are longitudinal.	
Male tail	Asymmetrical dorsal lobe in bursa.	Bursal lobes are symmetrical.	Bursal lobes are symmetrical.
	Spicules taper to barbed points.	Spicules vary with species.	Spicules vary with species.

Table 4.4 NEMATODES OF THE SMALL INTESTINES

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

CAECUM	***Trichuris***		
Mature size	Male 50 to 80 mm long. Female 35 to 70 mm long. The anterior end is very thin, the posterior end is thick. It is called the "whip-worm" because of its shape.		
Other features	Male has single spicule in spine-covered protrusible sheath. Female produces barrel-shaped eggs with a transparent plug at each end.		
COLON	***Chabertia***	***Oesophagostomum venulosum***	***Oesophagostomum colombianum***
Mature size	Male 13 to 14 mm long.	Male 11 to 16 mm long.	Male 12 to 16 mm long.
	Female 17 to 20 mm.	Female 13 to 24 mm.	Female 15 to 21 mm.
Other features	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.	Small buccal cavity surrounded by leaf crown. Cervical papillae are situated posterior to the oesophagus.	Small buccal cavity surrounded by leaf crown. Cervical papillae are situated opposite anterior region of oesophagus.

4.6 Post-mortem examination for trematodes

4.6.1 Introduction

For a more precise assessment of the liver fluke burden of an animal the liver can be examined post-mortem for its content of immature and adult flukes.

4.6.2 Equipment

- A sharp knife
- A cutting board
- A medium size tray
- A wash bottle
- Petri dishes
- A laboratory counter

4.6.3 Procedure

(a) Place the liver on a board and cut it into fine slices with a sharp knife.

(b) After each cut is made apply pressure to the liver to squeeze out the flukes and wipe these off gently before making the next cut.

(c) When the whole organ has been sliced, place all of the material in a tray and cover with water.

(d) Remove the pieces of liver, again squeezing each piece as it is removed from the water.

(e) Pour water and flukes into a sieve and wash parasites until clean.

(f) Pour into petri dishes and count the flukes present.

(g) If very large number of immature flukes are present, count by a dilution technique.

4.7 Post-mortem examination for cysticercosis

4.7.1 Introduction

The diagnosis of cysticercosis in carcasses is done during routine meat inspection according to rules and regulations which are specific for each country. The rules governing meat inspection attempt to accommodate the interest of the owner/butcher (carcasses should not be spoiled by too many incisions) and those of the veterinary public health service.

4.7.2 Equipment

- A sharp knife
- A cutting board
- A laboratory counter (optional)

4.7.3 Procedure

The routine inspection for *Cysticercus bovis* consists of the following:

(a) Two incisions parallel to the jaw of the external masseter muscles and one of the interior.

(b) A longitudinal incision of the ventral side of the tongue.

(c) Incision of the heart septum.

(d) Two parallel incisions in the triceps muscle.

(e) Visual inspection of all exposed muscle surfaces.

As the judgement of carcasses is related to the number of cysts, a quantification of cysts may be required if the routine inspection only reveals 1-5 cysts. The masseter muscles, the heart, the tongue, the triceps muscle and the diaphragm should be removed from the carcass and examined by cutting the muscles in 5 mm thin slices, inspecting all exposed surfaces for live and dead cysts.

---

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 L_3 from representative samples of herbage taken from defined grazing areas.

5.2.2 Application

This is a useful procedure for determining seasonal variations in L_3 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.

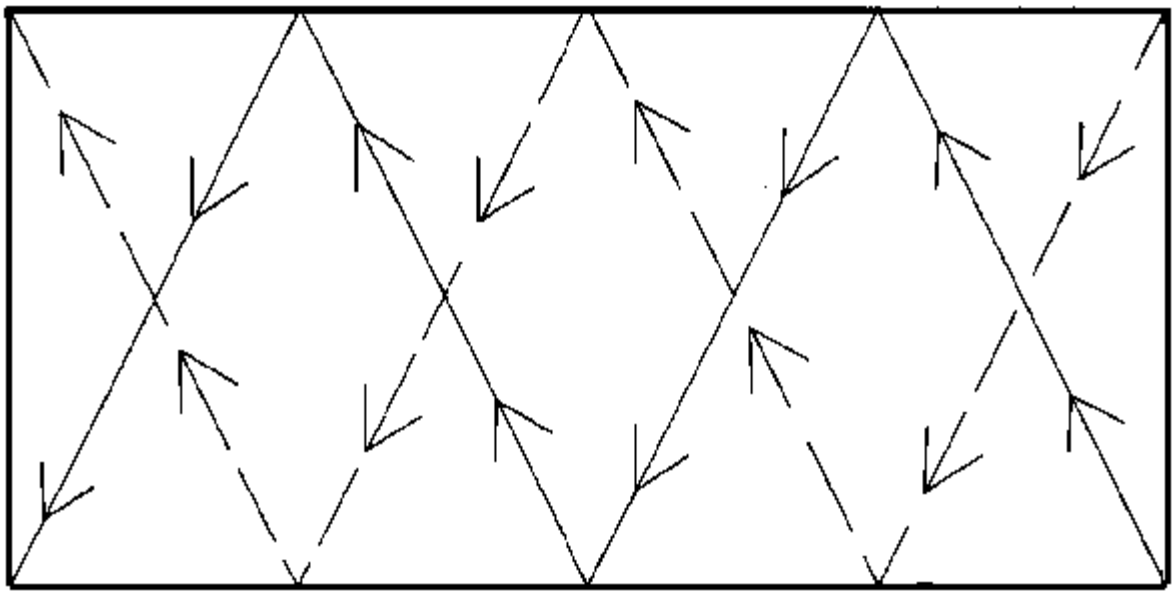
5.2.3 Equipment

- 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

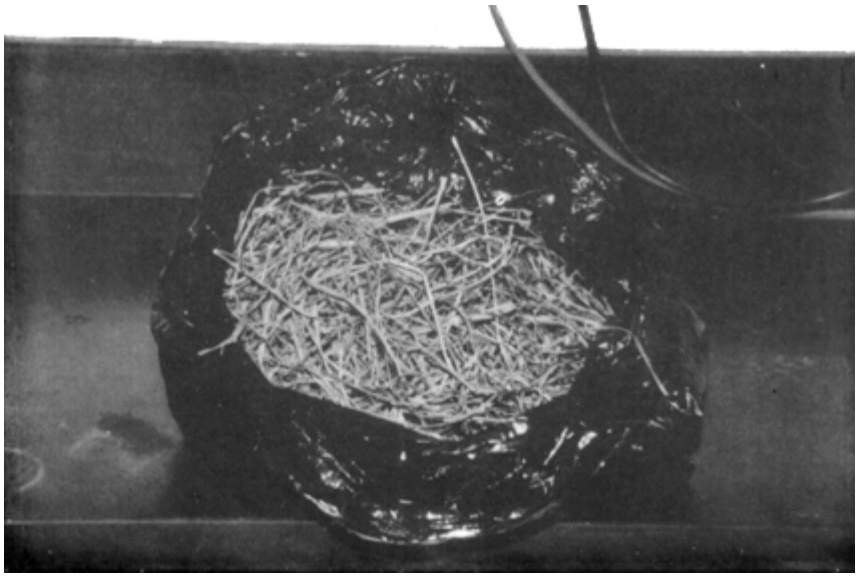
Dissecting microscope or microscope
- Balance

