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EPIDEMIOLOGY, DIAGNOSIS AND CONTROL OF HELMINTH PARASITES OF SWINE

Food and Agriculture Organization of the United Nations

EPIDEMIOLOGY, DIAGNOSIS AND CONTROL OF HELMINTH PARASITES OF SWINE

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FOREWORD

Helminth parasites of swine are ubiquitous and although no precise information is available on the economic impact of these to the pig producers, there is little doubt that they are important causing reduced feed conversion efficiency and slower weight gain. Both the commercial and the small-scale farmer continuously rank worm infections high among the health problems. The difficulties in substantiating the losses are associated with the fact that the infections are chronic and less dramatic than other diseases of swine which make these infections among the most neglected areas of veterinary care in developing countries. In addition, a few helminths are important in veterinary public health, as transmission to humans is possible through ingestion of raw or under-cooked meat. This handbook is written to assist animal health staff in prevention and control of these infections.

The handbook, in a simple style, reviews the epidemiology of economically important helminth parasites of swine and present procedures and techniques for their diagnosis, survey and control. The book is designed for routine use in all types of animal health institutions, including universities, research institutes and field laboratories where diagnostic parasitology is performed. It is hoped that it 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.

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CHAPTER 1

THE MAJOR HELMINTH SPECIES - AN INTRODUCTION

1.1 INTRODUCTION

As a background for evaluating prevalence and impact of helminthosis in swine, identification of the species present in a herd, area, country or region should be investigated. Different species have different pathogenic effects and bionomics. Some species are particularly important in the young animal, while others seem to accumulate in the older age categories. Furthermore, some species are relatively easy to control, while others are highly persistent in the environment and difficult to combat, unless more drastical measures are taken.

Unless the helminth spectrum in a given herd or area is already defined, a status of the helminth situation should be made initially. In the first run this should be kept simple, having the primary purpose to encircle the major species present. With this information at hand more detailed investigations should be designed to cover the major, relevant species in the particular herd or area.

Helminths in the pig are relatively easy to identify if one is aware of their size and gross morphology and the tissues and organs in which they are normally located. Macroscopically visible lesions typical for some species may provide supportive information.

Identification of the parasites may be based on *post-mortem* examination at slaughter or when animals have died from disease. Identification may also be based on examination of eggs excreted with faeces. Only rarely will clinical symptoms be sufficiently specific to point to a single, responsible species.

The helminths may be grouped according to their systematics (Section 1.2) or according to their location in the pig host (Section 1.3).

1.2 HELMINTH GROUPS

The most important helminth species, classified into major groups, are listed below.

1.2.1 Nematodes

Hyostrongylus (red stomach worm)
Gnathostoma
Ascaris (large roundworm)
Strongyloides (threadworm)
Globocephalus (hookworm)
Trichostrongylus
Oesophagostomum (nodular worm)
Trichuris (whipworm)
Metastrongylus (lungworm)
Stephanurus (kidney worm)
Trichinella

1.2.2 Trematodes

Fasciolopsis (intestinal fluke)
Gastrodiscus
Opistorchis
Fasciola (liver fluke)
Schistosoma (blood fluke)

1.2.3 Cestodes

Cysticercus cellulosae
Cysticercus tenuicollis
Echinococcus granulosus

1.2.4 Acanthocephala

Macracanthorhynchus hirudinaceus

1.3 LOCATION IN ORGANS/TISSUES OF THE HOST

Helminths usually have typical host locations that may help isolation and identification. Adult worms in particular are confined to distinct organs or parts of these, whereas larvae of many species may be encountered in several organs or tissues in connection with their migration.

For parasite identification attention should be paid to the young animal, and animals which have not recently received anthelmintic treatment. Usually pigs in the age category 2-6 months have the largest worm burdens and may excrete many eggs with faeces. However, for some species like *Oesophagostomum* spp. and *Hyostrongylus* worm populations tend to accumulate in older animals. One must also bear in mind that some species have rather long prepatent periods, i.e. time interval between infection and start of egg excretion, for which reason faecal egg counts may be negative for a long period of time even in the presence of high parasite burdens in the host. *Stephanurus*, the kidney worm, has a prepatent period as long as 6-11 months.

1.3.1 Alimentary tract

The majority of worm species develops and lives in distinct, characteristic sites of the alimentary tract. In some situations the infections are accompanied by macroscopical gut lesions, but in some cases there are no overt lesions even at high parasite burdens. Larvae, which are less easily identifiable, may in many instances be more pathogenic than adults. Table 1.1 lists the location and effects of the most common helminths.

TABLE 1.1 Helminths in different sections of the alimentary tract

Site	Helminth	Effects
Stomach	*Hyostrongylus*	Mucosal damage
	Ascarops	Mucosal damage
	Physocephalus	Mucosal damage
	Gnathostoma	Mucosal damage
Small intestine	*Ascaris*	Adults: mucosal changes Larvae: liver and lung lesions
	Strongyloides	Mucosal damage
	Globocephalus	Blood sucking
	Trichostrongylus	Mucosal damage
	Macracanthorhynchus	Mucosal damage
	Fasciolopsis	Mucosal damage
Large intestine	*Oesophagostomum*	Mucosal damage, nodules
	Trichuris	Blood sucking
	Gastrodiscus	Mucosal damage

1.3.2 Liver

Parasites in the liver usually produce macroscopical lesions indicative of their migration and presence. Large transparent cysts attached to the visceral surface of the liver will most likely show to be

Cysticercus tenuicollis, the metacestode of *Taenia hydatigina* (a dog tapeworm). Deeper, partially superficial cysts may be metacestodes - socalled hydatids of *Echinococcus granulosis*, also a dog tape worm. Migratory lesions (tracts) in the parenchyma may be caused by immature *Fasciola* and *Cysticercus tenuicollis* larvae. *Ascaris* and *Schistosoma* produce fibrotic or granulamatous lesions, typical for each of these helminths. Following incision adult *Fasciola* may be observed and collected from the bile ducts.

TABLE 1.2 Helminths in the liver

Helminth	Effect
Ascaris (larvae)	Fibrotic lesions: "white" or "milk" spots
Schistosoma (eggs)	Fibrosis, granulomas
Fasciola (larvae, adults)	Fibrosis, bile duct enlargement. Blood sucking
Cysticercus tenuicollis (metacestodes)	Tissue damage, fibrosis
Echinoccocus granulosus (metacestodes: hydatid cysts)	Pressure atrophy

1.3.3 Lungs

The most important helminths that invade the lung are the pig lungworms, of which there are three common species belonging to *Metastrongylus*. These are primarily found in the bronchioli. In addition, *Ascaris* larvae pass the lung as part of their host migration - and may produce traumatic lesions. Hydatid cysts may occasionally be observed in the lung parenchyma.

TABLE 1.3 Helminths in the lung

Helminth	Effect
Metastrongylus	Bronchitis, pneumonia
Ascaris	Traumatic lesions
Hydatid cysts	Tissue atrophy

1.3.4 Other organs and tissues

Helminths may be found in a number of tissues outside the alimentary tract, liver and lungs. Accidental findings may simply reflect aberrant migration of parasites belonging to the above organs. However, other parasites have primary, natural predilection sites, e.g. in muscle tissue and in the kidney, see Table 1.4 below.

TABLE 1.4 Helminths in various organs

Site	Helminth	Effects
Muscle	*Trichinella* (larvae) *Cysticercus cellulosae*	Minimal Minimal
Kidney	*Stephanurus*	Damage, moderate
Blood vessels	*Schistosoma*	Intestinal and hepatic damage

CHAPTER 2

LIFE CYCLES AND EPIDEMIOLOGY OF HELMINTH PARASITES

2.1 INTRODUCTION

This chapter primarily describes life cycles, epidemiology and pathogenecity of those helminths having highest prevalences and greatest economic impacts. The description therefore mainly comprises the helminths listed in Chapter 1 (Sections 1.2.1-1.2.3, Tables 1.1-1.4). A more comprehensive list of helminths in pigs, with focus on life cycles, transmission and host relationships will be found at the end of the present chapter (Tables 2.1 and 2.2).

2.2 NEMATODES OF THE DIGESTIVE TRACT

With a few exceptions, these nematodes have direct life cycles, i.e. transmission from host to host without development in intermediate hosts. The adult worms are located in different parts of the gastrointestinal tract, where females after mating produce eggs that are passed out in the faeces. In some species the eggs embryonate and hatch into larvae which after two moults develop into the infective third-stage larvae (L_3). In other species like *Ascaris suum* and *Trichuris suis* eggs do not hatch and the infective stage is reached within the egg. In the following the major species will be dealt with separately, starting with species located in the anterior part of the alimentary tract.

2.2.1 *Hyostrongylus rubidus* (red stomach worm)

Fig. 2.1 illustrates the life cycle of this nematode, which is confined to the pig. The eggs, which are of the typical "strongyle"-type, indi-

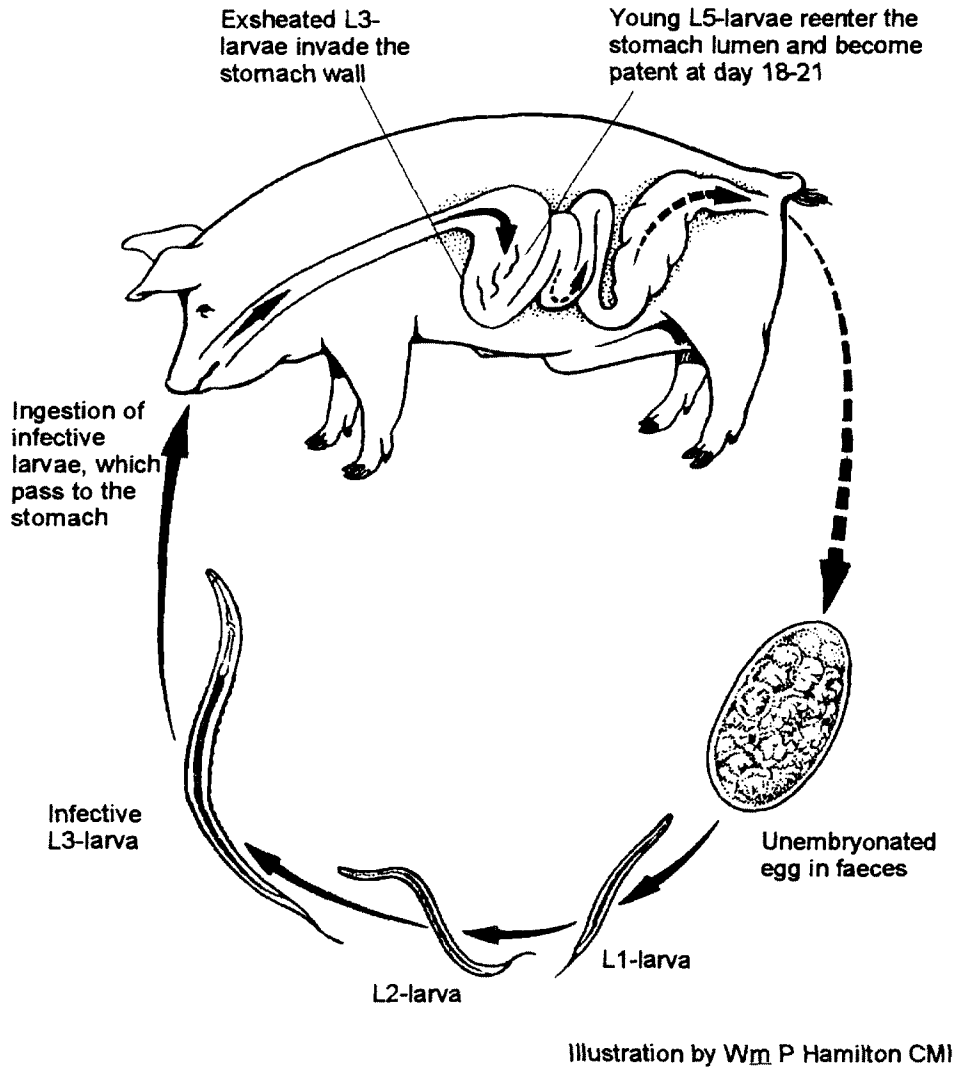


FIGURE 2.1 *Hyostrongylus rubidus* life cycle

stinguishable from those of e.g. *Oesophagostomum* species (see below), are passed with faeces. Here they hatch within a few days, and infective third stage larvae are developed within 1-2 weeks at optimal temperatures (15-25°C). They are very motile and are not only localized to faecal material but also to surrounding soil and herbage (see Chapter 5). Pigs are infected by ingestion of the infective larvae, developing into fourth stage larvae which inhabit the stomach mucosa. The adult worms establish on the mucosal surface and in the stomach lumen, and start egg production already after 18-21 days (viz. prepatent period). Egg excretion per female worm is generally low, compared with other nematodes (Chapter 3). Under certain circumstances in temperate regions, the larvae may be arrested in their development in the mucosa (hypobiosis) for periods up to several months.

During the prepatent period there may be severe damage to the gastric glands, leading to lowered acidity, mucosal hyperplasia, nodule formation and haemorrhage. Clinical signs include inappetance, loss of condition and anaemia, but usually not diarrhoea.

The infections are mainly confined to outdoor reared pigs due to the biological requirements for larval development. In some temperate regions it is practice to mainly keep breeding stock, gilts and sows, on pasture, which is the reason why these age categories are most severely affected. The so-called "poor sow syndrome" is usually attributed to *Hyostrongylus rubidus* often together with *Oesophagostomum* spp. infection.

2.2.2 *Ascarops strongylina* and *Physocephalus sexalatus*

Fig. 2.2 shows the principle life cycle of these species, belonging to the spiruid nematodes. The relatively small eggs already contain well-developed embryos when passed in the faeces. For further development the eggs are swallowed by coprophagous beetles. In these, the larvae develop up to the infective larvae (L_3) and pigs be-

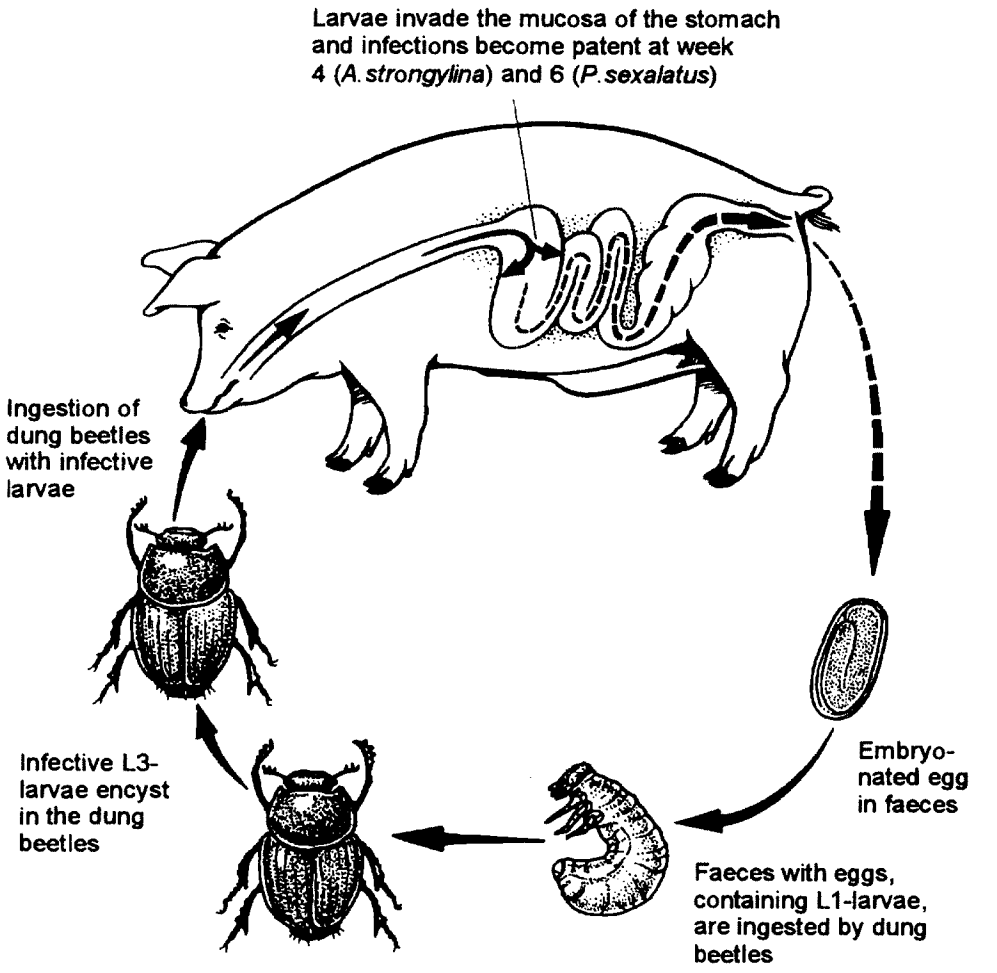


FIGURE 2.2 *Ascarops strongylina* and *Physocephalus sexalatus* life cycles

come infected when ingesting infected dung beetles. After uptake of beetles, the larvae are released and develop further to adults in or on the gastric mucosa. Egg excretion is initiated after approximately 1-2 months.

Heavily infected animals have severe gastric inflammation with ulcerations. Symptoms are more or less comparable with those caused by *Hyostrongylus rubidus*.

Acquisition of these spiruid nematodes almost exclusively takes place outdoors. A relatively wide range of beetles may serve as intermediate hosts in tropical and subtropical areas, but infections do also occur in some temperate regions of North America and Europe. Infections are likely to be more severe where pigs are fed at a restricted level and hence more dependent on scavenging.