5.2.4 Procedure

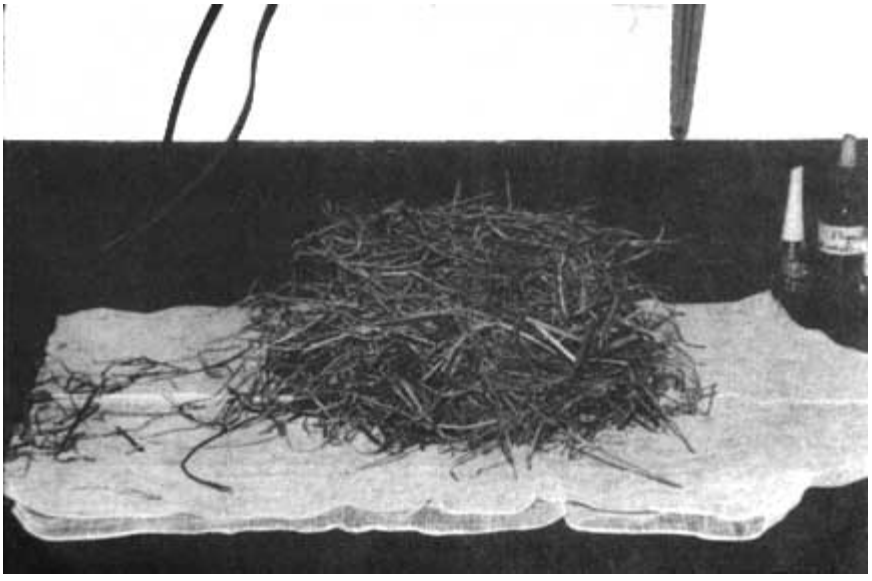
(a) Collect small samples of grass from a large number of sites randomly scattered throughout the area to be sampled, using a "W" or "N" collecting route. Avoid areas of heavy faecal contamination and avoid collecting soil.



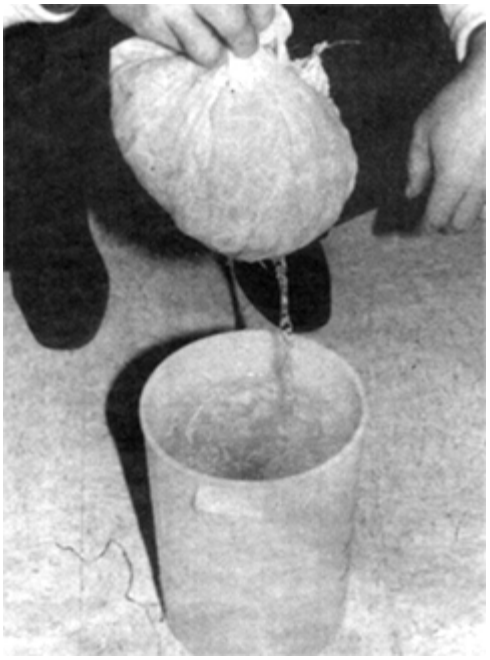
(b) Grass can be collected by hand. Where grasses are coarse, scissors may be used. Grass samples of approximately 300-600 g should then be placed in a plastic bag.



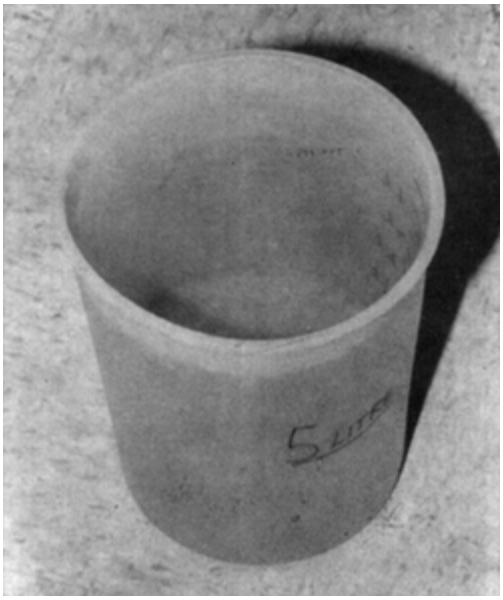
(c) Place the collected grass sample inside a gauze bag and immerse the bag in water in a bucket but keep the bag clear of the bottom of the bucket.



(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.

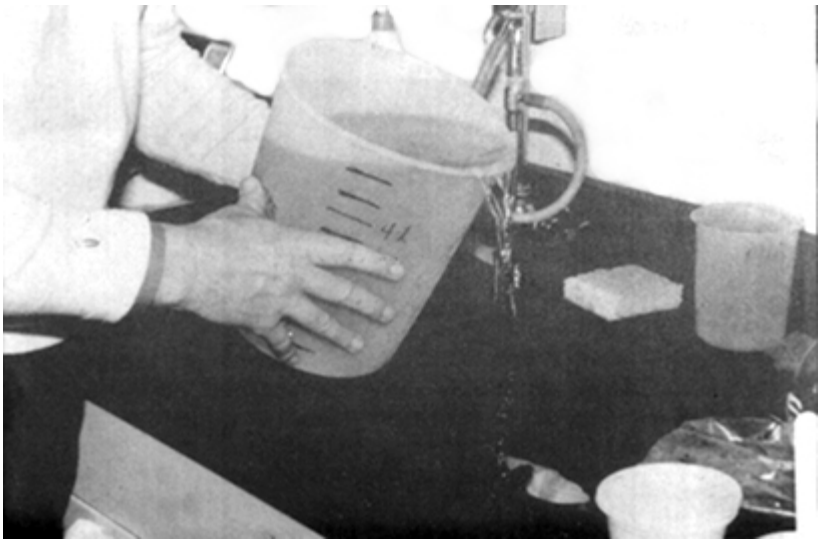


(e) The next morning, remove the bag and run fresh tap water over it and into the bucket. Leave the contents of the bucket to sediment for about 1 hour.

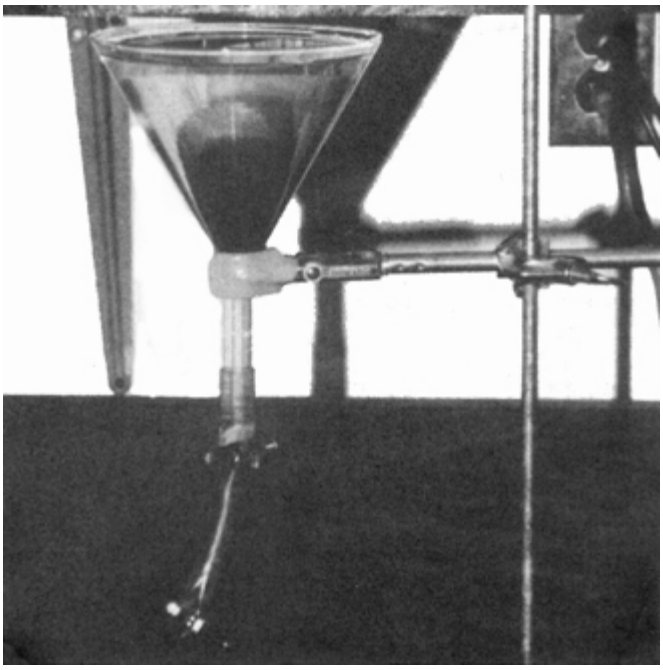


(f) The bag of grass should be dried (sun/oven/incubator) and weighed when completely dry.

(g) Carefully decant or syphon off the supernatant, leaving about 1 litre containing all the sediment.

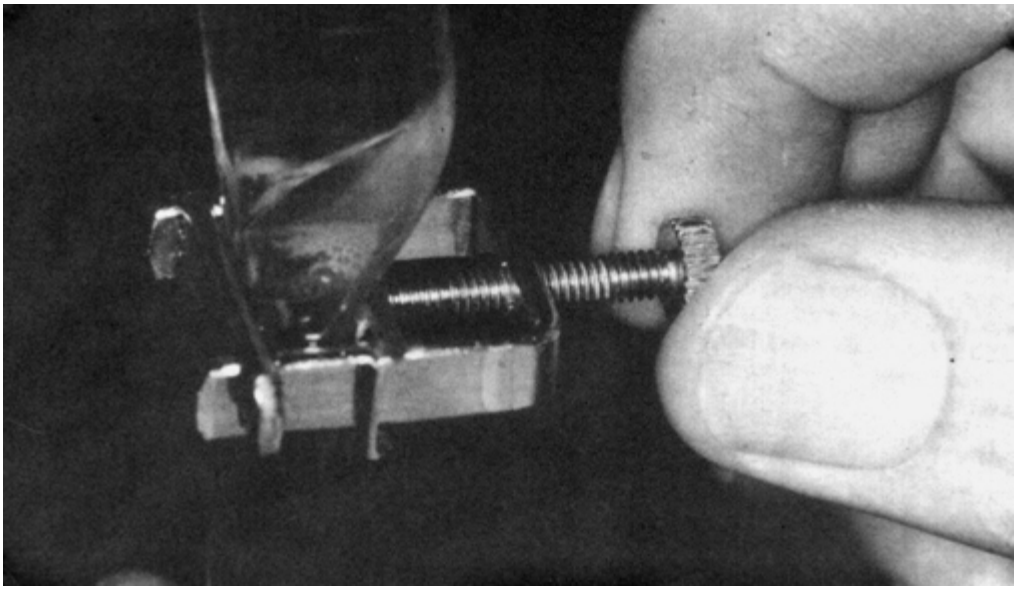


(h) Suspend the sediment and pour it into the large funnel in its stand with the bottom clamp fastened. Wash out the bucket into the funnel, but discard any heavy debris that sediments rapidly.

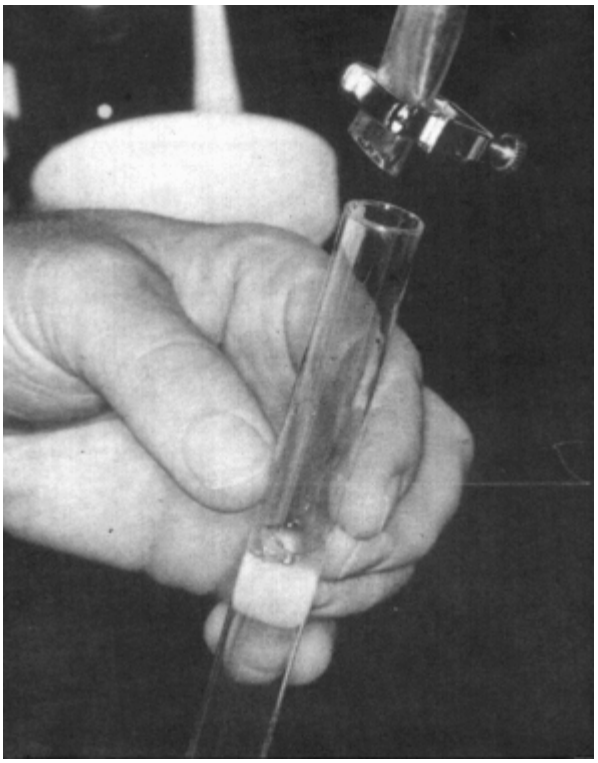


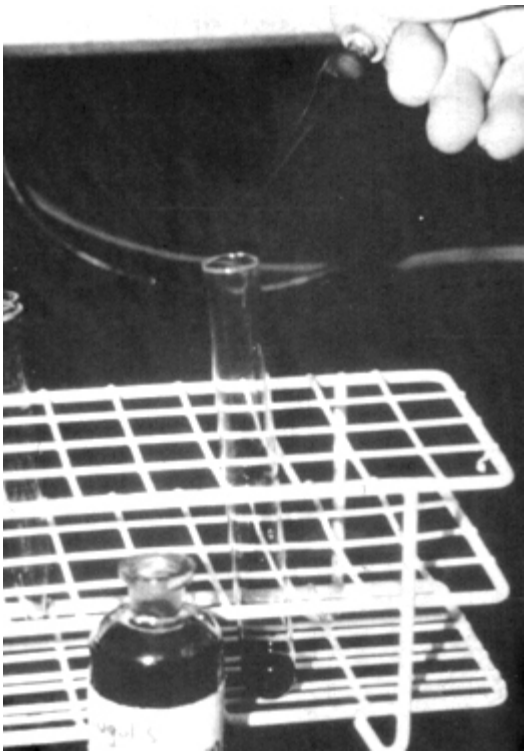
(i) Leave the funnel to stand for another hour.