2.2.3 *Ascaris suum* (large roundworm)

Fig. 2.3 illustrates the life cycle of this highly prevalent nematode. The eggs which pass with faeces, usually in high numbers, immediately start embryonating at temperatures above 15°C in the dung or soil, and may reach the infective stage within 1 to 3 months (or more) depending on temperature. Following uptake by the pig host, the L_3-larvae hatch in the small and large intestine, and penetrate the large intestinal wall, from where they travel to the liver. There they pass in the bloodstream to the lungs and hence to the small intestine via the bronchi and trachea. The final moults take place in the intestine, and patency (egg production) is reached after 6-8 weeks.

The initial penetration of the gut wall is apparently harmless, but the migration in the liver causes local lesions ("white spots", "milk spots"), which may cause condemnation of livers at slaughter. Yet, the liver lesions are usually not rather pathogenic. However, the arrival of the larvae to the lungs may lead to transient pneumonia even with clinical symptoms if larval challenges are high. The imma-

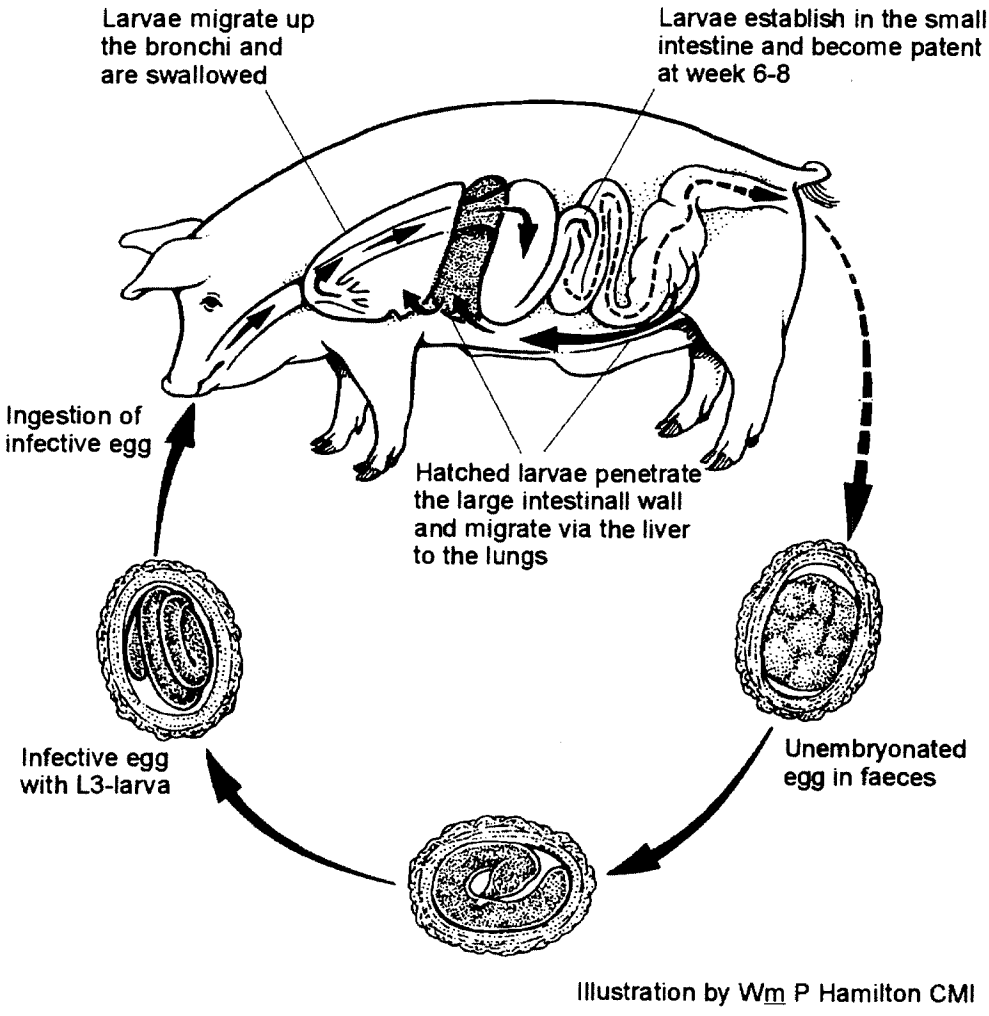


FIGURE 2.3 *Ascaris suum* life cycle

ture and adult worms in the small intestine may lead to intestinal disturbances, depending on the worm loads. Poor feed conversion and slower weight gains resulting in an extension of the fattening period have been recorded.

Infections with *Ascaris suum* are associated with both outdoor and indoor production. Among all swine helminths, *Ascaris* is possibly the most persistant and the most resistant to adverse environmental conditions, due to its thick and resistant egg shell, which protects against adverse environmental factors, desiccation and chemicals. In the most modern and hygienic production enterprises, worm problems are often left with this species only. In the pasture environment eggs may maintain infectivity for up to 6-7 years. Infections usually stimulate the development of a rather strong protective immune reaction, which under practical conditions means that older animals have less worms and excrete fewer eggs than the younger ones. This has important implications for the targeting of control programmes.

Ascaris suum may occasionally infect other hosts, in the form of larval migratory lesions in liver and lung (ruminants), or even resulting in patent infections (man and ruminants). The zoonotic nature of *Ascaris suum* is as yet not fully defined.

2.2.4 *Strongyloides ransomi* (pig threadworm)

Fig. 2.4 illustrates the rather complex life cycle of this parasite, which includes free-living generations of adult males and females, and parasitic parthenogenetic females in the small intestine of the pig. The female worms in the small intestine produce larvated eggs by parthenogenesis, and these are excreted with faeces and will soon hatch. After hatching, the larvae may develop into free-living adult male and female worms, and this may lead to successions of free-living generations. But under certain temperature and moisture conditions the third stage larvae (L_3) may become infective to the pig by oral ingestion (penetration of oral mucosa) or skin penetration.

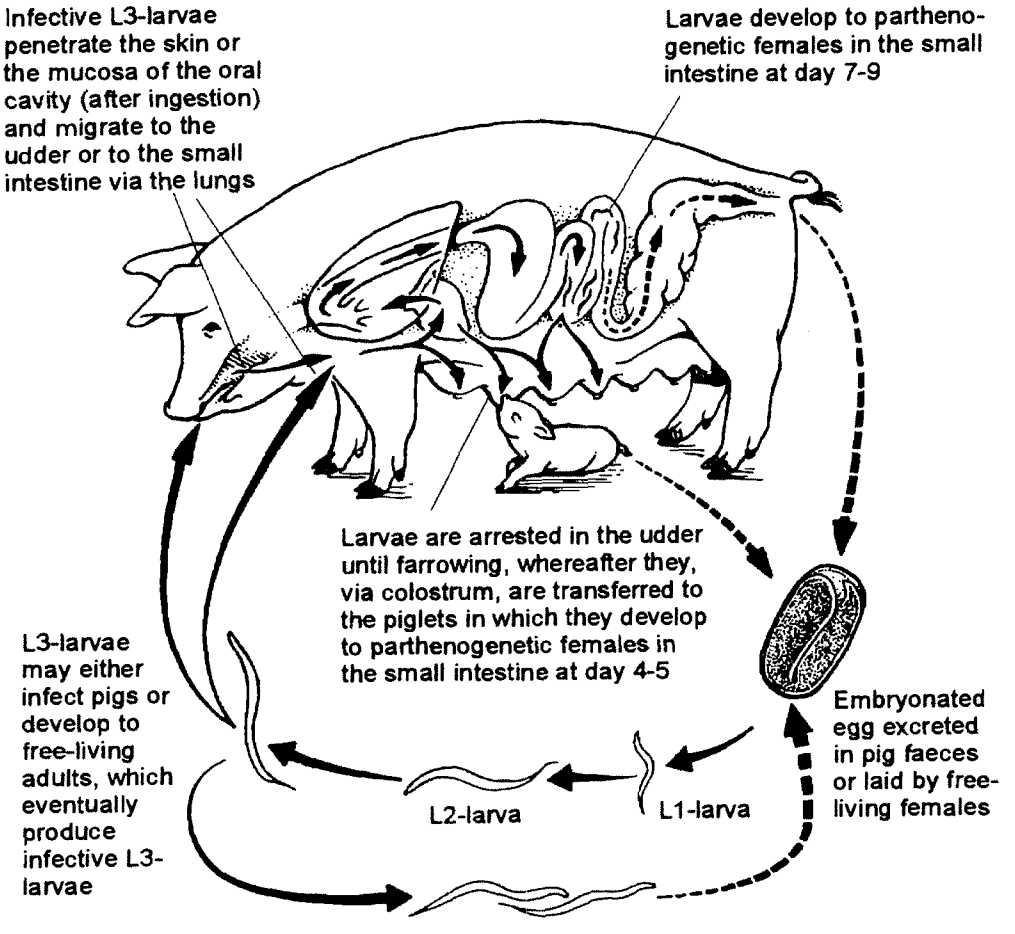


FIGURE 2.4 *Strongyloides ransomi* life cycle

In the pig, the larvae migrate via the venous system, lungs, and trachea to mature into adult females in the intestine. In addition, piglets may acquire parasites immediately after birth from the mobilisation of tissue-dwelling larvae in the sow which are subsequently excreted in the milk (colostrum). The time from infection to egg excretion, i.e. the prepatent period, is only 7-9 days (or 4-5 days after lactogenic transmission to piglets).

Large intestinal burdens of *Strongyloides ransomi* may cause inflammation with erosion of the epithelium. Severe diarrhoea in the neonatal animal leads to weight loss, dehydration and in some cases death.

Conditions with high temperature and humidity in connection with poor hygiene favour development and accumulation of large numbers of larvae that may severely affect young animals. In addition, the neonatal pig may suffer from larvae transmitted through suckling. Fatal cases may occur before eggs are excreted with faeces. The infection induces strong immunity which explains that older age categories are usually not clinically affected.

2.2.5 *Oesophagostomum* spp. (nodular worms)

Fig. 2.5 shows the life cycle of the different species of this parasite located as adults in the large intestine. These are commonly represented by two co-existing worms in the large intestine, i.e. *Oesophagostomum dentatum* and *O.quadrispinulatum*. Species like *O.brevicaudum* may also occur. Adult females produce large numbers of eggs of the "strongyle"-type, similar in morphology to those of e.g. *Hyostrongylus rubidus*. The eggs hatch within a few days after deposition in faeces, and through the first and second stage larvae the third and infective stage (L_3) may be reached within 10 days, provided high temperature and humidity prevail. These larvae have a low motility and tend to be localized in faeces and surrounding soil, to a lesser extent in herbage. Pigs ingest larvae from their environment, and the infective stages enter the mucosa of particular-

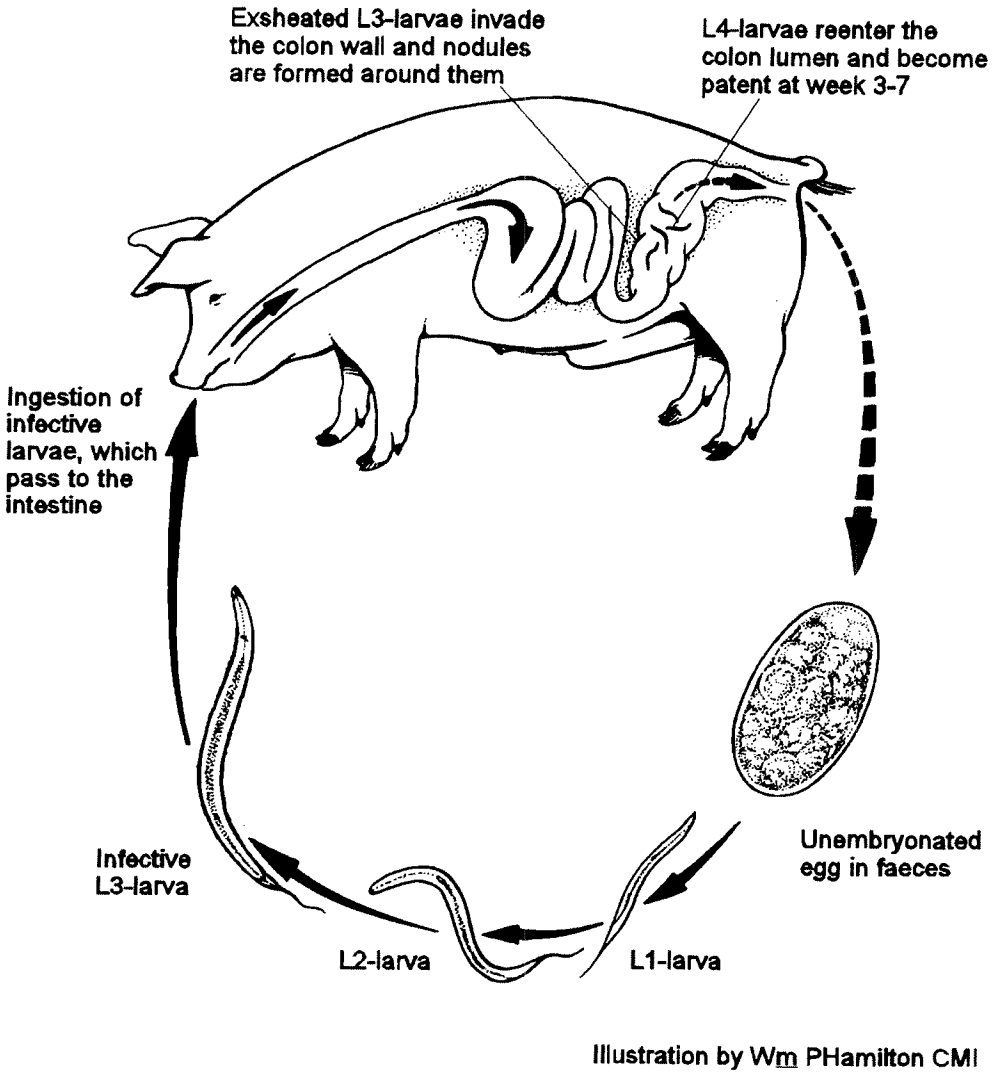


FIGURE 2.5 *Oesophagostomum* spp. life cycle

ly the large intestine where they become enclosed in nodules and moult. Then they emerge to the surface of colon and caecum and develop to adult, egg-producing worms within 3 to 8 weeks (different prepatent periods for different strains and regions are listed in the literature).

The nodular worms cause enteritis and nodular formations in the gut wall. Hyperplasia and ulcerations are prominent features at high infection pressure. Since the parasite is only moderately immunogenic, worm loads tend to accumulate in older age categories, and clinical disease is most often encountered in the sow, showing inappetence, weight loss and reduced milk production, only rarely accompanied by diarrhoea. The "poor sow syndrome" is a clinical condition usually associated with *Hyostrongylus rubidus* and *Oesophagostomum* spp. (see Section 2.2.1).

Transmission of the infection is favoured by the high egg excretion and humid and unhygienic conditions. Besides *Ascaris suum* this is one of the most difficult worms to control, even under modern conditions with strict control measures, including use of anthelmintics. Some investigations have shown that sows may sometimes exhibit a periparturient egg-rise coincident with farrowing, a phenomenon which will favour transmission to the offspring.

2.2.6 *Trichuris suis* (whipworm)

Fig. 2.6 shows the life cycle of *Trichuris suis*. The adult worms, located in caecum and in the anterior colon, produce eggs that are excreted with faeces. The larva embryonates and develops within the thick-shelled egg to the first stage, which is infective. As with *Ascaris suum*, eggs do not hatch. After ingestion by the pig host, the larva is released and penetrates the caecal mucosa and moults. Subsequently, the remaining moults up to the adult stage take place on mucosal surfaces of caecum and colon. Egg production occurs after approximately 6 weeks.

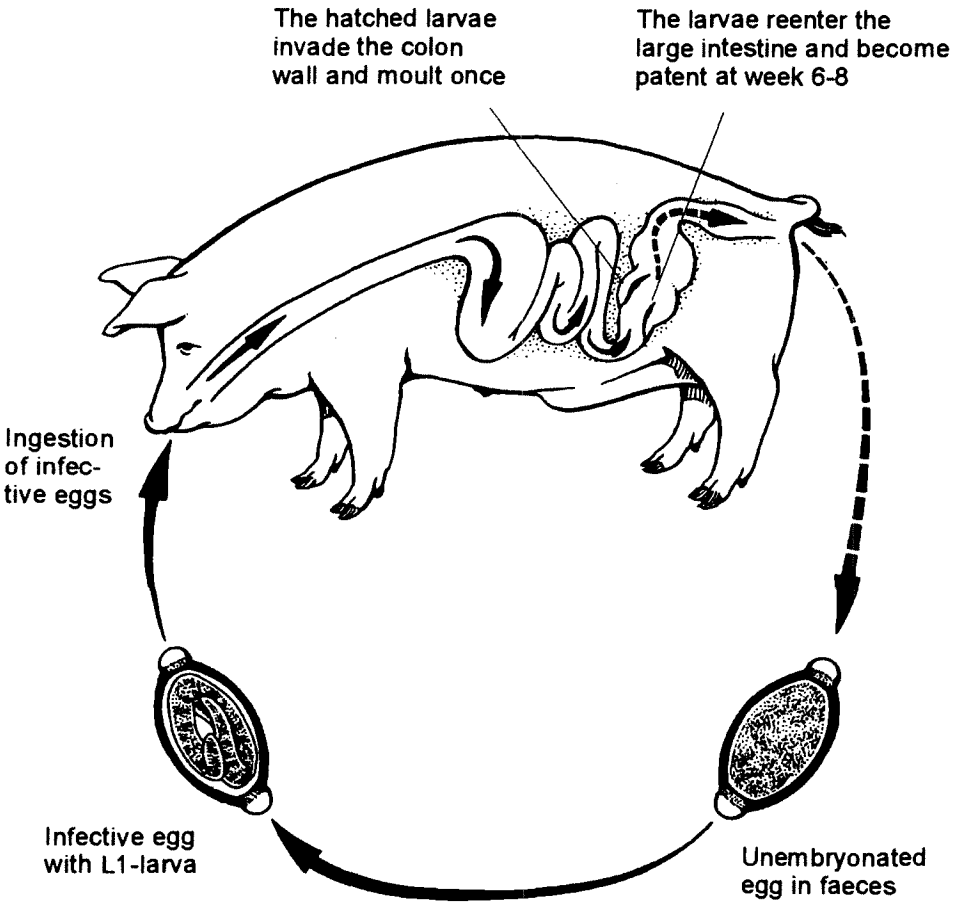


FIGURE 2.6 *Trichuris suis* life cycle

Many infections are subclinical, but high worm burdens in young animals may cause blood-stained diarrhoea due to the inflammatory mucosal reactions, and the blood sucking activities of the worms. Severe infections may lead to weight loss, dehydration and in some cases death.