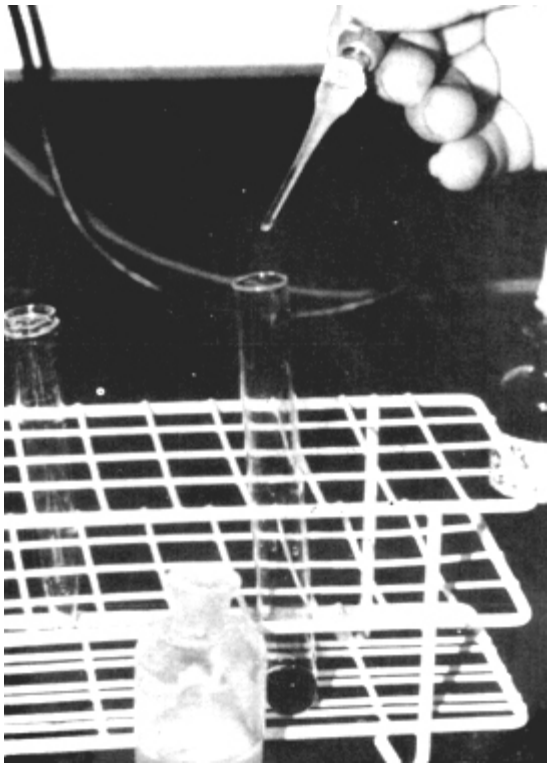
(j) Close the top clamp and collect the trapped sediment with about 15 ml of fluid in a test tube. Procedures (h) and (i) may need to be repeated when large volumes of sediment have to be processed, or when only a small funnel is available. Leave to cool at 4 °C for at least 1 hour.



(k) Siphon off the supernatant to leave 35 ml, which is stained with 35 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 coloration of the iodine for 30-40 minutes after decolouration with sodium thiosulphate.

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

(n) Number of larvae per kg of dry herbage = count x 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, Trichuris* and *Fasciola* can cause anaemia. In acute haemonchosis and fascioliasis, 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* and other blood-sucking parasites in a parasite problem. It is useful as an early aid to the diagnosis of haemonchosis and

fascioliasis. 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 needles 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 or tail) 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 x 1.5 mm capillary tube for ¾ 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.

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 units 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 diarrhea 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

Number in herd/flock	Number to sample
1-10	All
11-25	At least 10 animals
26-100	At least 20 animals
101-200	At least 30 animals
Over 200	At least 15 per cent
Over 500	At least 10 per cent

(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 of 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 of 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 or 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 (6-9-month-old calves; 3-4-month-old lambs) 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 L_3 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 (L_3).

(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.

---

7. Treatment and control strategies

7.1 Principles of control: Nematodes

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 programmers should reduce the effect of parasites to sub-economic levels.

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

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 gastrointestinal parasites. The pathogenicity is usually high when *Haemonchus, Mecistocirrus, 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

If possible data should be acquired on the number of animals, the broad age structure of the herd/flock and the time of calving/lambing in relation to the rainy season(s). Furthermore information should be obtained on the grazing management practiced as the control measures may differ for animals grazing communal lands, confined animals (zero grazing), tethered animals, animals in crop rotation systems and herds/flocks under transhumant/nomadic systems.

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 (L_3) can be established for any particular pasture. Similarly, trends in relative abundance of (L_3) 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 (L_3) on the pasture. This is strongly dependent upon climate. Climatic data from each study site or from local meteorological stations are therefore required.

7.1.5 Genetic resistance

With the widespread development of resistance to anthelmintics and the high cost of developing new drugs, the interest in exploiting and developing animals that are genetically resistant to helminth parasites has increased considerably. Studies have shown that resistance is heritable in cattle, sheep and goats but this approach to the control of helminths is currently being exploited principally in sheep. Two strategies can be followed in the development of genetically resistant sheep; one is the selection for resistant individuals within breeds; the other is the exploitation of sheep breeds which appear to have increased helminth resistance.

7.1.5.1 Parasite resistance within breeds

It has been shown that within any one breed, lambs of certain rams or ewes have increased

resistance to helminth parasites and some selection for resistance is feasible. Based on the results from breeding programmes for resistance against helminths, it has been concluded that faecal worm egg counts are a reasonable comparative indicator on which to base selection of resistant animals with heritabilities similar to those for production. This allows a simple and practical method of-breeding for resistance.

7.1.5.2 Parasite resistance between breeds

A number of studies have shown that some sheep breeds are more resistant to helminth infections, specifically *Haemonchus contortus,* than other breeds. The Red Maasai, the Barbados Blackbelly and the St. Croix are examples of breeds which have demonstrated resistance traits.

7.1.6 Control of gastro-intestinal nematodes

The ideal approach is an integration of:

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

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

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

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

With a number of anthelmintics available in several formulations (ready-to-use drench, paste, powder, mineral premix, urea/molasses/anthelmintic blocks, pour on, injectable, slow release and pulse release devices), it is recommended that the ideal formulation is selected by considering availability, price, parasite species to be treated, type of livestock, livestock numbers and type of management.

7.1.6.1 Control in savannah-type climates with one or more distinct dry season(s)

In many cases a separation of young newly weaned animals from the older animals is not possible and it is important that all animals in the herd/flock are treated. An effort should be made to convince livestock owners using communal grazing areas to include their animals in the programme. It is recommended to treat animals at the beginning of the dry season in order to eliminate the parasite burden, enabling them to better cope with the nutritional stress during the dry season. A treatment prior to the rainy season using a larvicidal drug will prevent the "rains rise" and contamination of pastures at a time when conditions are becoming favourable for egg and larval development.

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 gastro-intestinal mucosa. If not treated, this "rains rise" results in a heavy contamination of the pasture with eggs, and subsequently L_3 which when ingested by young, susceptible animals may result in severe clinical disease.

More than one treatment at the beginning of the rainy season may be necessary in the following cases:

- all animals in the herd/flock (communal grazing) were not treated and contamination of pasture continues resulting in rapid reinfection of animals
- drugs not effective against arrested larvae were used
- the pasture was not parasitologically safe after the dry season

The second treatment if necessary should be given three weeks after the pre-rains treatment. Two successive treatments three weeks apart should prevent livestock from acquiring parasite burdens.

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

7.1.6.2 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.

7.1.6.3 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.

7.1.7 Control of lungworms

The introduction of control programmes for gastro-intestinal nematodes using grazing management and strategic use of modern anthelmintics will often result in partial control of

lungworms as well *(Dictyocaulus* species, *Protostrongylus).* This effect is enhanced by the use of anthelmintics with a prolonged period of activity (such as ivermectin) or slow-release and pulse-release devices. Several drugs are available in these formulations, including most of the benzimidazoles, immidazothiazoles and ivermectin and these are effective against both adults and larvae.

The pathogenic effect of *Mullerius capillaris* is usually limited to small sub-pleural nodules and the infection only rarely requires specific treatment. The benzimidazoles are efficient but at 3-5 times the normal dose.

Irradiated L_3 vaccines have been developed for the control of *D. viviparus* in cattle and *D. filaria* in sheep and goats. The attenuated larvae are administered orally in 2 doses 4 weeks apart. The animals should preferably be confined during the treatment and for 2 weeks after to allow time for an adequate resistance to develop. The vaccines produce a strong immunity which is maintained if animals are continuously exposed to reinfection.

7.1.8 Control of filarial nematodes

The principle of control of the filarial nematodes is based on either reducing the number of microfilaria, eliminating the adults, or both.

The most common treatment of *Stephanofilaria* infections is local application of organophosphate compounds. Broad-spectrum benzimidazoles and ivermectin have been reported to be efficacious.

Onchocerciasis treatment is usually directed towards the microfilaria; diethylcarbamazine and ivermectin both have high activity against these.

Animals infected with *Parafilaria* can be treated with nitroxynil or high doses of fenbendazole or levamisole, given daily for four days. This will eliminate the adult parasites and reduce the lesions significantly.

The treatment of epidemic cerbrospinal nematodiasis caused by migrating larvae of *Setaria* species is very difficult. Diethylcarbamazine and levimisole have been used but the results are not conclusive. Early treatment is crucial. Treatment with ivermectin has been shown to be efficacious against adult *Setaria.*

7.1.9 Control of Toxocara vitulorum

Newborn calves are infected with *T. vitulorum* larvae (L_3) through the colostrum and milk. The majority of larvae are excreted during the first 10 days post partum. In some parts of the world calves may become infected ingesting infective eggs from the environment. If calves survive the infection they develop a strong immunity against the parasite and the adult parasites are eliminated from the intestine. The principle of control is to prevent the parasites from having an effect on the calves and from contaminating the environment with eggs. Both objectives can be met by treating calves between 10–16 days of age, eliminating the immature parasites before they can harm the calves and start egg production.

Farmers should be advised to treat all calves between 10–16 days of age, repeating the procedure as more calves reach the age for treatment. With large herds this may require a regular weekly treatment programme.

Several efficient drugs are available including piperazine, pyrantel/morantel, levamisole and fenbendazole. The treatment can be administered either as a drench or as a paste. The efficacy of treating cows in an attempt to eliminate somatic migrating larvae of *T. vitulorum* has

not been adequately demonstrated, and is not considered justifiable.

7.2 Principles of control: Trematodes

Effective control of most trematode infections is based on strategically applied chemotherapy. Improvements in current farm management can reduce the chances of infections by limiting the contact between intermediate and final hosts. Furthermore, direct action may be taken to reduce or eliminate intermediate host populations. The use of one or more of these measures in an integrated strategy should be based on sound economic assessments of the diseases and the relative merits of control options. Some animal husbandry systems such as zero-grazing (cut and carry) and tethering of animals may minimize the risk of trematode diseases.

7.2.1 Fasciola hepatica and Fasciola gigantica

Efficient control of fascioliasis requires a well planned and executed, integrated control programme designed for each farm, area, country or region. The available strategies which can be used individually or in combination are:

- Strategic application of anthelminthics, eliminating the parasites from the host at the most appropriate time for effective prevention of pasture contamination.
- Reduction in the number of intermediate host snails by chemical or biological control.
- Reduction in the number of snails by drainage, fencing and other management practices.
- Reduction in the risk of infection by planned grazing management.

7.2.1.1 Strategic chemotherapy of ruminants

Seasonal strategic application of effective anthelmintics specific for trematodes, as well timed prophylactic and curative treatments, play an important role in the control of liver fluke infections. Strategic treatments have been developed for several regions of the world based on meteorological data. However, it is advisable to supplement meteorological data with sound epidemiological information in order to improve the timing, and thereby the efficiency, of treatments. The basic principles of strategic anthelminthic application (treatment/prophylaxis) are:

(a) Prophylactic treatment of ruminants towards the end of a period of ecologically reduced activity of the parasites and the intermediate hosts.

One treatment is therefore recommended towards the end of a period when larval development in the fluke eggs or in the snails has been retarded, and when the reproductive rate of snails is low or their activity is impaired (such as during a

prolonged dry season, or extreme cold). At that time, a prophylactic effect can be achieved by reducing the pasture contamination of eggs before favourable climatic conditions for larval development and snail activity resume.

(b) Curative treatment about one to two months after the expected peak infection of the hosts.

A curative effect can be achieved by one treatment to remove the residual fluke burden acquired from metacercariae which had survived on the herbage.

(c) Additional treatment in highly contaminated areas where seasonal variations do not significantly affect the life cycle of the flukes.

These additional treatments may be required occasionally, when the seasonal climatic conditions are favourable for parasite and snail development, or in areas where high metacercariae intake often occurs as a result of restricted grazing of wet areas during dry seasons.

The most important prerequisite for efficient chemotherapy and chemoprophylaxis is a prior knowledge of the epidemiology of the disease based mainly on meteorological data and seasonal surveys in hosts.

The economics of chemotherapy should be evaluated for each farm, area and country, including assessments of the availability of anthelminthics, their price and the economics of the livestock production system in which they are to be used. More treatments are necessary if the drugs available (or selected on the basis of cost) are those that are only effective against mature flukes. Efficient control programmes can be developed based on less frequent treatments with drugs effective against early immature and immature flukes. However, the price of these drugs is considerably higher than those effective only against older flukes and their use may therefore be restricted to the more intensive livestock production systems.

If animals are grazing communal areas, it is important to achieve a synchronized reduction in pasture contamination of eggs, if possible. Ideally all animals in the area should receive treatment within a short period of time.

7.2.1.2 Chemical control of snails

The use of molluscicides for the control of snail intermediate hosts is a potential tool for the control of fluke infections. Before considering chemical control of snails it should be noted that:

- many habitats are topographically unsuitable for the use of molluscicides and it is often very difficult to apply them effectively.
- they are toxic to the environment
- cooperation between neighbouring properties is required for effective cover
- regular (at least yearly) application is required because rapid repopulation of snails may occur
- they are not species-specific and may destroy edible snails highly valued as food in some communities.
- they are expensive

7.2.1.3 Biological methods of snail control

Reports from several parts of the world indicate that a number of plants have molluscicidal properties. Planting of these trees and shrubs along streams and irrigation channels can reduce the number of snails in a population. The efficacy of this method for control of flukes has not yet been assessed.

The introduction of large numbers of ducks into rice fields after harvest has been used to reduce the snail population. The ducks eat the snails and the fluke species specific to the ducks compete with the fluke species of ruminants in the infection of snails. It is reported that snails infected with duck flukes will not become infected with flukes of livestock.

The introduction of edible snail species unsuitable as intermediate hosts into the habitat of the host snails may prevent the flukes from completing their life cycle.

7.2.1.4 Managemental methods of snail control

The important management methods of controlling fluke infections are:

(a) To prevent snail habitats from developing by regular clearing of drainage channels in vegetation which provides suitable sites for snail development. Good drainage and the building of dams at appropriate sites in marshy and low lying areas may reduce the snail problem.

(b) To keep livestock away from pastures contaminated with metacercariae. This may only be possible when the number of animals involved is small.

(c) Establish proper watering facilities to prevent animals from drinking from lakes, ponds and streams.

7.2.2 Control of paramphistomes

Most of the principles described for the control of liver flukes also apply to the control of intestinal and ruminant flukes.

Outbreaks of clinical paramphistomiasis caused by the immature flukes in calves, sheep and goats often pass undiagnosed, and the importance of this disease may be considerably underestimated.

7.2.3 Control of schistosomes

Control of schistosomes is based on control of the snail intermediate host and treatment of infected animals and humans.