Infections with *Trichuris suis* are mainly associated with outdoor rearing of pigs. The eggs of this helminth species have much in common with eggs of *Ascaris suum*, as they are highly resistant and may remain infective for years (up to 11 years). A relatively strong immunity develops, and older animals are usually not carrying high worm burdens, and hence they contaminate the environment only to a minor degree.

2.2.7 Other nematodes of the digestive tract

These are briefly listed in Tables 2.1 and 2.2 with information on type of life cycle, transmission and host relationship.

2.3 NEMATODES OF THE LUNGS

Fig. 2.7 presents the life cycle of the common lungworms in swine, i.e. *Metastrongylus* spp. There are three widely distributed species, *M.apri*, *M.salmi* and *M.pudendotectus*, which often occur simultaneously in the same individual. The adults are located in the small bronchi and bronchioles. They produce eggs that pass trachea, are swallowed, and then excreted with faeces. These eggs may hatch immediately after being ingested by earthworms which serve as intermediate hosts. In the earthworm the development to the infective (L_3) takes place within 1-2 weeks at optimal temperatures of 22-26°C. The longevity of the L_3 in the earthworm may be several years. The pigs become infected by eating earthworms, and the L_3 are released and migrate to the mesenteric lymph nodes and moult, and from there they migrate via the lymphatic-route to the lungs. The

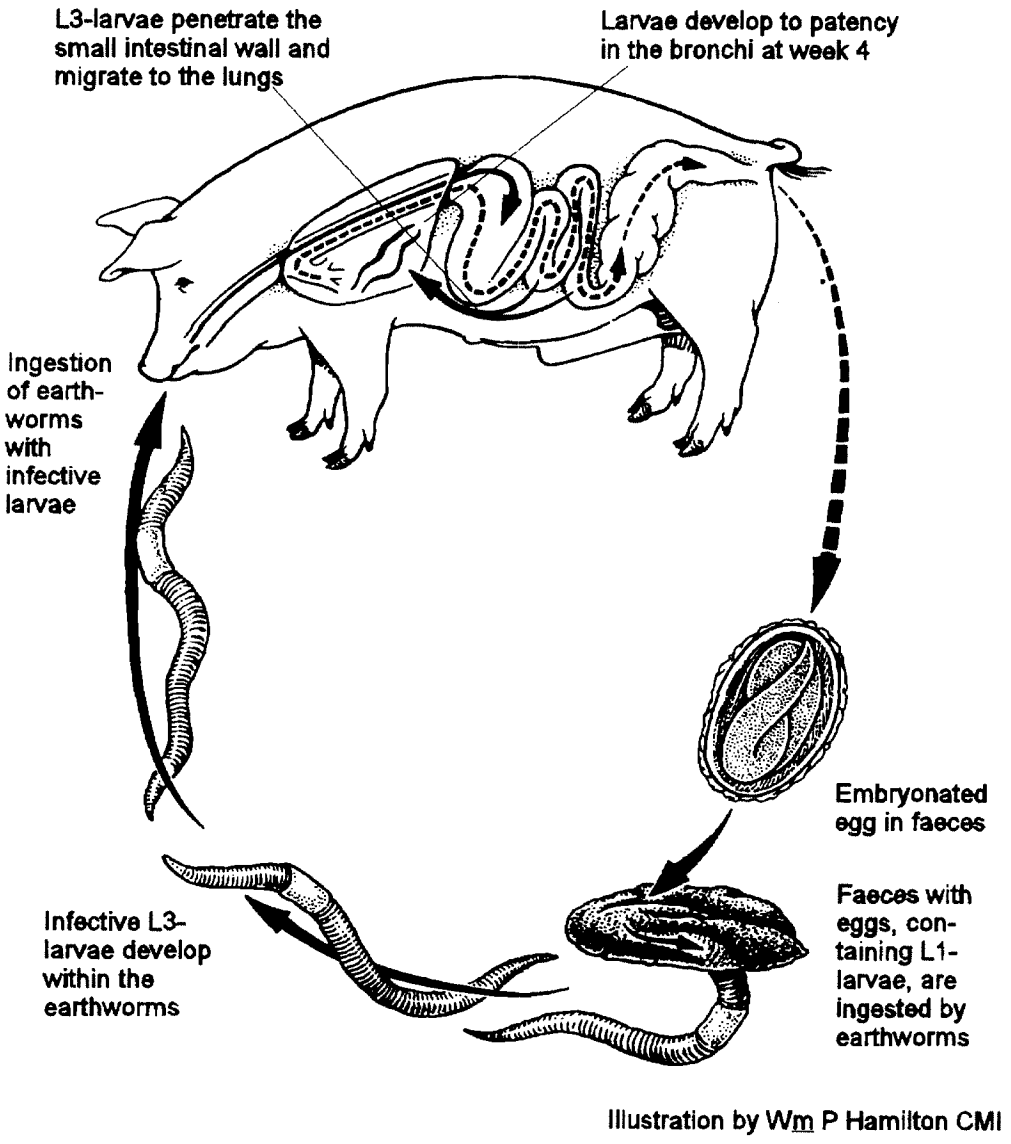


FIGURE 2.7 *Metastrongylus* spp. life cycle

prepatent period is approximately 4 weeks.

The lungworms may cause traumatic lesions during their migration in the lungs in the form of peribronchial lymphoid hyperplasia, and when the worms mature, chronic bronchitis and emphysema develop. In heavy infections coughing is marked, accompanied by nasal discharge and dyspnoea. Fatal cases do occasionally occur.

Lungworm infections in swine seem to be most severe in young pigs 4-6 months old. Older age categories are normally protected from clinical attacks due to acquisition of immunity, although they may suffer subclinically. In a given herd the exposure to infection may be long-lasting due to the environmental earthworm reservoir.

2.4 NEMATODES IN OTHER ORGANS

2.4.1 *Stephanurus dentatus* (kidney worm)

Fig. 2.8 shows the life cycle of the kidney worm, which occurs in tropical and subtropical regions of all continents. Eggs are passed with the urine, and develop into third-stage infective larvae (L_3) in the environment. Thereafter there are three modes of transmission to the pig: by ingestion of the free L_3, ingestion of earthworms carrying L_3, and percutaneous infection with free L_3. Irrespective of mode of infection the larvae develop further in the liver parenchyma, and thereafter they migrate in the peritoneal cavity to the perirenal region. There, as adults they become enclosed in a cyst which they communicate with the ureter, allowing worm eggs to be excreted in the urine. The prepatent period ranges from 6-11 months. Quite many worms migrate erratic in the body of the pig.

The pathological lesions due to the migration of the parasite are most severe in the liver, where hepatic failure may occur in very serious

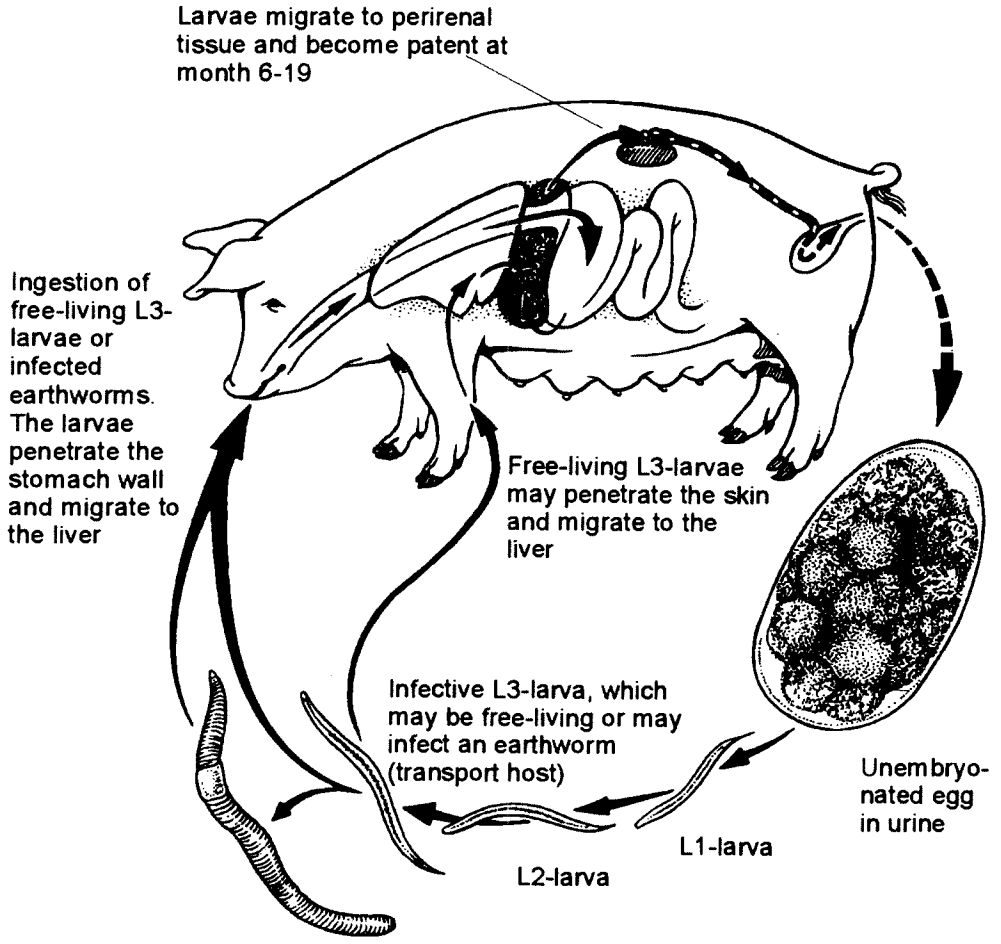


FIGURE 2.8 *Stephanurus dentatus* life cycle

cases. In general, overt clinical signs are rare, unless animals are exposed to heavy challenge.

The L_3 are highly susceptible to desiccation and are favoured by damp ground. Since pigs often are lying in wet areas they may be exposed to percutaneous infection. Infected earthworms ensure persistence of infection in the environment.

2.4.2 *Trichinella spiralis*

Fig. 2.9 illustrates the life cycle of this nematode, having a unique transmission biology. The parasite is able to infect a large range of mammals, but from the zoonotic aspect (transmission to man) pig is the most important host. The infective larvae are encysted in striated muscles with particular high larval densities in *Musculus masseter* and diaphragma. Development is resumed when the larvae are ingested by another host as a result of cannibalism, cryptocannibalism or predation. The larvae are then liberated, and in the small intestine of the new host it undergoes several moults to become sexually mature within a few days. The adults produce larvae which enter the lymphatic vessels of the gut and travel via the bloodstream to the skeletal muscles, where they become encapsulated after 3-4 weeks.

Infection in pigs is normally not associated with clinical signs, opposite the infection in man who may suffer severe illness. Fatal human cases are not rarely reported from endemic areas.

The epidemiology of trichinellosis is determined by one animal eating another (or offal from this). There are sylvatic and arctic *Trichinella* life cycles, where animals of the wild fauna maintain transmission through predation, cannibalism, carrion feeding etc. Usually these cycles involve other species than *T.spiralis*, e.g. *T.britovi*, *T.nelsoni*, *T.nativa* and others. In the domestic pig cycle, transmission is maintained by feeding pigs on food waste containing flesh of infected pig. Rats in piggeries can also maintain a secondary cycle

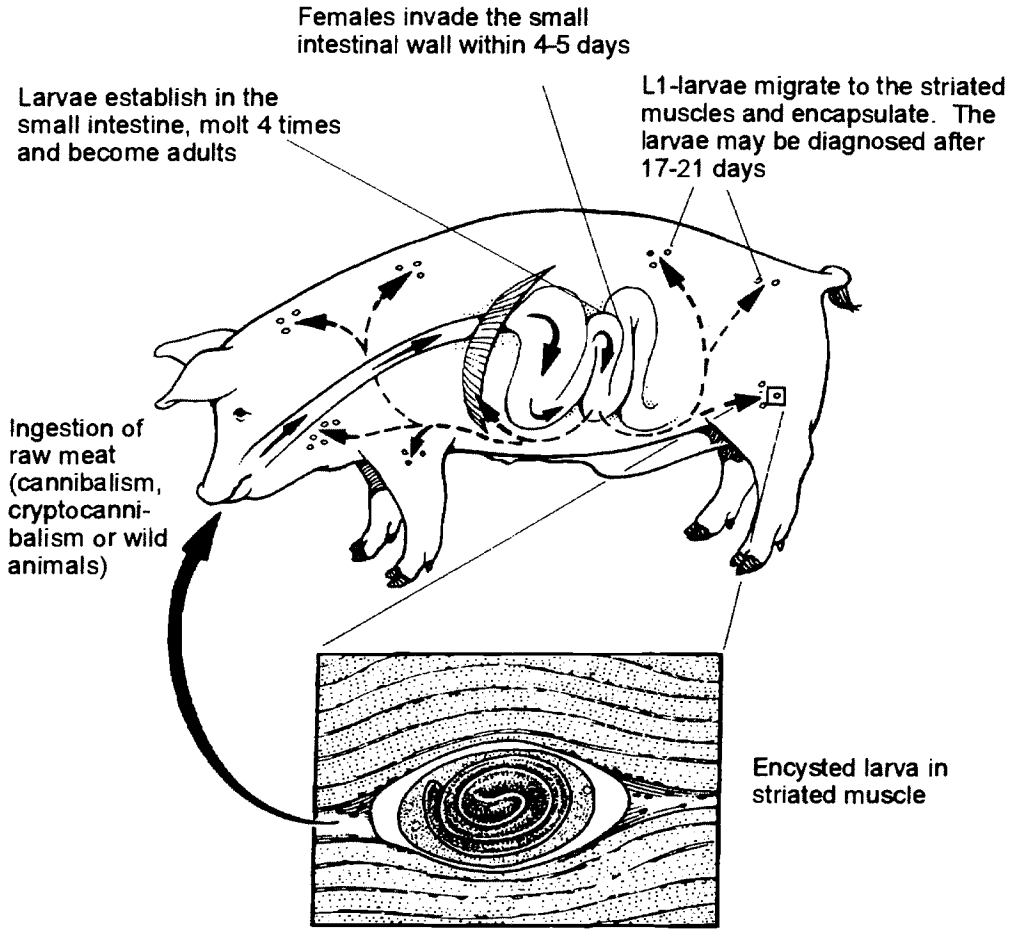


FIGURE 2.9 *Trichinella spiralis* life cycle

which on occasions may pass to pigs or *vice versa*. *Trichinella* species from the wild fauna may accidentally be introduced to pig herds, but it is at present unknown to which extent they are able to maintain a continuous transmission within the pig population. Until now, there is overwhelming evidence that *Trichinella spiralis* is the predominant species in the pig industries. The zoonotic importance of this species is furthermore emphasized by the observation that it may possibly be more pathogenic to man than the other species.

2.5 TREMATODES

There are several trematodes that may infect pig, as it will appear from Tables 2.1 and 2.2 in this chapter, e.g. *Fasciolopsis buski* in the small intestine, *Fasciola hepatica*, *Opistorchis noverca* and *Dicrocoelium dendriticum* in the liver, and *Schistosoma japonicum* (and other schistosome species) in the blood vessels. They have in common that they have a broad final host range, among which man may be an important and vulnerable host. In this way pig may serve as a zoonotic reservoir host, where *Schistosoma japonicum* and *Fasciolopsis buski* are the most important examples.