The proposed control strategies for the control of snails outlined in the section on *F. hepatica* and *F. gigantica* are also applicable to the control of the intermediate host snails of schistosomes. The managemental methods recommended in section 7.2.1.4, points a and c, may also contribute to the control of schistosomiasis.

7.3 Principles of control: Cestodes

The principles of control of tapeworms are to prevent livestock from becoming infected, and

when infection has occurred, to limit the pathogenic effect. The strategies available to achieve these differ according to whether the ruminants act as the final host for the tapeworm or the intermediate host for the larval stages.

7.3.1 Ruminants as final hosts

7.3.1.1 Intestinal tapeworms

As infections with *Moniezia, Thysaniezia* and *Avitellina* species are considered virtually non-pathogenic, specific treatment for these parasites are not recommended. Furthermore, young animals which are not treated develop an immunity to reinfection, whereas regular treatment may interfere with the development of immunity and result in repeated re-infection. In the case of severe infections, it is advisable to modify the treatment and control strategies selected for nematodes to include the use of a broad spectrum anthelminthic that controls both tapeworms and roundworms. A number of the benzimidazoles, such as albendazole, cambendazole and mebendazole, effectively control tapeworms.

Control may also be directed at the intermediate host, the oribatid mites. It has been shown that the habitat of the mites can be destroyed by ploughing and where this is feasible, newly sown pastures can be kept free of tapeworms for several years.

7.3.1.2 Hepatic tapeworms

The apparent lack of pathogenic effects and the absence of diagnostic techniques render treatment of hepatic tapeworms unjustified.

7.3.2 Ruminants as intermediate hosts

7.3.2.1 Cysticercosis

With the availability of sensitive and specific serological tests, it is now possible to diagnose cysticercosis in living ruminants. However, at present it is not considered feasible to treat animals due to the high cost and the possible public health significance of dead, calcified cysts in meat and organs. Drugs which have shown efficacy against larval cestodes include praziquantel, mebendazole and albendazole. Control of cysticercosis and the adult tapeworms is therefore based on the prophylaxis of the infections.

To prevent infection of cattle, sheep and goats, all final hosts, namely man *(Taenia saginata)* and dogs *(T. ovis, T. hydatigena)* should be treated according to need. In addition humans should be educated in the use of high standards of personal hygiene and latrines to prevent the spread of *T. saginata* eggs.

Standardized methods of meat inspection should be implemented to detect infections at slaughter, and infected meat and organs should be condemned, treated (by cooking until grey) frozen to prevent infected meat reaching humans and dogs.

Considerable progress has been made in the development of vaccines against cestode larvae in ruminants, and a commercial vaccine is now available in Australia against *Cysticercus ovis.* It is anticipated that vaccines against other larval cestodes may become available in the future.

7.3.2.2 Coenurosis

Clinical diagnosis of coenurosis is difficult and clinical signs can be confused with other disorders of the central nervous system, such as hypocalcaemia, listeriosis, cerebral abscess, tumours and *Oestrus ovis* infection (in sheep).

No specific treatment is available for this infection and slaughter of the animal is usually recommended.

Prevention of the disease includes the treatment of dogs with taenicidal drugs and the education of farmers and butchers so that offal and condemned material are not fed to dogs after slaughtering a parasitized animal.

7.3.2.3 Hydatidosis/echinococcosis

Immunodiagnostic methods, radiology and ultrasound scanning are used routinely in diagnosing hydatidosis in humans, but these methods are rarely if ever applied in veterinary medicine.

Surgery to remove cysts is still the only effective treatment in human cases. Various drugs, such as mebendazole and praziquantel, are presently being tested; it appears that high doses are required, administered over a long period of time. Animals are not treated for hydatidosis.

Control of hydatidosis and *Echinococcus granulosus* is therefore based on prophylaxis of the infection in man, livestock and dogs.

- Public education programmes should convey the message that dogs infected with *E. granulosus* present a danger to the human population and their livestock. Farmers and other dog owners must be aware that uncooked viscera containing hydatid cysts should not be given to dogs, that dogs should be dewormed regularly, and that the handling of infected dogs may increase the risk of becoming infected.
- Several anthelminthics are available for the treatment of dogs but a number of these cause segments to disintegrate, allowing the eggs to remain viable and thus spread after being passed. If possible, faeces passed by dogs after treatment should be buried or burned. However, experience gained during several control programmes has shown that these eggs are of minor importance in the epidemiology of hydatidosis. An alternative treatment is to use the purgative drug, arecoline hydrobromide; this is administered following 12 hours of fasting. The parasites are expelled during the subsequent 6 hours, during which time the animal should be confined. The faeces should be destroyed.
- All abattoirs, slaughterhouses and slaughter slabs should rigorously prevent dogs from gaining access to the premises; all offal and condemned material containing hydatid cysts should be destroyed.

7.3.2.4 Regional/national hydatidosis control programmes

Several control programmes in different parts of the world have reduced transmission to the stage where infection of humans is rare. The best known are on the islands of New Zealand,

Tasmania, Cyprus and the Falkland Islands. A successful programme has also been undertaken in two regions of Chile. These control programmes have all been based on effective cooperation between veterinarians, technicians and dog owners and have had a strong educational component to each programme.

The objective of control is to reduce the transmission of echinococcosis/hydatidosis from animals to humans. The objective of eradication is to eliminate the transmission between animal hosts. It is very important to assess the potential for eradication/control and it is advisable to exercise extreme caution before decisions are made regarding the preference for eradication over control. Among other factors the following should be considered:

(a) Socioeconomic importance (prevalence, severity of disability, risk of mortality).

(b) Epidemiological features of the infection

(c) Annual losses due to infections in livestock

(c) Availability of adequate operational and financial resources

(d) Feasibility of control

Based on evaluations of effective control programmes the following four phases can be distinguished:

(a) *Planning.* The planning phase includes (1) appointment of the appropriate control authority supported by the necessary legislation. The majority of successful programmes has been implemented by the Ministry of Agriculture or its equivalent. (2) Collection of data from which a benefit-cost analysis can be made and appropriate control strategies identified. Data should be collected on the size of rural dog populations, the incidence of hydatidosis in humans by age group, the reinfection rate of rural dogs and the number of veterinarians and technicians needed to treat and test every 10,000 rural dogs. (3) Development of a computer-based surveillance programme from which progress in control can be determined and cost-effective modifications can be made. (4) Selection and training of staff. (5) Securing of appropriate funding for the programme before entering the next phase.

(b) *Attack.* The attack phase is labour-intensive and therefore very costly. It involves the use of arecoline surveillance and/or 6-weekly dog dosing. The duration of this phase depends on the strategy in use but according to experiences gained takes at least 10 years. To secure success it is important that funding is identified for the whole period before the programme is started. This phase requires constant monitoring to determine when a transfer can safely be made to the next phase.