Fig. 2.10 shows the principle life cycle of *Schistosoma japonicum*, which has a high prevalence in large areas of South-East Asia. The relatively small eggs pass out with the faeces, and hatch in the presence of suitable light and temperature, when they come in contact with water. The short-lived miracidium, a free-swimming larva, swims around in search of an *Oncomelania* snail, which it penetrates and infects. Two generations of sporocysts develop, and finally fork-tailed cercariae are formed. They break out of the snail tissue into fresh water, approximately 4-8 weeks after the snail has become infected. They rise to the surface and "hang" for some days in the surface film. This exposes them to the pig skin, and they quickly penetrate this. They reach the circulatory system and are carried to the lungs from where they migrate to the liver where they mature. Young adult pairs subsequently migrate in the hepatic portal

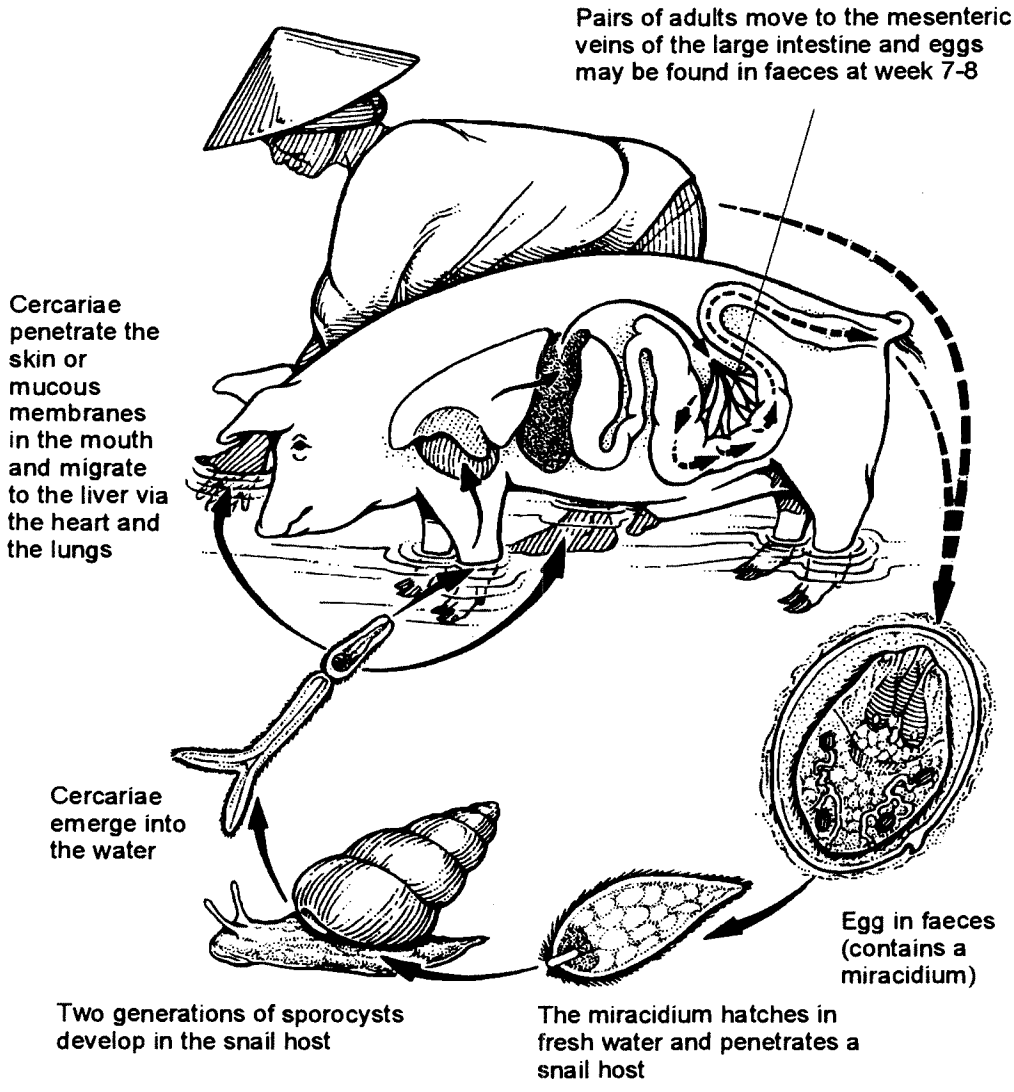


FIGURE 2.10 *Schistosoma japonicum* life cycle

system and settle primarily in the mesenteric veins of the large intestine, in close distance to the gut wall. Here they produce eggs, which penetrate the wall to the intestinal lumen, and are passed with faeces. It usually takes 7-8 weeks from infection until eggs appear in the faeces.

Acute disease, characterized by anorexia, dullness and diarrhoea, occasionally bloody, may occur 7-10 weeks after heavy infection due to inflammatory reactions related to the passage of eggs through the gut wall. With time there appears to be a shift away from intestinal reactions to hepatic lesions caused by the entrapment of eggs that accidentally are released from the worms and pass via the hepatic portal system to the liver. This phenomenon is particularly prominent in the human.

The epidemiology is totally influenced by water as a medium for the free-swimming larvae and the intermediate host snail. Percutaneous infection implies that the potential hosts, e.g. pig and man, must have close water contact for shorter or longer periods of time. Fertilization with human or animal sludge to crops that are grown under humid, wet conditions (rice, certain vegetables) favour heavy transmission. The relative importance of human or animal excreta to infection of respectively humans and animals (e.g. pigs) is poorly determined.

2.6 CESTODES

The principal larval cestodes (metacestodes) found in pigs are those of *Taenia solium* (*Cysticercus cellulosae*), *Taenia hydatigena* (*Cysticercus tenuicollis*) and *Echinococcus granulosus* (hydatids), cf. Table 2.2. The most important cestode specifically related to the pig is *Taenia solium*/*Cysticercus cellulosae*.

Fig. 2.11 shows the life cycle of *Taenia solium*, which is highly prevalent in Latin America, India, and many parts of South-East Asia.

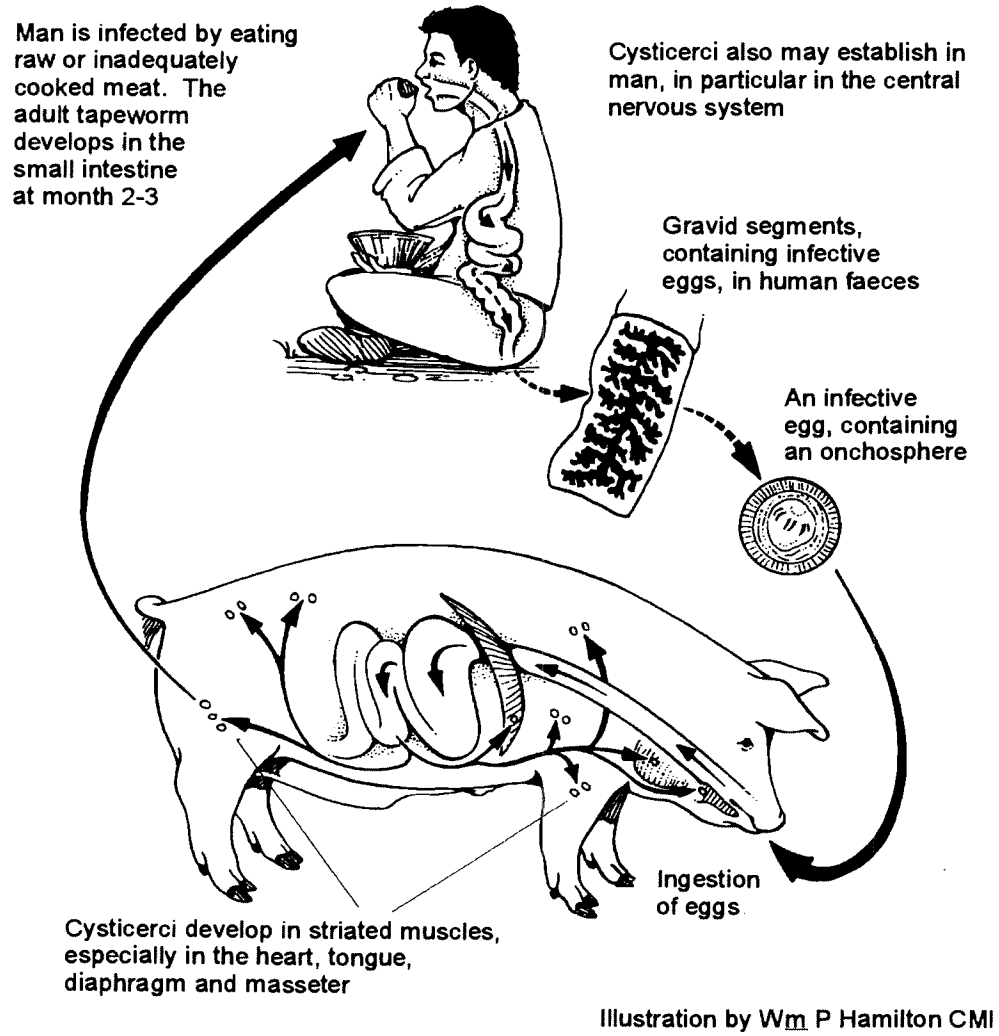


FIGURE 2.11 *Taenia solium* life cycle

Man as the final host harbours the adult worm in the small intestine. The tapeworm segments containing infective eggs, are excreted with faeces. The eggs are ingested by pigs, and onchospheres hatch in the small intestine. They penetrate the gut wall and via the blood they are distributed all over the body, but the metacestodes (cysticerci) almost exclusively develop in the striated muscles, in particular in the heart, tongue, masseters and diaphragm. They appear as fluid-filled cysts, 6-8 mm, containing an invaginated scolex. Man becomes infected through consumption of raw or inadequately cooked pork meat, and the adult tapeworm is developed in the small intestine after 2-3 months. A peculiar and severe feature is occasional establishment in man of metacestodes, in the form of cerebral cysticercosis, a condition caused by the parasite in the central nervous system. This may occur either from the ingestion of tapeworm eggs, or possibly more likely from adult worms in the gut, which release onchospheres to the stomach by reverse peristalsis. This may be called autoinfection.

Clinical disease is inapparent in pigs harbouring the metacestodes and in man with adult tapeworms. However, if man is infected with metacestodes, e.g. in the form of cerebral cysticercosis a range of severe neurological disorders may follow.

The epidemiology depends primarily on the close association of rural pigs with man, in particular pigs with unrestricted access to human faeces. As mentioned, man may acquire cerebral cysticercosis from ingesting eggs, e.g. adhering to vegetables fertilized with night soil or sewage sludge.

Among the other tapeworm infections, *Echinococcus granulosis* established as hydatid cysts in liver and lungs, has great zoonotic importance and severe implications for human health. Usually infections in ruminants play a more important role than infections in the pig (see Hansen and Perry, 1994).

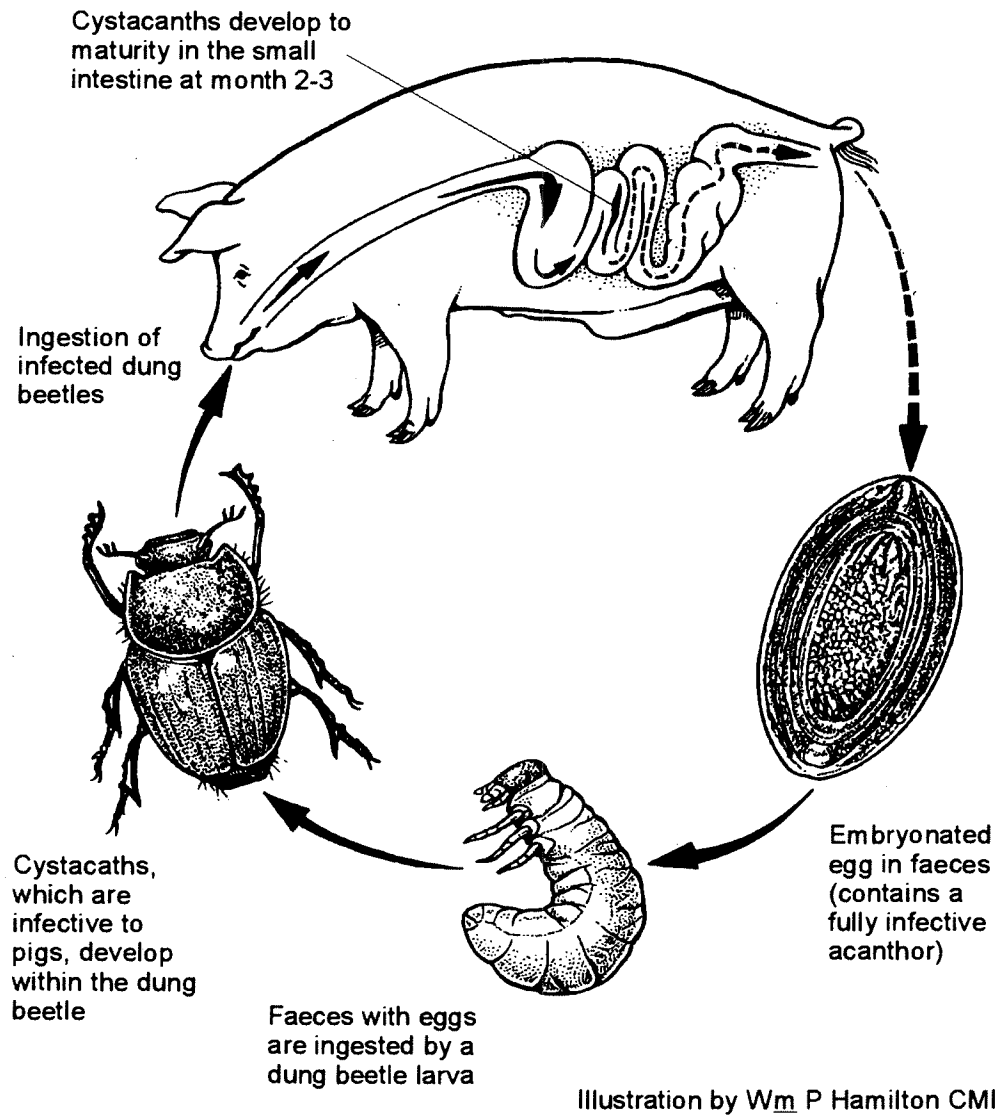


FIGURE 2.12 *Macracanthorhynchus hirudinaceus* life cycle

2.7 ACANTHOCEPHALA

Fig. 2.12 shows the life cycle of *Macracanthorhynchus hirudinaceus*, the only important species of this phylum. It is found worldwide except for certain temperate areas, e.g. Western Europe. This large worm (up to 65 cm) lives in the small intestine. Embryonated eggs are passed with faeces, and after ingestion by dung beetles an infective stage, the cystacanth, is developed in approximately 3 months. Pigs become infected by eating dung beetles, and the cystacanth develops in the small intestine to the adult stage in 2-3 months.

Infections are usually subclinical, but heavy infections may cause gut lesions, and in rare cases penetration of the gut wall, and fatal peritoneitis.

The epidemiology is determined by pigs' contact with beetles and biotic factors favouring beetles. Eggs may survive for many years in the faeces/soil environment. Pig is the only final host.

TABLE 2.1 An overview of gastro-intestinal helminth species in pigs

Organ	Helminth	Life cycle	Transmission	Final hosts
Oeso-phagus	*Gongylonema pulchrum*	indirect	I: dung beetles etc.	wide host range
Stomach	*Trichostrongylus axei*	direct	L3 orally	ruminants + horse
	Hyostrongylus rubidus	direct	L3 orally	pig
	Ollulanus tricuspis	direct?	'vomiting'?	Felids and canids
	Ascarops strongylina *Physocephalus sexalatus* *Simondsia paradoxa*	indirect	I: dung beetles	mainly pig
	Gongylonema pulchrum	indirect	I: dung beetles etc.	very wide host range
	Gnasthostoma hispidum *G.doloresi*	indirect	I-1: aqua. crustaceans I-2: small vertebrates	wide range of domestic animals
Small Intestine	*Trichostrongylus vitrinus* *T.colubriformis*	direct	L3 orally	primarily ruminants
	Globocephalus urosubulatus *G.longemucronatus*	direct	L3 orally	domestic animals
	Strongyloides ransomi	direct	L3 orally, colostral, percutan	pig
	Ascaris suum	direct	L3 in eggs	mainly pig
	Trichinella spiralis	indirect	L1 in striated muscles	wide host range
	Fasciolopsis buski	indirect	I: fresh water snail	man (primarily)
	Macracanthorhynchus hirudinaceus	indirect	I: dung beetles	pig
Large Intestine	*Oesophagostomum dentatum*[1] *O.quadrispinulatum*[2] *O.brevicaudum*	direct	L3 orally	pig
	Trichuris suis	direct	L1 in eggs	pig

1: *O.granatensis* and *O.georgianum* regarded as variant forms of *O.dentatum*
2: *O.longicaudum* synonymous with *O.quadrispinulatum*
I: Intermediate host

TABLE 2.2 An overview of non-gasto-intestinal helminths in pigs

Organ	Helminth	Life cycle	Transmission	Final hosts
Lungs	*Metastrongylus apri*[1] *M.salmi* *M.pudendotectus*	indirect	I: earthworms (L3)	pig
Blood system	*Schistosoma japonicum* *S.mansoni* *S.incognitum*	indirect	I: freshwater snails	large variety of mammals
Kidneys	*Stephanurus dentatus*	direct	L3: oral, percutan (transport:earthworm)	pigs
Liver	*Taenia hydatigena* (metacestodes)[2]	indirect	eggs orally	canids
	Echinococcus granulosus (metacestodes, hydatids)	indirect	eggs orally	canids
	Dicrocoelium dendriticum	indirect	I-1: landsnail I-2: ants	primarily ruminants
	Fasciola hepatica	indirect	I: freshwater snails	large variety of mammals
	Opisthorchis noverca	indirect	I-1: aquatic snails I-2: fish	fish eating mammals
	Ascarid migrating larvae	direct	L3 in eggs	pigs
	Schistosoma spp. (eggs)	indirect	I: freshwater snails	large variety of mammals
Connective tissue	*Taenia hydatigena* (cysticercs)[2]	indirect	eggs orally	canids
Muscles	*Trichinella spiralis* (larvae)	indirect	L1 in striated muscles	very wide host range
	Taenia solium (metacestodes)[3]	indirect	eggs orally	man

1: *M.elongatus* synonymous with *M.apri*
2: The metacestodes of *T.hydatigena* are called *Cysticercus tenuicollis*
3: The metacestodes of *T.solium* are called *Cysticercus cellulosae*
I: Intermediate host

CHAPTER 3

FAECAL EXAMINATIONS FOR PARASITES

3.1 INTRODUCTION

Examination of faecal samples for helminth eggs is an easy way to diagnose many helminth infections and to get an impression of the infection level. All helminths which use the pig as a final host, must find a way for their eggs to become available for the next host. Most often, the eggs simply pass with the faeces, and this applies to the large majority of helminths in the gastro-intestinal tract and associated organs, such as the liver and the lungs, and even schistosomes living in the mesenteric veins use the same outlet to the surrounding world.

However, few helminths in the pig do not make use of faeces for egg transport. Thus, the eggs of *Stephanurus dentatus* pass with the urine, while the larvae of *Trichinella spiralis* migrate directly to the striated muscles to become encysted there, and infective larvae of *Ollulanus tricupis* are apparently transmitted in vomit.

Newly deposited pig faeces may contain unembryonated eggs or eggs with well-developed embryos (e.g. *Metastrongylus* spp. and *Strongyloides ransomi*), but no hatched larvae. Therefore, a complete examination of fresh porcine faeces does not include the Baermann technique for isolation of parasite larvae.

This chapter presents laboratory techniques suitable for identifying and quantifying parasite infections on the basis of examination of faecal material.

3.2 COLLECTION OF FAECAL SAMPLES

It is important that the examinations are carried out on fresh faeces, as eggs of many helminths hatch within 24 hours (e.g. *Oesophagostomum* spp.) or even after 8 hrs (*Strongyloides ransomi*) at room temperature, and the young larvae will not be detected by the standard laboratory techniques.

Equipment

* Plastic gloves (cheap plastic gloves are often preferable for the more expensive latex gloves)
* Marking pen (waterproof)
* Plastic bags
* Insulated cooling box (storage temperature: 0-8°C), if the transport time to the laboratory exceeds 1-2 hrs
* 3% formalin and plastic containers with tight lids, if a long transport time is expected, and a cooling box is not available

Procedure

Faecal samples are preferably collected directly from the rectum, to ensure that they are completely fresh. This will also allow for registration of the pigs age, sex, reproductive status etc., and repeated samples from the same individual may be avoided.

If rectal samples cannot be obtained, freshly deposited faeces may be collected from the pens/pastures.

The samples may be stored in the plastic gloves by turning their

inside out. Each sample should be unambiguously labelled with animal identification, date and localization in waterproof ink directly on the plastic glove. The amount of faecal matter required depends on the analyses, but at least 4 g is needed for most egg count procedures, and >20 g is preferred if more than one kind of analysis is needed, or if an unexpected result must be confirmed by repeated analyses.

The samples are gathered in larger plastic bags. If the transport time to the laboratory is expected to exceed 1-2 hours (depending on temperature), the samples should be packed in a cooling box to avoid hatching of the eggs. The storage temperature should be 0-8°C, and care should be taken to avoid freezing, as this may damage the eggs and invalidate later results. If larval cultures are to be done, the faecal samples may not be cooled, as even 24 hours at 5°C may interfere with larval development.

When a cooling box is not available, the samples may be placed in plastic containers with tight lids, and 3% formalin should be admixed to the faeces (approx. 1 ml formalin to 4 g faeces). This will preserve the sample and the parasite eggs, but it should be noted that quantitative egg counts will not be completely correct, and that formalin-fixed faeces cannot be used for faecal cultures.

In the laboratory, the samples for egg counts should immediately be placed in a refrigerator (approx. 4°C) until they are processed. Samples may be stored at this temperature for more than 3 weeks without significant changes in egg counts. If faecal cultures are to be set up, a storage in a refrigerator cannot be recommended at all, but the cultures should be set up immediately. Note again that faecal samples should never be kept in a freezer.

3.3 QUALITATIVE TECHNIQUES FOR FAECAL EXAMINATIONS

A large number of different procedures is available for demonstrating

eggs in faeces of pigs. Three methods will be described below, all of them providing results that are only qualitative (or, at the highest, semiquantitative), because the egg recovery may be rather low and highly variable.

The most widely used principle for concentration of parasite eggs is flotation. As most nematode eggs (and coccidia oocysts) have a specific gravity which is lower than that of plant residues in the faeces, the eggs may be separated from other faecal particles by mixing the faeces with a fluid in which the eggs flotate, while the plant particles sink.

Unfortunately, the specific gravity of helminth eggs varies. While most nematode eggs will flotate in saturated NaCl, some nematode species have eggs which will flotate only in fluids with higher specific gravities, such as saturated $MgSO_4$ or saturated NaCl + glucose (as used below). Among porcine helminths, this applies to, for example, *Metastrongylus* spp. and to some degree *Trichuris suis*, and therefore it is recommended to use only one of the heavy flotation fluids.

Trematode eggs, on the other hand, are in general so heavy that the flotation principle does not work at all. Therefore, these eggs are concentrated by different sedimentation techniques, which, however, are not fully efficient and highly variable.

3.3.1 Test tube flotation

This is a simple qualitative flotation technique for the detection of nematode eggs (and coccidia oocysts) in the faeces.

Equipment

* 2 beakers or plastic containers (disposable or recycling)
* Balance or a precalibrated teaspoon (3 g)

* Flotation fluid: Saturated NaCl with 500 g glucose per litre
* Measuring cylinder or another container graded by volume
* Stirring device (fork, tongue depressor)
* Nylon tea strainer or a single layer of cotton gauze
* 10-12 ml test tube
* Test tube rack
* Coverslips and microscope slides
* Microscope with 40-100x magnification

Procedure

The *Test Tube Flotation* procedure is illustrated in Fig. 3.1.

Transfer approximately 3 g faeces (weigh out or measure with precalibrated teaspoon) to plastic container 1.

Pour 50 ml flotation fluid into plastic container 1 by means of the measuring cylinder.

Mix faeces and flotation fluid thoroughly with a stirring device.

Immediately after stirring, pour the faecal suspension through a tea strainer or a single layer of cotton gauze into plastic container 2.

Discard the retained faecal debris, and immediately pour the sieved faecal suspension from plastic container 2 into a test tube, which is placed in a precisely vertical position in a test tube rack.

The test tube should be topped up with the faecal suspension, so that it has a convex meniscus at the top. Place a coverslip on the top of the test tube.

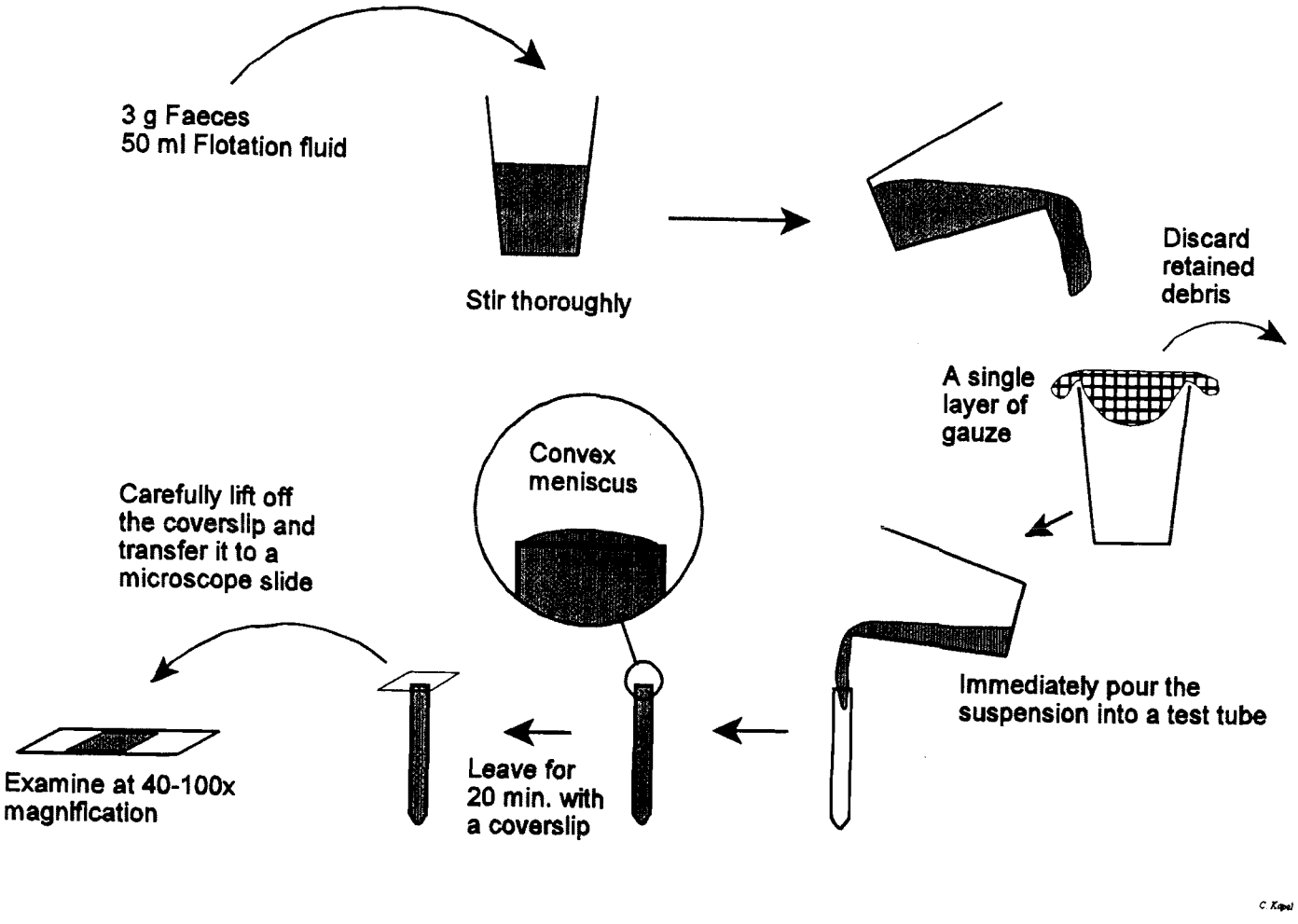


FIGURE 3.1 Test Tube Flotation

Leave the test tube for about 20 minutes. The helminth eggs will flotate and thus accumulate just beneath the coverslip.

Lift off the coverslip vertically from the tube together with the adhering flotation fluid. Some of the accumulated helminth eggs will now be within the adhering fluid, and the transfer of the coverslip must be done very carefully in order to retain as many eggs as possible. Place the coverslip on a microscope slide, and examine the sample at 40-100x magnification in a microscope.

Note: this method is qualitative and not quantitative.

3.3.2 Simple flotation

The principle for simple flotation is identical to that of the above test tube flotation. The only difference is that the flotation takes place in a beaker.

Equipment

* 2 beakers or plastic containers (disposable or recycling)
* Balance or a precalibrated teaspoon (3 g)
* Flotation fluid: Saturated NaCl with 500 g glucose per litre
* Measuring cylinder
* Stirring device (fork, tongue depressor)
* Nylon tea strainer or a single layer of cotton gauze
* Test tube (dry)
* Coverslips and microscope slides
* Microscope with 40-100x magnification

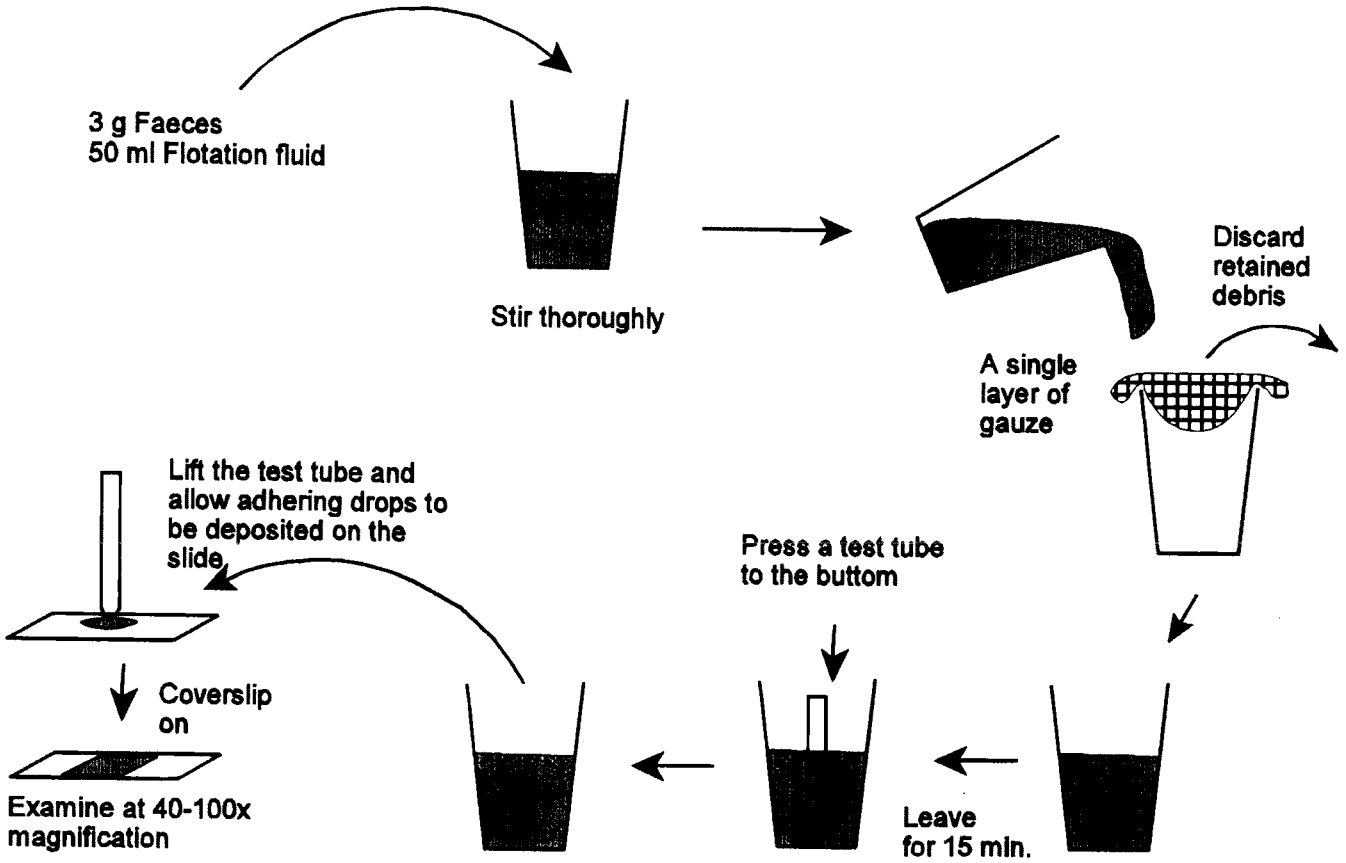


FIGURE 3.2 Simple Flotation

Procedure

The *Simple Flotation* procedure is illustrated in Fig. 3.2.

Transfer approximately 3 g faeces (weigh out or measure with precalibrated teaspoon) to plastic container 1.

Pour 50 ml flotation fluid into plastic container 1 by means of the measuring cylinder.

Mix faeces and flotation fluid thoroughly with a stirring device.

Immediately after stirring, pour the faecal suspension through a tea strainer or a single layer of cotton gauze into plastic container 2.

Discard the retained faecal debris and leave the container undisturbed on the table for 10-15 minutes, during which helminth eggs will flotate and thus accumulate in the surface layer.

Press a dry test tube to the bottom of the faecal suspension, while a microscope slide is ready for use. Some of the helminth eggs, accumulated in the surface layer of the suspension, will now be adhering to the test tube.

In one movement, the test tube is carefully lifted out of the fluid, and adhering drops of faecal suspension are transferred to the microscope slide. The bottom end of the test tube must rest on the slide for several seconds for the drops to run off.

Place a coverslip on the microscope slide, and examine the sample at 40-100x magnification in a microscope.

Note: this method is qualitative and not quantitative.

3.3.3 Sedimentation (Trematode eggs)

As mentioned previously, trematodes eggs have high specific gravities, and therefore the eggs do not flotate in common flotation fluids, but they may be concentrated by sedimentation. Among porcine trematodes, this applies to *Fasciola hepatica*, *Dicrocoelium dendriticum*, *Fasciolopsis buski* and *Opisthorchis noverca*, while special techniques are elaborated for *Schistosoma* spp. (not dealt with in the present guidelines).

The technique described below is merely a combination of washing and sieving of faeces, to remove the smallest and the largest faecal particles. The technique utilizes the high gravity of the eggs, which facilitates their sedimentation in beakers with steeply sloping sides.

Equipment

* 1 or 2 beakers or plastic containers (disposable or recycling)
* Balance or a precalibrated teaspoon (3 g)
* Measuring cylinder
* Stirring device (fork, tongue depressor)
* Nylon tea strainer or a single layer of cotton gauze
* Test tubes and test tube racks. These may preferably be replaced by a conic sedimentation beaker of glass, but these are rather expensive (the conic beakers are also very useful in the Baermann technique described later in this chapter). Fig.3.3 illustrates the technique using conical beakers
* Methylene Blue (1% solution) or Malachite Green (1% solution)
* Coverslips and microscope slides
* Microscope with 40-100x magnification

Procedure

The *Sedimentation Procedure* is illustrated in Fig. 3.3.

Transfer approximately 3 g faeces (weigh out or measure with precalibrated teaspoon) to plastic container 1.

Pour 50 ml tap water into plastic container 1 by means of the measuring cylinder.

Mix faeces and tap water thoroughly with a stirring device.

Immediately after stirring, pour the faecal suspension through a tea strainer or a single layer of cotton gauze into a conic sedimentation beaker, and fill up the beaker with tap water. Alternatively: pour the faecal suspension through a tea strainer or a single layer of cotton gauze into plastic container 2, and transfer approx. 10 ml of the filtered suspension into a test tube which is placed in a test tube rack.

Allow the faecal particles, including the trematode eggs, to sediment for 10 minutes.

Remove the supernatant carefully in one steady movement (conic sedimentation beakers) or with a pipette (test tube sedimentation). Care should be taken not to resuspend the sediment during the process. The supernatant is discarded.

Resuspend the sediment in tap water. The sedimentation beaker should be almost filled up (or alternatively: the test tube should be almost filled up).

Allow the faecal particles, including the trematode eggs, to sediment for 10 minutes.

Remove the supernatant carefully in one steady movement (conical sedimentation beakers) or with a pipette (test tube sedimentation). Care should be taken not to resuspend the sediment during the process. The supernatant is discarded.