(c) *Consolidation.* The consolidation phase transfers activities from nondiscriminatory dog dosing to quarantine of infected farms (or high risk farms). This transfer is often accompanied by the introduction of penalties for keeping infected dogs.

(d) *Maintenance of eradication.* During this phase all special activities cease and the normal resources of the meat inspection services of the Ministry of Agriculture are used to prevent reintroduction.

7.4 Anthelmintics

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

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

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

7.4.1 Characteristics and selection of anthelmintics

The ideal anthelmintic has the following properties:

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

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

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

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

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

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

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

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

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

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

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

7.4.2 Administration of anthelmintics

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

WARNING: Many formulations of anthelmintics are easily adulterated and it is strongly recommended that only registered drugs from authorized sources be purchased.

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

7.4.2.1 Dosing by mouth

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

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

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

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

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

7.4.2.2 Dosing by injection

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

7.4.2.3 Dosing by external application

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

7.4.3 Testing of anthelmintics

Anthelmintics marketed by international pharmaceutical companies are usually well tested for efficiency and toxicity and can be safely applied according to the manufacturer's instructions. In cases where the origin of the drug is unknown or when it has been obtained from a source without an established reputation, it may be advisable to test the efficiency of the drug before using it in large scale control programmes. A rapid and cheap method of assessing the efficacy of an anthelmintic is to determine the effect on worm egg counts before and after treatment.

7.4.4 Summary of anthelmintics for the treatment of gastro-intestinal

Nematodes, Lungworms, Tapeworms and Flukes

Table 7.1 ANTHELMINTICS AND THEIR APPLICATION

Generic name	Route of administration*	Dose rate (mg/kg)	Spectrum of activity**
Benzimidazoles			
Albendazole	O	5-7.5	GI, L, T
Cambendazole	O	20-25	GI, L, T
Febantel	O	5-10	GI, L
Fenbendazole	O	5-7.5	GI, L, T
Mebendazole	O	12.5	GI, L, T
Oxfendazole	O/IR	4.5-5	GI, L, T
Oxibendazole	O	10-15	GI
Parbendazole	O	20-30	GI
Thiabendazole	O	44-110	GI
Thiophanate	O	50-80	GI, L
Immidazothiazoles			
Tetramisole	O	15	GI, L
Levamisole hydrochloride	O/S0/SC	7.5	GI, L
Levamisole phosphate	O/SC	8-9	GI, L
Organophosphates			
Coumaphos	O/F	8-15	GI
Haloxon	O	40-50	GI
Naphtalophos	O	30	GI, T
Trichlorfon	IM/SC	10-15	GI
Tetrahydropyrimidines			
Morantel	O	10	GI
Pyrantel tartrate	O	25	GI

Miscellaneous			
Ivermectin	D/SC/SO	200 mcg/kg 500 mcg/kg	GI, L

**O: Oral*
SC. Subcutaneous
SO: Spot-on
IM: Intramuscular
IR: Intraruminal
F: Feed
***GI: Gastro-intestinal nematodes*
L: Lungworms
T: Tapeworms

Table 7.2 ADDITIONAL INFORMATION ON ANTHELMINTICS FORMULATIONS

Generic name	Preparation
Albendazole	Liquid suspension
Cambendazole	Ready-to-use suspension, paste
Fenbendazole	Ready-to-use suspension, granules, tablets, mineral pre-mix, licking blocks
Mebendazole	Ready-to-use suspension, granules, paste, mineral pre-mix
Oxfendazole	Ready-to-use suspension, tablets
Thiabendazole	Ready-to-use suspension, powder, granules, tablets, paste
Tetramisole	Suspensions, granules, tablets, injectable
Levamisole	Pour-on preparation
Morantel	Ready-to-use suspension, tablets, granules, paste
Pyrantel	

Table 7.3 ANTHELMINTICS FOR THE TREATMENT OF INHIBITED LARVAE

Cattle	Sheep
Albendazole	Albendazole
Febantel	Febantel
Fenbendazole	Fenbendazole
Oxfendazole	Oxfendazole
Thiophanate	Levamisole
Ivermectin	Ivermectin

Table 7.4 ANTHELMINTICS AND FORMULATIONS FOR STRATEGIC PROPHYLACTIC PROGRAMMES

Generic name	Formulation	Animal
Albendazole	Slow release bolus, active 90–120 days	Cattle Sheep
Albendazole	Pulse release bolus	Cattle
Oxfendazole	Pulse release bolus	Cattle
Morantel tartrate	Slow release bolus active 60 + days	Cattle

Table 7.5 ANTHELMINTICS FOR THE TREATMENT OF LIVER FLUKES

Generic name	Route of Administration	Dose rate (mg/kg)		Minimum age of fluke in weeks efficiency [3] 90%	
		Sheep	Cattle	Sheep	Cattle
Hexachlorophene*	O	15	20	12	>20
Hexachloroethane	O	250–300	300	12	12
Tribromsalan	O	20	20	12	>12
Bithionol*	O	75	30	>12	>12
Hexachloroparaxylene	O	150	130	12	12
Bromophenophos	O	16	12	12	>12
Clioxanide*	O	20	NR	12	NR
Oxyclozanide*	O	15	13–16	12	>14
Niclofolan*	O	4	3	12	>12
	SC	NR	0.8	NR	<12
Nitoxynil	SC	10	10	8	10
Brotianide*	O	5.6	NR	12	NR
Rafoxanide*	O	7,5	7.5	6	12
	SC	NR	3	NR	12
Closantel	O	7.5-10	NR	8-6	NR
	SC	NR	3	NR	>12
Diamphenetide	O	80–120	100	1 day–6 weeks	1 day–7 weeks
Albendazole	O	4.75	10	>12	>12
Triclabendazole	O	10	12	1	1
Clorsulon	O	-	7	-	8
	SC	-	2	-	>12

O = Oral
SC = Subcutaneous
NR = Not Recommended
** = Also effective against paramphistomes (see Table 7.6)*

Table 7.6 ANTHELMINTICS FOR THE TREATMENT OF PARAMPHISTOMES

Generic name	Route of Administration	Dose rate (mg/kg)		Efficiency (%)		
		Sheep	Cattle	Immature in small intestine + abom.		Mature in rumen
				Sheep	Cattle	Sheep/Cattle
Clioxanide*	O	20–40	NR			
Niclosamide	O	50–100	50–100	95	0-96	0
		NR	160 (2 doses)	NR	92	

Niclofolan*	O	6	6	76-95	–	42
Oxyclozanide*	O	15	15	85–100	61–96	73–100
		NR	18 (2 doses)	NR	99–100	100
Rafoxanide*	O	75–100	25–50	99–100	9–100	63–98
Bithionol S03*	O	40	40	–	–	97–100
Resorantel	O	65	65	80–90	62-99	85–100

O = Oral
NR = Not Recommended
** = Also effective against liver flukes (see Table 7.5)*

7.5 Anthelmintic resistance

Increased productivity in ruminants through the control of helminth parasites will to a large extent depend upon the availability of low cost, effective anthelmintics. It is therefore of great concern that the regular use of anthelmintics has lead to the selection of drug-resistant helminths. This has become a serious problem in many countries, and resistance of parasites to one or more anthelmintics is now widespread, particularly in sheep. The loss of inexpensive drugs against helminths due to problems of anthelmintic resistance may leave many countries without a means of controlling helminth parasitism.