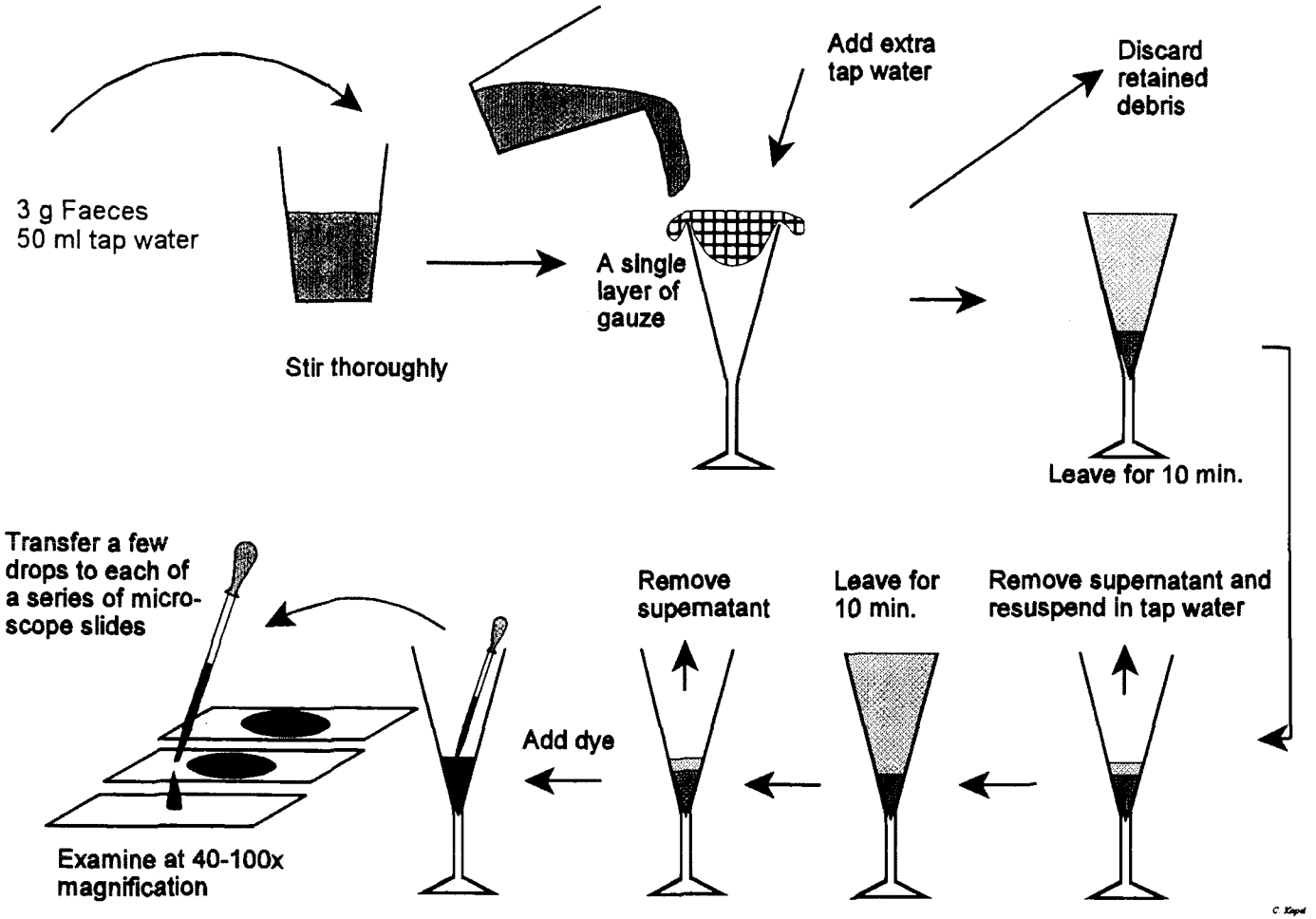


FIGURE 3.3 Sedimentation

Add 1-2 drops of Methylene Blue or Malachite Green. Both dyes will stain the faecal particles deeply blue/green, while the trematode eggs remain unstained. This contrast staining allow the brownish eggs to be discovered more easily. Use a weaker dye solution if the staining is too heavy.

Transfer a few drops of the stained sediment to a microscope slide with a pipette, place a coverslip on the microscope slide, and examine the sample at 40-100x magnification in a microscope.

Repeat the last step until all the sediment has been examined. If nematode eggs are present in the faecal sample, some of them may be found in the sediment, but the recovery rate is very low, and sedimentation cannot replace flotation where nematodes are concerned.

3.4 QUANTIVATIVE TECHNIQUES FOR FAECAL EXAMINATIONS

The qualitative flotation techniques above, which are used for nematode eggs (and coccidia oocysts), have been elaborated to become quantitative, when the eggs are allowed to flotate in a special counting chamber, called the McMaster chamber. Many modifications exist, and a *Simple McMaster Technique* and slightly more elaborated *Concentration McMaster Technique* will be presented in the following.

3.4.1 Simple McMaster technique

No concentration of eggs is carried out in this procedure, and the sensitivity is 50 eggs per gram faeces.

Equipment

* 2 beakers or plastic containers (disposable or recycling)
* Balance
* Measuring cylinder
* Flotation fluid: Saturated NaCl with 500 g glucose per litre
* Stirring device (fork, tongue depressor)
* Nylon tea strainer or a single layer of cotton gauze
* Pasteur pipettes and rubber teats
* McMaster counting chamber. Several designs exist, but the traditional design with two counting fields and a permanently fixed, solid upper glass with a counting grid on the inside is recommended. This design may be found in expensive models of glass and cheap models of plastic. If many samples are to be examined by skilled personnel, the glass chambers are recommended, as they do not become scratched so easily, and as the visual fields in general are more clear. On the other hand, the plastic chambers are recommended when only a low number of samples is to be examined, or when students and trainees are learning the technique. The plastic chambers will quickly become scratched, but they are so cheap that they may then be discarded; or, alternatively, the scratched outer surface may be polished (e.g. by a watchmaker)
* Filtering paper cut into approx. 1 cm wide strips
* Microscope with 40-100x magnification

Procedure

The *Simple McMaster Technique* is illustrated in Fig. 3.4.

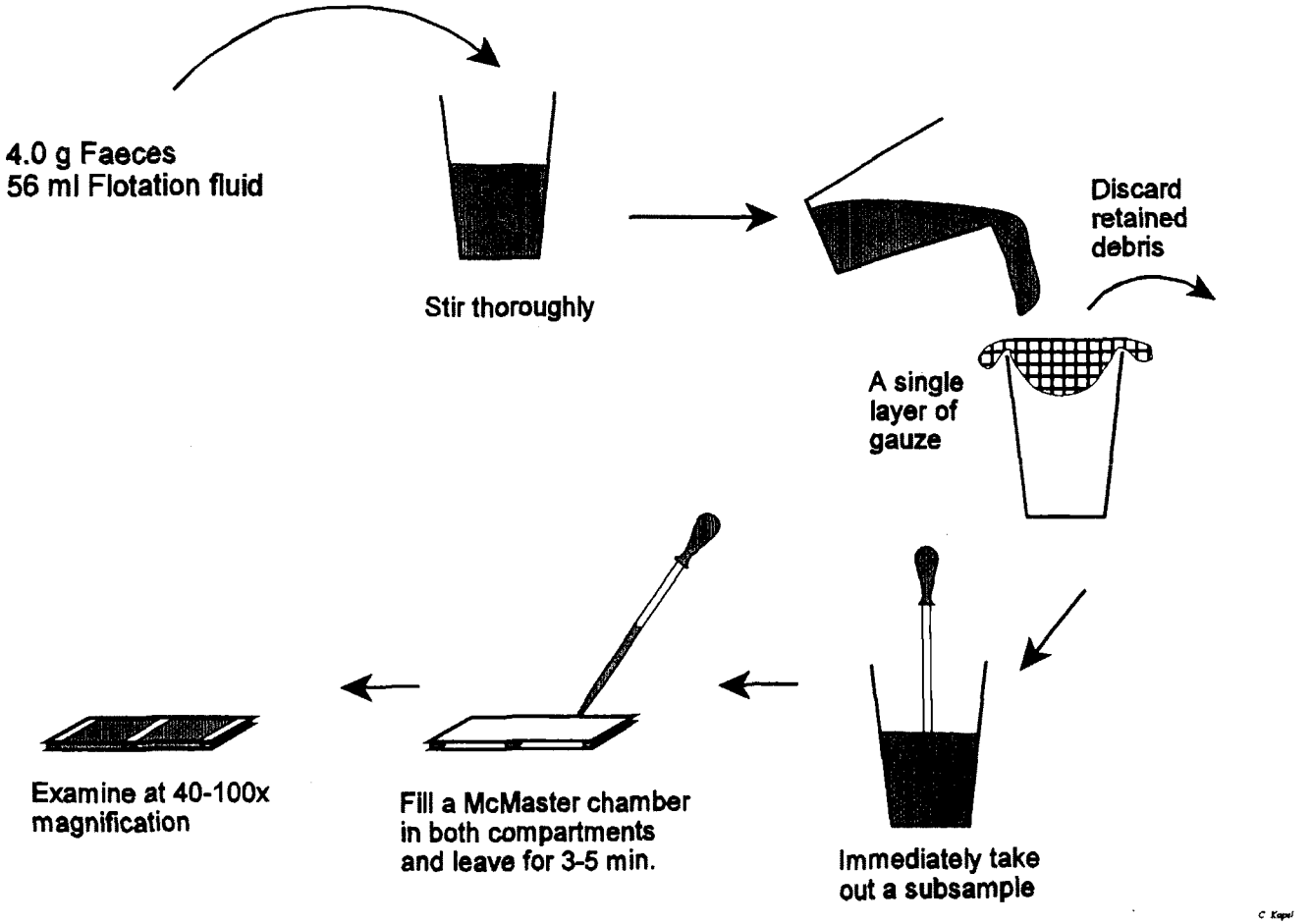


FIGURE 3.4 Simple McMaster Technique

Weigh out 4.0 g faeces and transfer it to container 1. The container should be unambiguously labelled (disposable containers may be labelled in waterproof marking ink).

Add 56 ml flotation fluid by means of the measuring cylinder. If another amount of faeces is weighed in the former step (i.e. more or less than 4.0 g), the volume of flotation fluid should be adjusted correspondingly (ratio: 14 ml flotation fluid to 1.0 g faeces). This ratio ensures that 15 ml of the resulting faecal suspension correspond to 1.0 g faeces.

Mix faeces and flotation fluid thoroughly with a stirring device.

Pour the faecal suspension through a tea strainer or a single layer of cotton gauze into container 2, immediately after stirring. The retained debris is discarded. If disposable containers are used, container 2 may be placed into container 1, which is still labelled.

A subsample is taken with a pasteur pipette immediately after the filtering procedure, when the suspension is still well mixed.

Fill both sides of the McMaster counting chamber with the faecal suspension. Be careful to avoid air bubbles.

Leave the filled McMaster chamber to rest on the table for 3-5 minutes before counting (minimum 3 minutes to allow all eggs to flotate, and maximum 10 minutes, as some eggs may be distorted in the flotation fluid).

Count the number of eggs in both counting fields, and calculate the number of eggs per gram of faeces by multiplying the number of eggs by 50 (see Section 3.4.3 *Counting the McMaster chamber*).

After counting, the McMaster chamber should be washed under a stream of tap water, shaken to remove most of the water, and dried with a cotton cloth on the outside and with a strip of filter paper inside the chamber.

3.4.2 Concentration McMaster technique

This technique is slightly more complicated than the *Simple McMaster Technique*, but the recovery of eggs is higher and the sensitivity is better (20 eggs per gram of faeces). This technique is, therefore recommended if a centrifuge is available. Furthermore, the procedure may be more flexible when many samples are handled simultaneously.

Equipment

* 2 beakers or plastic containers (disposable or recycling)
* Balance
* Measuring cylinder
* Stirring device (fork, tongue depressor)
* Nylon tea strainer or a single layer of cotton gauze
* Test tube with 4 ml and 10 ml marks
* Test tube stopper
* Test tube rack
* Centrifuge
* Flotation fluid: Saturated NaCl with 500 g glucose per litre
* Pasteur pipettes and rubber teats
* McMaster counting chamber
* Filtering paper cut into approx. 1 cm wide strips
* Microscope with 40-100x magnification

Procedure

The *Concentration McMaster Technique* is illustrated in Fig. 3.5.

Weigh out 4.0 g faeces and transfer it to container 1. The container should be unambiguously labelled (disposable containers may be labelled in waterproof marking ink).

Add 56 ml tap water by means of the measuring cylinder.

Some people prefer to weigh out between 4.0 and 6.0 g faeces in the first step, and then add the corresponding volume of tap water (the ratio should always be 14 ml tap water to 1.0 g faeces. This ratio ensures that 15 ml of the resulting faecal suspension corresponds to 1.0 gram of faeces).

Mix the faeces and the tap water thoroughly with a stirring device.

Allow the faecal suspension to rest for 30 minutes at room temperature, and again mix the faeces and the tap water thoroughly with a stirring device.

The 30 minutes' soaking and the repeated mixing ensure that even firmer pieces of faeces will be completely dissolved, but the step may not be necessary if the faeces are soft diarrheia. A similar soaking step cannot be used in the previously mentioned flotation techniques, as faeces were dissolved directly in the flotation fluid, which may distort the eggs during a prolonged exposure.

Pour the faecal suspension through a tea strainer or a single layer of cotton gauze into container 2, immediately after stirring, and discard the retained debris. If disposable containers are used, container 2 may be placed into container 1, which is still labelled.

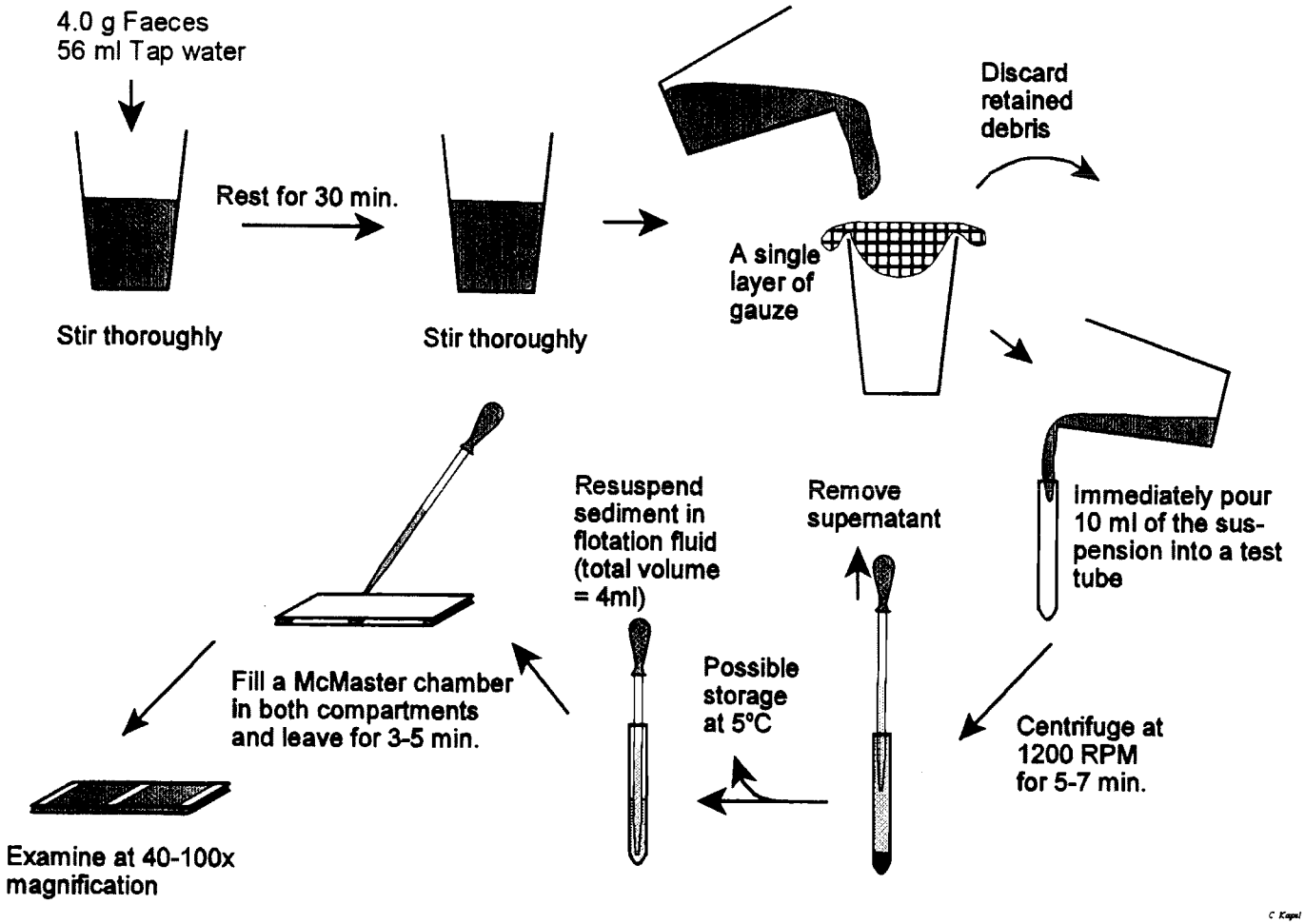


FIGURE 3.5 Concentration McMaster Technique

Immediately after the filtering procedure, pour faecal suspension into a test tube to the 10 ml mark. As 15 ml faecal suspension represents 1 g faeces, the 10 ml suspension will represent 2/3 g.

Centrifuge the test tube for 5-7 minutes at 1200 RPM (revolutions per minute).

Remove the supernatant with a pipette or another vacuum device, but be careful not to resuspend the sediment. Correctly done, the sediment still represents 2/3 g faeces.

At this step it is possible to interrupt the procedure by stoppering the tube and storing it in a refrigerator (approx. 4°C) for up to 7 days without any significant reduction in the egg counts. If many samples are to be handled simultaneously, this possibility for storage makes the laboratory work more flexible and rational, as 50-100 samples may be sieved and centrifuged in one step, whereafter they are stored until they are counted one by one.

A similar storage is not possible in the simple McMaster technique, as the faeces are dissolved directly in the flotation fluid.

Shortly before counting, flotation fluid is added to the 4 ml mark (i.e. the total volume of faecal sediment and flotation fluid is 4.0 ml). These 4 ml now represent 2/3 g faeces.

Resuspend the sediment very carefully, using a pasteur pipette several times. Avoid making bubbles in the suspension, as these will make the egg counts less reliable.

Fill both sides of the McMaster counting chamber with the faecal suspension, immediately after resuspension of the sediment. Be careful to avoid air bubbles.

Leave the filled McMaster chamber to rest on the table for 3-5 minutes before counting (minimum 3 minutes to allow all eggs to flotate, and maximum 10 minutes, as some eggs may be distorted in

the flotation fluid). Count the number of eggs in both counting fields and calculate the number of eggs per gram of faeces by multiplying the number of eggs by 20 (see Section 3.4.3 *Counting the McMaster chamber*).

After counting, the McMaster chamber should be washed under running tap water, shaken to remove most of the water, and dried with a cotton cloth on the outside and with a strip of filter paper inside the chamber.

3.4.3 Counting the McMaster chamber

The filled McMaster chamber must rest on the table for at least 3-5 minutes to allow all eggs to flotate, i.e. accumulate just below the upper glass of the chamber. It is important that the eggs have enough time to flotate in order to avoid underestimating the egg count. On the other hand, the sample becomes less clear, and some egg types may be distorted and sink, if the sample rests for too long (15-20 minutes) in the chamber before microscopical examination. This can be prevented by keeping the filled McMaster slides in the refrigerator prior to counting.

Focus on the counting grid (or parasite eggs) and count the different nematode eggs within the engraved area of both sides of the chamber. Skilled personnel often prefer a 4x10 magnification, while all others are recommended to use a 10x10 magnification until they are completely familiar with all types of eggs. If coccidia oocysts are counted, a 10x10 magnification should always be used, as porcine coccidia have rather small oocysts (as small as 12 μm, i.e. much smaller than the oocysts of most ruminants).

When counting the engraved areas, the general rules for counting should be followed: all eggs inside the grid should be counted plus all eggs touching two sides of the grid (e.g. the upper and the left borderlines), while excluding all eggs touching the two other sides of the grid (e.g. the lower and the right borderlines).

Every type of nematode egg should be counted separately.

The distance between the upper and the lower glass of the McMaster chamber is 0.15 cm, and the two counting fields each measure 1x1 cm. Therefore, the faecal suspension under the two counting fields has a volume of 2 x 0.15 ml = 0.3 ml.

In the *Simple McMaster Technique*, 15 ml faecal suspension represents 1 g faeces, and therefore 0.3 ml represents 1/50 g faeces. The number of eggs per gram of faeces can now be calculated as follows: The total number of eggs in both sides of the chamber should be multiplied by 50. This gives the number of 'Eggs Per Gram of faeces', usually abbreviated EPG.

In the *Concentration McMaster Technique*, 4 ml of the final faecal suspension in the test tube represents 2/3 g faeces, and therefore the counted volume of 0.3 ml faecal suspension represents 1/20 g faeces. The number of eggs per gram of faeces (EPG) can now be calculated by multiplying the total number of eggs in both sides of the chamber by 20.

Example: 18 eggs are counted in side 1 of the chamber, and 22 eggs are counted in side 2.
If the *Simple McMaster Technique* has been used, EPG = (18+22)x50 = 2000.
If the *Concentration McMaster Technique* was used, EPG = (18+22)x20 = 800.

3.5 FAECAL CULTURES

Helminth eggs of the strongylid type (see Section 3.6 *Identification of eggs and larvae*) cannot be identified to species. They may belong to *Oesophagostomum* spp., *Hyostrongylus rubidus* or, very rarely, *Trichostrongylus* spp. In order to differentiate between these helminths, it is necessary to make faecal cultures in which the eggs

will hatch and the larvae develop to the infective third stage (L_3). These L_3 larvae may then be identified to genus by microscopical examination (see Section 3.6 *Identification of eggs and larvae*).

Several simple techniques for culturing L_3 larvae exist. The only precautions to be taken are that the physico-chemical conditions should be so favourable that eggs of all species are able to develop into larvae, and that a large number of larvae should be identified in order to detect all species present. The latter precaution is necessary for the detection of *Hyostrongylus rubidus*, since pigs infected with *H.rubidus* most often also harbour *Oesophagostomum* spp., which has a much higher fecundity.

It is important that the faecal samples are collected rectally, as faeces picked up from the ground is very often contaminated with free-living nematodes. The latter will multiply extremely rapidly in the cultures and totally outnumber the parasite larvae, which may then be difficult to find. Furthermore, the faecal samples should be very fresh, as even a few days of storage in a refrigerator may significantly reduce the larval development of some species.

One simple technique for faecal cultures and for harvest of larvae (Baermann technique) is described below.

Equipment

* 1 beaker or plastic container (disposable or reusable)
* Balance
* Stirring device (fork, tongue depressor)
* Vermiculite (an inert absorbing material which provides the cultures with a fine structure)
* Tap water (not chlorinated)

* Humidity chamber (e.g. a plastic tray within a large plastic bag, or a tray with a lid)
* Little stick and a rubber band
* Nylon tea strainer or a double layer of cotton gauze
* Conical sedimentation beaker (alternatively, other kinds of beakers may be used, but they do not concentrate the larvae as well because of the larger bottom area)
* Pipettes (e.g. 2 ml)
* Microscope slides. The best slides are large and equipped with a paraffin bank surrounding the sample area
* Microscope with 10-40x magnification

Procedure

The *Faecal Culture* technique is illustrated in Fig. 3.6.

Weigh out 10 g faeces and transfer it to a plastic container.

Add 4 g vermiculite (vermiculite is very light, so its volume is considerably larger than that of the faeces) and stir thoroughly.

Add tap water if necessary, and then stir thoroughly. The volume of water depends on the consistency of the culture. Dry faeces needs more water than normal faeces, and if the faeces is like diarrhoea, it may be necessary to add more vermiculite to absorb the water instead of adding water. Eventually, the culture should end up with a consistency where it does not appear wet, but still has a 100% relative humidity.

If non-chlorinated tap water is not available, it is necessary to use deionized or distilled water.

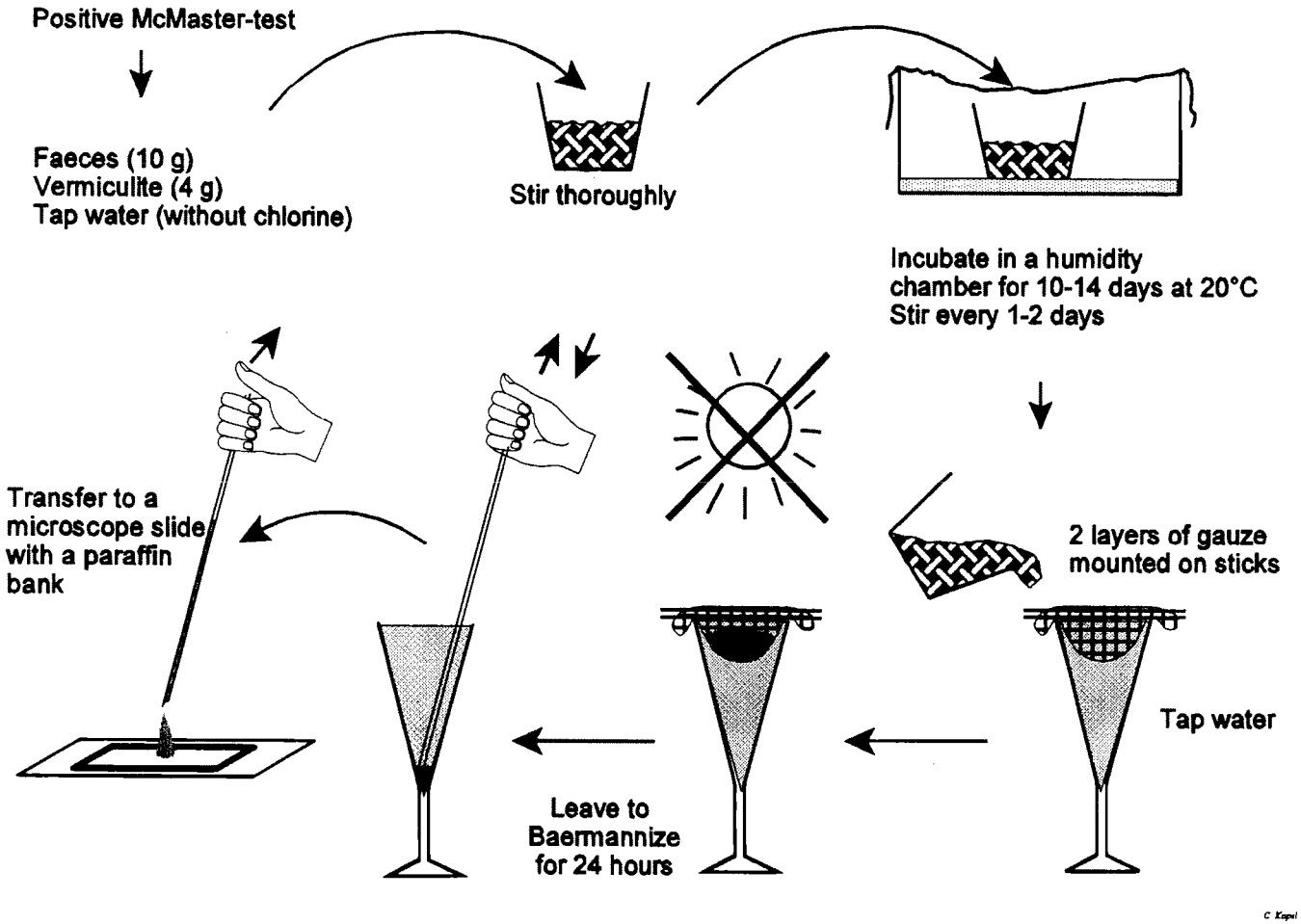


FIGURE 3.6 Faecal Culture

The beaker is placed in a humidity chamber in which the bottom is covered with water, to ensure a relative humidity of 100%.

The humidity chamber is stored at room temperature (20-21°C) or in an incubator at similar temperature for 10-14 days, by which time the larvae should have reached the L_3 stage. If the cultures are kept at lower temperatures (min. temperature 15°C), the incubation period should be considerably increased.

Stir the cultures every 1-2 days, and add water if they have become too dry. Stirring may help to break up fungal hyphae and thereby keep fungal growth at an acceptable level.

After the incubation period, the larvae are recovered by a Baermann technique. First, place the culture on a double layer of gauze.

Wrap up the culture and fasten it to a small stick of wood by means of a rubber band.

Submerge the culture in tap water in a conical sedimentation beaker and leave it there until the next day. During the night, the large majority of larvae will move out of the culture and sink to the bottom of the beaker.

Harvest the larvae with a 2 ml pipette by placing your fingertip firmly at the end of the pipette, move the tip of the pipette to the bottom of the beaker, relieve the fingertip pressure from the pipette and allow the larvae to be sucked up into the pipette. Do not suck by mouth, as larvae may accidentally be sucked into your mouth.

Transfer the larvae to one or more microscope slides equipped with a paraffin bank. At least 100 parasite L_3 larvae (if available) should be counted and differentiated under a microscope.

3.6 IDENTIFICATION OF EGGS AND LARVAE

Helminth eggs found in pig faeces may have a characteristic appearance, which permits an unambiguous identification, or they may be of the strongylid type which cannot be identified before the infective larvae have been developed in cultures.

Some of the most common eggs, and two types of infective larvae developed from eggs of the strongylid type, are shown below (Figs 3.7, 3.8 and 3.9 a+b). As coccidia are quite common in pigs, two types of oocysts are also shown (Fig. 3.9 c+d). Furthermore, some characteristics are briefly listed in Table 3.1.

Note that all photos are of the same scale and therefore may be compared directly.

3.7 INTERPRETATION OF FAECAL EGG COUNTS

It is difficult to present guidelines for the interpretation of egg counts. First of all, one should be aware of the possibility of false positive and false negative egg counts. Secondly, the egg counts are not clearly correlated with the worm burden. And finally, even if it were possible to predict the worm burdens from the egg counts, one would still have the problem of how to interpret this worm burden.

The latter topic is discussed in Chapter 4, where it is seen that the interpretation of estimated worm burdens depends on the helminth species and its properties, the specific host-parasite relationship (e.g. the effect of host age immunity), and the management. Therefore, only the false negative and positive results, and the relationship between egg counts and worm burdens, will be discussed below.

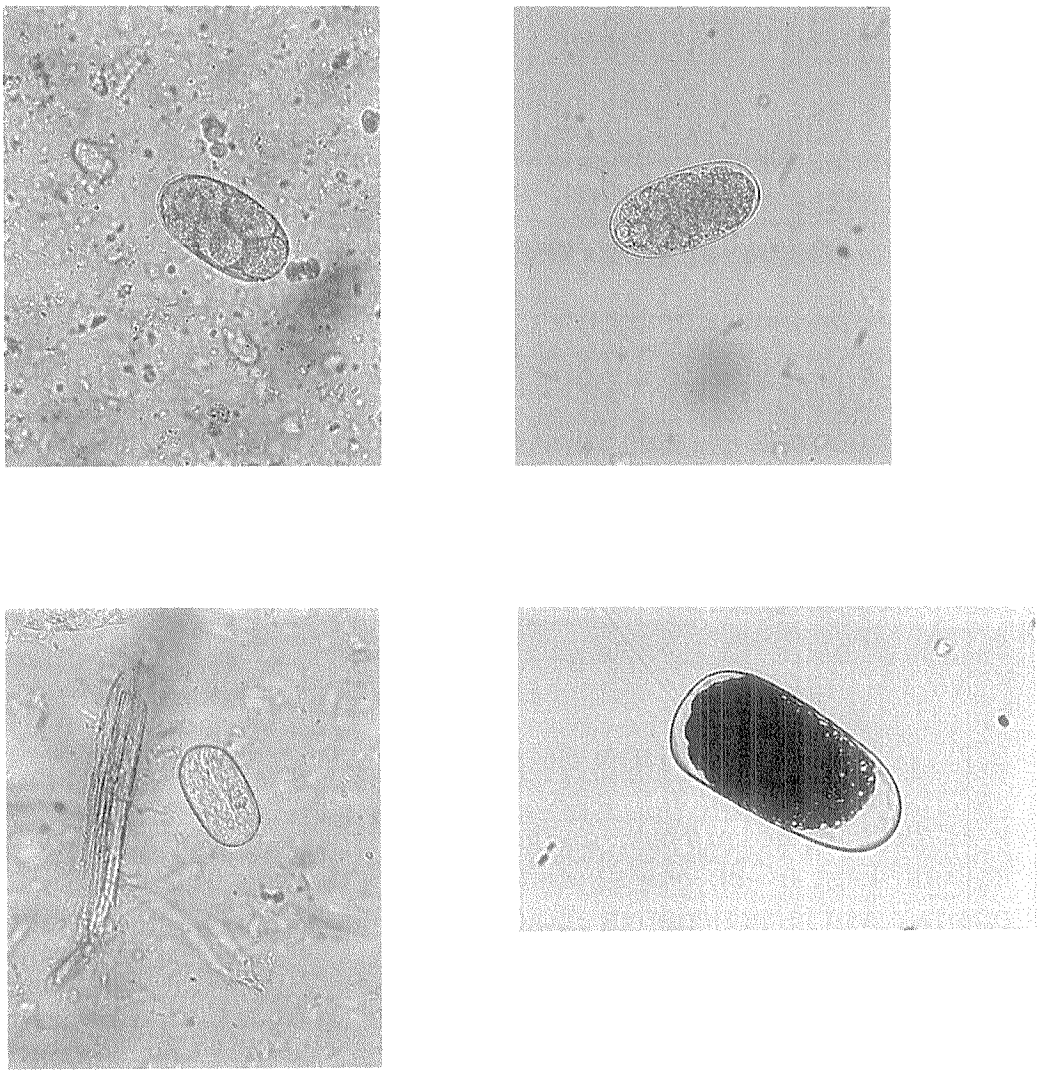


FIGURE 3.7 Some of the most common 'strongylid' eggs in pig faeces. The two upper figures show *Oesophagostomum* sp. (left) and *Hyostrongylus rubidus* (right) which cannot be discriminated. The two lower figures show the larvated, small egg of *Strongyloides ransomi* (left) and the big egg of *Stephanurus dentatus* (right).