7.5.1 Detection of resistance

Anthelmintic resistance is often first suspected in cases of apparent anthelmintic failure, but it should be kept in mind that several other factors can be responsible for the lack of efficiency of a drug. These include:

(a) *Underdosing.* Most farmers usually estimate (guess) the weights of their animals and many surveys have shown that such estimates are often considerably below the actual weight. In addition farmers often use the "average weight" to establish the dose. This automatically results in underdosing. This can be further compounded if a manufacture recommend dosages for a broad weight range.

(b) *Rapid reinfection.* If animals are grazed on heavily contaminated pastures, reinfection occurs immediately and this may give the impression of drug failure. This is particularly relevant where *Haemonchus contortus* is dominant, as it develops rapidly and is very pathogenic.

(c) *Inefficiency against arrested or dormant larvae.* Arrested larvae which are unaffected by the anthelmintic being used may continue development immediately after treatment.

(d) *Drug resistant parasites are present.* Frequent regular treatments using the

same anthelmintic given at low dosages over a prolonged period of time, will predispose to the development of drug resistance.

7.5.2 Testing for anthelmintic resistance

A simple method to test for the presence of drug-resistant nematodes which can be applied in the field is as follows.

(a) Collect faecal samples from a representative group of animals for faecal egg counts prior to treatment. There should be 10-15 animals in the group.

(b) The animals should be carefully weighed and dosed according to weight with the drug under suspicion.

(c) A control group of the same size should be identified which is not treated, and these should be sampled.

(d) All animals should be sampled again 7-10 days later for faecal egg counts.

A reduction in egg counts of less than 85% strongly suggests that resistant nematodes are present.

It is then necessary to determine to which drugs the parasites are susceptible and immediately change to an efficient drug. Other tests are also available, such as the egg hatch test, but these require laboratory equipment and are more sophisticated. To confirm that resistance is present, experimental infections in lambs can be established with subsequent treatment, slaughter and worm counts, but these are expensive and time consuming. Thus in many cases the egg count reduction test will be the only feasible test available.

A fuller account of methods for detecting anthelmintic resistance recommended by the World Association for the Advancement of Veterinary Parasitology can be found in Coles *et al., Veterinary Parasitology* (1992) 4, 35-44.

7.5.3 Preventing the development of anthelmintic resistance

There is an urgent need for the development and adoption of strategies to prevent the spread of anthelmintic resistance, particularly in nematodes of sheep, and prevent it from becoming a problem in cattle. The following practical measures can be taken to delay the occurrence.

(a) *Use the correct dose.* It is very important that farmers use a scale or a measure tape to establish the weights of animals in each age group (lambs, weaners, young ewes, older ewes, rams). The dose for each group is then established for the heaviest animal in each group; the use of an "average weight" should be discouraged as this results in underdosing of the heavy animals. This rule also applies to cattle.

(b) *Maintain drenching equipment.* A common cause for incorrect dosing is faulty dosing equipment. It is very important that equipment is tested for accuracy before the start of dosing. Some drenching guns have a low compatibility with certain preparations, and it is recommended that the appropriate equipment is used. Thorough cleaning of equipment after use is important, as some preparations destroy the piston if not removed. In general, drenching guns with a plastic or plexiglass cylinder are recommended.

(c) *Reduce dosing frequency.* Studies have shown that the risk of anthelmintic resistance increases with increased dosing frequency. It is therefore important to

establish the epidemiology of the helminth infections and introduce strategic deworming programmes based on a few well-timed treatments given when it is most advantageous.

(d) *Establish treatment and quarantine for all animals introduced to the farm.* The source of anthelmintic resistant nematodes can be from purchased animals, such as introduced breeding stock. If nothing is known about the previous deworming history of animals, it is recommended that they be treated prior to introduction with a double dose of levamisole and a modern benzimidazole (fenbendazole, albendazole). It is advisable to keep the newly introduced animals isolated for 72 hours after arrival and treatment.

(e) *Alternate anthelmintics.* Present information recommends continued use of an anthelmintic for at least a whole season (one year for many tropical and sub-tropical countries) provided it is effective. When changing the anthelmintic, a drug from a different class (see Table 7.1) should be selected.

In addition farmers should consider establishing grazing management practices which reduce the parasite burden and subsequently the need for treatment.

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 gives 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

NOTE. 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

1.	Armour, J. 1980. The epidemiology of helminth disease in farm animals. *Veterinary Parasitology* 6: 7-46.
2.	Armour, J. 1982. An approach to the epidemiology of helminthiasis in grazing ruminants. In: *Proceedings of Symposium on Nuclear Techniques in the Study of Parasitic Infections,* Vienna, 1981. Vienna, Austria: International Atomic Energy Agency, pp. 167-177.
3.	Brunsdon, R.V. 1980. Principles of helminth control. *Veterinary Parasitology* 6: 185-215.
4.	Campbell, W.C. and Rowe, R.S. 1986. *Chemotherapy of Parasitic Diseases.* New York: Plenum Press, pp. 239-503.
5.	Dunn, A.M. 1978. *Veterinary Helminthology,* Second Edition. London: Heinemann Medical.
6.	Gibbs, H.C. 1984. Effects of parasites on animal and meat production. In: S.M. Gaafar, W.E. Howard and R.E. Marsh, eds. *Parasites, Pests and Predators.* Amsterdam: World Animal Science, Elsevier, pp. 7-25.
7.	Gibbs, H.C. 1986. Hypobiosis in parasitic nematodes: an update. *Advances in Parasitology* 25: 129-166.
8.	Levine, N.D. 1978. The influence of weather on the bionomics of the free-living stage of nematodes. In: T.E. Gibson, ed. *Weather and Parasitic Animal Disease.* Technical Note No. 159. Geneva, Switzerland: World Meteorological Organization, pp. 51-57.
9.	Michel, J.F. 1976. The epidemiology and control of some nematode infections in grazing animals. *Advances in Parasitology* 14: 355-397.
10.	Morley, F.H.W. and Donald, A.D. 1980. Farm management and systems of helminth control. *Veterinary Parasitology* 6: 105-134.
11.	Skerman, K.D. and Hillard, J.J. 1966. *A Handbook for Studies of Helminth Parasites of Ruminants. Near East Animal Health Institute, Teheran. Handbook No.* 2. Rome: Food and Agricultural Organization of the United Nations.
12.	Soulsby, E.J.W. 1982. *Helminths, Arthropods and Protozoa of Domesticated Animals,* Seventh Edition. Bailliere Tindall, London: Lea and Febiger, Philadelphia, pp. 212-258.