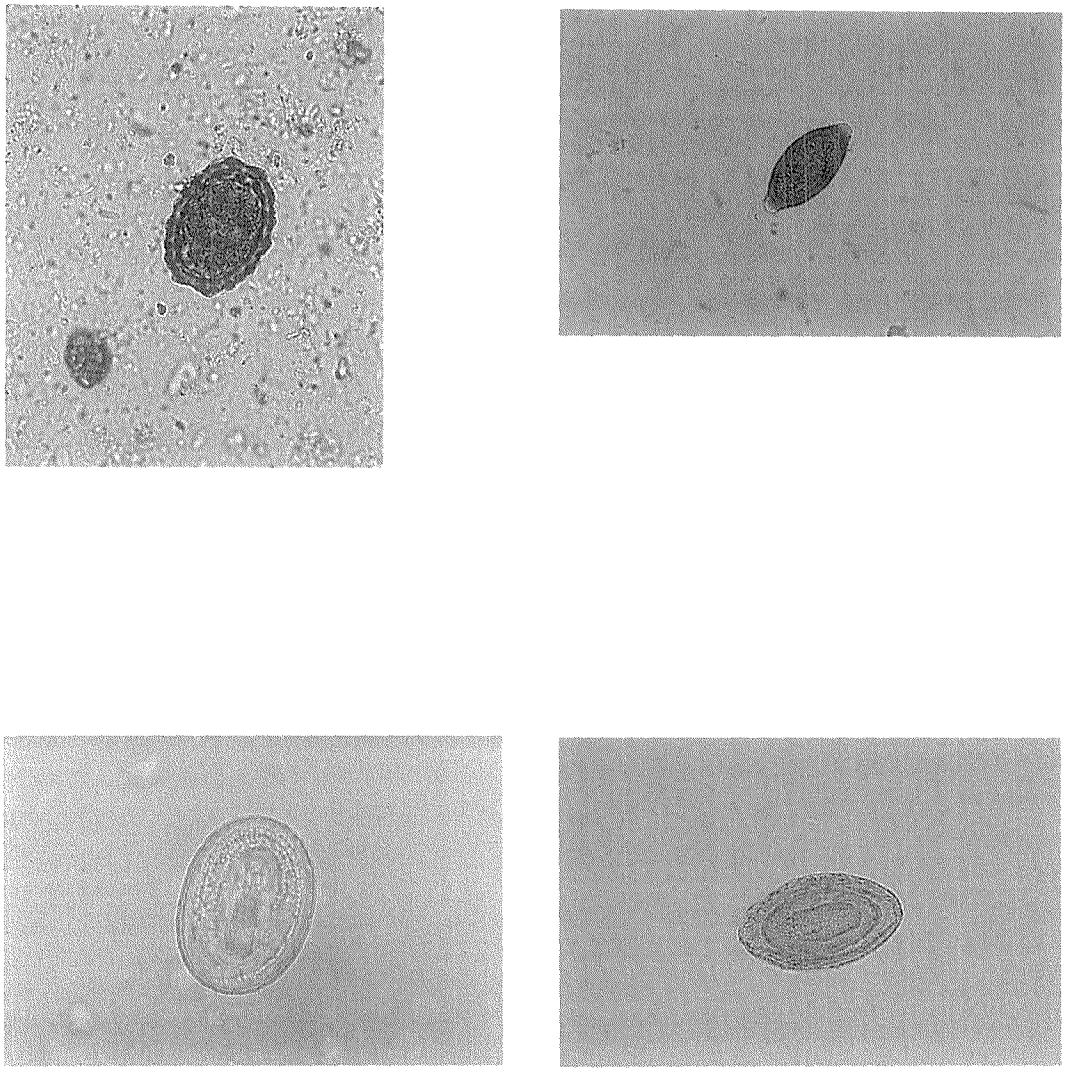


FIGURE 3.8 Some characteristic thickshelled eggs in pig faeces. The two upper figures show eggs of *Ascaris suum* (left) and *Trichuris suis* (right) that are unembryonated when excreted, while the two lower figures show newly excreted, embryonated eggs of *Metastrongylus* sp. (left) and *Macracanthorhynchus hirudinaceus* (right).

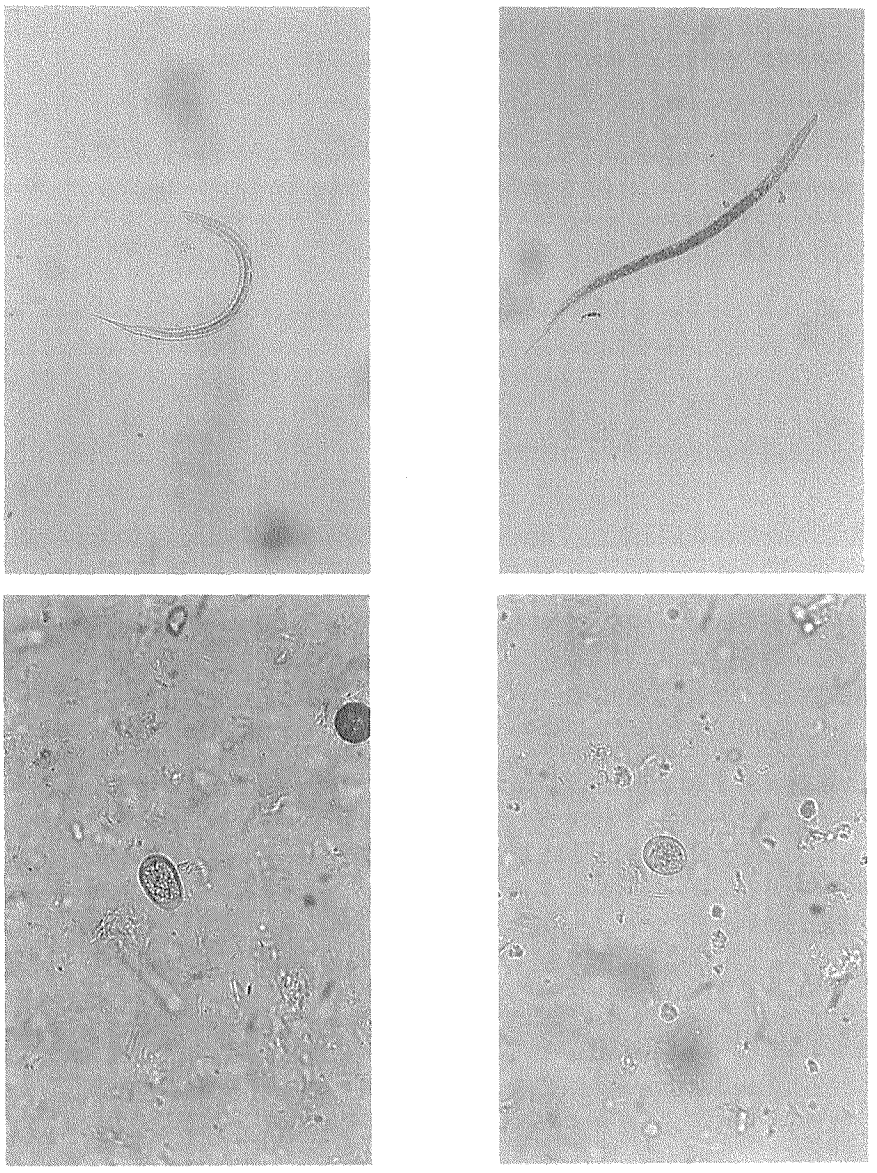


FIGURE 3.9 Two types of infective L_3-larvae developed from eggs of the strongylid type: the thick, sluggish, long-tailed larva of *Oesophagostomum* sp. (upper, left) and the slender, 'swimming', short-tailed *Hyostrongylus* larva. The two lower figures show the small oocysts of *Eimeria* sp. (left), typically found in older pigs, and the even smaller oocysts of *Isospora suis* (right), typically found in piglets.

TABLE 3.1 A list of characteristics of the most common helminth eggs, L_3-larvae (developed from 'strongylid' eggs), and coccidia oocysts (to be continued)

Parasite	width x length (μ)	contents of the fresh egg	morphological characteristics
EGGS			
Gongylonema pulchrum	30-34 x 57-59	well-developed embryo	elliptical egg, smooth surface
Hyostrongylus rubidus	31-38 x 60-76	32-64 cleavage cells	thin-shelled, transparent egg, smooth surface ('strongylid')
Ascarops strongylina	22-26 x 41-45	well-developed embryo	small, elliptical egg, slightly flatte-ned at each pole, smooth surface
Physocephalus sexalatus	22-26 x 41-45	well-developed embryo	small, elliptical egg, slightly flatte-ned at each pole, smooth surface
Simondsia paradoxa	15 x 20-30	well-developed embryo	oval or ellipsoidal
Gnathostoma spp.	39-42 x 72-74	one cell - early embryo	thick-shelled egg,
Trichostrongylus spp.	30-55 x 70-125	16-32 cells	thin-shelled, transparent egg, smooth surface ('strongylid')
Globocephalus spp.	40 x 68-72	2 cells	oval, thin-shelled, transparent egg, smooth surface
Strongyloides ransomi	30-34 x 53-57	well-developed embryo	small, thin-shelled, transparent egg, smooth surface
Ascaris suum	50-76 x 68-84	one cell (with granula)	thick-shelled, rounded or elliptical egg with brown sculptured surface[1]
Macracantho-rhynchus	65 x 110	acanthor larva with hook and spines	thick-shelled, oval egg with brown colour
Oesophagostomum spp.	38-53 x 61-83	16-32 cleavage cells	thin-shelled, transparent egg, smooth surface ('strongylid')
Trichuris suis	28-31 x 60-68	one cell (with granula)	thick-shelled, barrel-shaped egg, brown colour, a clear knob protruding at each pole
Metastrongylus spp.	38-45 x 43-64	well-developed embryo	thick-shelled, elliptical egg, rough surface (small mammillations)
Stephanurus dentatus	53-65 x 91-114	32-64 cleavage cells	large, thin-shelled, transparent egg in the urine
Dicrocoelium dendriticum	30 x 45	a miracidium (some structures visible)	small, dark brown, operculate egg, one side flattened
Fasciola hepatica	90 x 150	a miracidium (granular mass)	large elliptical yellow-brown, operculate egg

Table 3.1 Continued

Parasite	width x length (μ)	contents of the fresh oocyst	morphological characteristics
L_3-larvae			
Hyostrongylus rubidus	22 x 715-735[2]	-	long, slender L_3, the posterior end of L_3 with a small digitiform process, tail of sheath short (moves quickly and can swim)
Oesophagostomum spp.	26 x 500-532[2]	-	shorter, thick L_3, no digitiform process, tail of the sheath long and filamentous (sluggish, cannot swim)
Coccidia oocysts			
Isospora suis	17 x 20	1-2 cells	small oval, transparent oocyst
Eimeria spp.	12-23 x 13-32	one cell	spherical-elliptical transparent oocysts

1 *Ascaris suum* eggs may be atypical, as sterile eggs are much longer than wide, or as they may be immature without the outer sculptured, brown layer. The latter eggs are typically seen when mature females are expelled from the pig due to anthelmintic treatment or self-cure.
2 Widths and lengths of the L_3 larva inside the sheath.

3.7.1 False negative and false positive egg counts

The detection of a helminth infection by examination of faecal samples depends on the egg production of the parasites. If a pig has recently been moved from a clean to a contaminated area, it may harbour a heavy worm burden of young immatures which do not produce eggs, and the faecal examination will be false negative. It is evident that the likelihood of false negative results increases with the duration of the prepatent period, e.g. *Strongyloides ransomi* (prepatent period: 4-7 days) < *Oesophagostomum* spp. and *Hyostrongylus rubidus* (18-21 days) < *Ascaris suum* and *Trichuris suis* (7-8 weeks) < *Stephanurus dentatus* (9 months) (see Chapter 2 for prepatent periods).

When the transmission rate is high, the pigs will respond immunologically to the parasites, and a depression in egg output may take place. This is particularly common when the helminth species is strongly immunogenic. *Ascaris suum* is a good example, as often only 20-40% of the growing pigs (and a much lower percentage of older animals) harbour patent infections, even if all pigs are continuously exposed to infective eggs, and their livers may be badly affected by migrating larvae. But also lightly immunogenic species, such as *Oesophagostomum* spp., may at extreme transmission rates have ceased producing eggs temporarily, despite the fact that a substantial number of adult (stunted) worms may be found in the intestine.

False negative egg counts may also be found when a few adult worms are all either males or females (unisex infections, common when worm burdens in general are low, such as *Ascaris suum*), or when adult worms have a low fecundity (e.g. *Hyostrongylus rubidus*), or when the test used is not sufficiently effective.

The phenomenon of false negative egg counts is generally accepted, whereas the opposite phenomenon, 'false positive egg counts', is more or less overlooked. False positive egg counts may be found when unembryonated helminth eggs are eaten by an uninfected host and then passed with faeces. For such passage to occur, the eggs must remain unhatched in the environment and during the intestinal passage, and the host should eat faeces. Pigs are exceptionally good candidates for false positive samples, as they may eat significant quantities of faeces or contaminated soil, and as especially *Ascaris suum* and *Trichuris suis* produce huge numbers of eggs which remain unembryonated for considerable periods of time. As a rule of thumb, *Ascaris* EPG <200 may be regarded as false positive (one single mature female normally produces 400-800 EPG in growing pigs, although considerable variations exist).

3.7.2 The relationship between egg counts and worm burdens

When evaluating faecal egg counts, one should be aware of the fact that EPG nearly always fluctuates considerably over time (weeks, days), and that even in one large faecal sample (from one day) countings on subsamples vary considerably, indicating that the eggs are not evenly distributed in the faeces. Also the faecal consistency may have some influence, as dry, hard faeces with relatively little content of water will generally have higher EPG values than softer and more watery faeces.

As mentioned above, pigs respond immunologically to helminth infections, and the fecundity of the females seems to be the most sensitive target. Thus, *Ascaris* females seem to have the highest egg output when only one single pair is present, while the individual fecundity decreases with increasing worm burdens. For less immunogenic species, such as *Oesophagostomum* spp., there seems to be a reasonably good correlation between EPG and the size of the worm burden as long as this is below a certain level, whereas at higher infection levels the individual egg laying becomes reduced or may even stop completely.

CHAPTER 4

POST-MORTEM DIFFERENTIAL WORM COUNTS

4.1 INTRODUCTION

Post-mortem worm counts provide a much more precise assessment of parasite worm burdens than parasite egg counts or counts of larval cultures, because eggs and to a certain degree larvae may not always be identified to species, because there is no clear correlation between egg output and worm burden, and because results from counts of eggs and larvae may be false positive or false negative.

For worm counts in pigs, the following organs are required: The *gastro-intestinal tract* from oesophagus to anus, the *liver*, the *kidneys*, the *lungs*, a piece of the *diaphragm* and/or a piece of *musculus masseter*. Frozen organs may be used for most analyses, but fresh organs are much more convenient to handle, and isolation of parasite larvae by incubation or baermannization can only be carried out on fresh organs.

First of all, it is important to record all abnormalities and lesions, bearing in mind that alternative causes of illness or death may occur. Thereafter, adult and larval nematodes should be isolated, counted and identified as described below. Light helminth infections in pigs occur very often, and these infections do not in general cause clinical symptoms. Presence of several species at the same time (polyparasitism) may precipitate disease due to additive or even synergistic effects. Therefore it is important not only to identify the species present, but also to assess the numbers of all species.

Suitable methods for differential worm counting under field or laboratory conditions, using simple, easily obtainable and inexpensive equipment, are described below.

Counts of gastro-intestinal helminths are most conveniently done first by examination of the organs from the outside to count eventual

metacestodes, and thereafter by examination of the stomach, the small intestine, the large intestine, the muscles, the lungs, the liver, and the kidneys, separately.

4.2 THE CONTENTS OF THE STOMACH

The stomach may be infected by a number of species belonging to Trichostrongyloidea (*Hyostrongylus rubidus*, *Trichostrongylus axei*, *Ollulanus tricuspis*) and Spiruroidea (e.g. *Ascarops strongylina*, *Physocephalus sexalatus*, and others). Nearly all species are small, slender worms (10-30 mm), and the larvae, and for some species the adults, live in the gastric wall, e.g. in gastric nodules. Therefore, careful observations should be carried out on the stomach wall, mucus should be scraped off when examining the gastric contents, and the gastric mucosa should be scraped off and digested if deeply imbedded larvae are to be isolated and counted.

Equipment

* One or two trays of about 40 x 60 x 15 cm. The precise size is not important. Suitable plastic trays are easily procurable. Rectangular trays are easier to pour from
* Blunt knife
* Pair of scissors
* Two wide-mouthed plastic buckets with a capacity of at least 12 litres. These are used to collect and mix the contents of the different parts of the intestine before subsamples are taken. The inner walls of the buckets should be calibrated in litres
* 500 ml spoon for taking subsamples. It may be home-made by mounting a beaker with a handle - the beaker should contain exactly 500 ml when completely full
* Sieve of 100-220 μm. A nylon net or another mesh of

equivalent size may be mounted with appropriate sides

* Physiological saline, 0.9% (9 g of NaCl in 1 litre of tap water)

* Wash bottle

* Jet stream of water. It may be a water pistol mounted on a hose

* Tight 500 ml containers, some with a calibrated scale on the outside

* Petri dishes, about 9 cm in diameter

* Aqueous solution of iodine to stain the samples. A strong solution may for example be: 80 g I_2 and 400 g KI dissolved in distilled water to a total volume of 1 litre

* Aqueous solution of sodium thiosulphate to decolourize the faecal matter, while the worms remain stained. The solution could for example be 30%, i.e. 300 g sodium thiosulphate crystals dissolved in water to a total volume of 1 litre

* Light table/box. This may be a commercially available lamp or a home-made construction. In principle, an electric bulb is mounted inside a wide shallow box. The top of the box is made of translucent white plastic or ground glass. When samples in clear petri dishes are placed on top, the diffuse white light shining up through the petri dishes provides a strong contrast for the stained worms. Be careful to construct the light board so that it is to some degree waterproof

* Dissection microscope

* Needles or fine forceps to handle worms during counting

* Bucket for handling of waste water and one bucket for discarded organs may be useful

* 70% ethanol with 10% glycerol for eventual storage of isolated worms

* Small tubes (glass or plastic) with tight lids for eventual storage of isolated worms

Procedure

The handling of *Stomach contents* is illustrated in Fig. 4.1.

Separate the stomach from the oesophagus and the duodenum. If necessary, ligate the stomach from the duodenum with a string.

Place the stomach in a tray and open it along the greater curvature with scissors, so that all contents fall into the tray. Empty the tray into the 12-litre bucket.

Wash the empty stomach thoroughly with water, preferably a jet stream, carefully cleaning between the folds of the mucous membrane.

Pour the washing into the 12-litre bucket with stomach contents.

Scrape off the mucus of the stomach wall with a blunt knife.

Repeat the washing of the stomach wall one more time.

The mucus and the washing water is poured into the 12-litre bucket with the stomach contents.

Open the oesophagus and wash it twice. Pour the washing water into the stomach bucket with the stomach contents.

Total the volume of stomach contents in the bucket up to 10 litres with water.

Stir vigorously until all food materials, mucus and water are well mixed, and transfer one spoonful (500 ml, equal to 5% of the volume) to the 100-220 μm sieve.

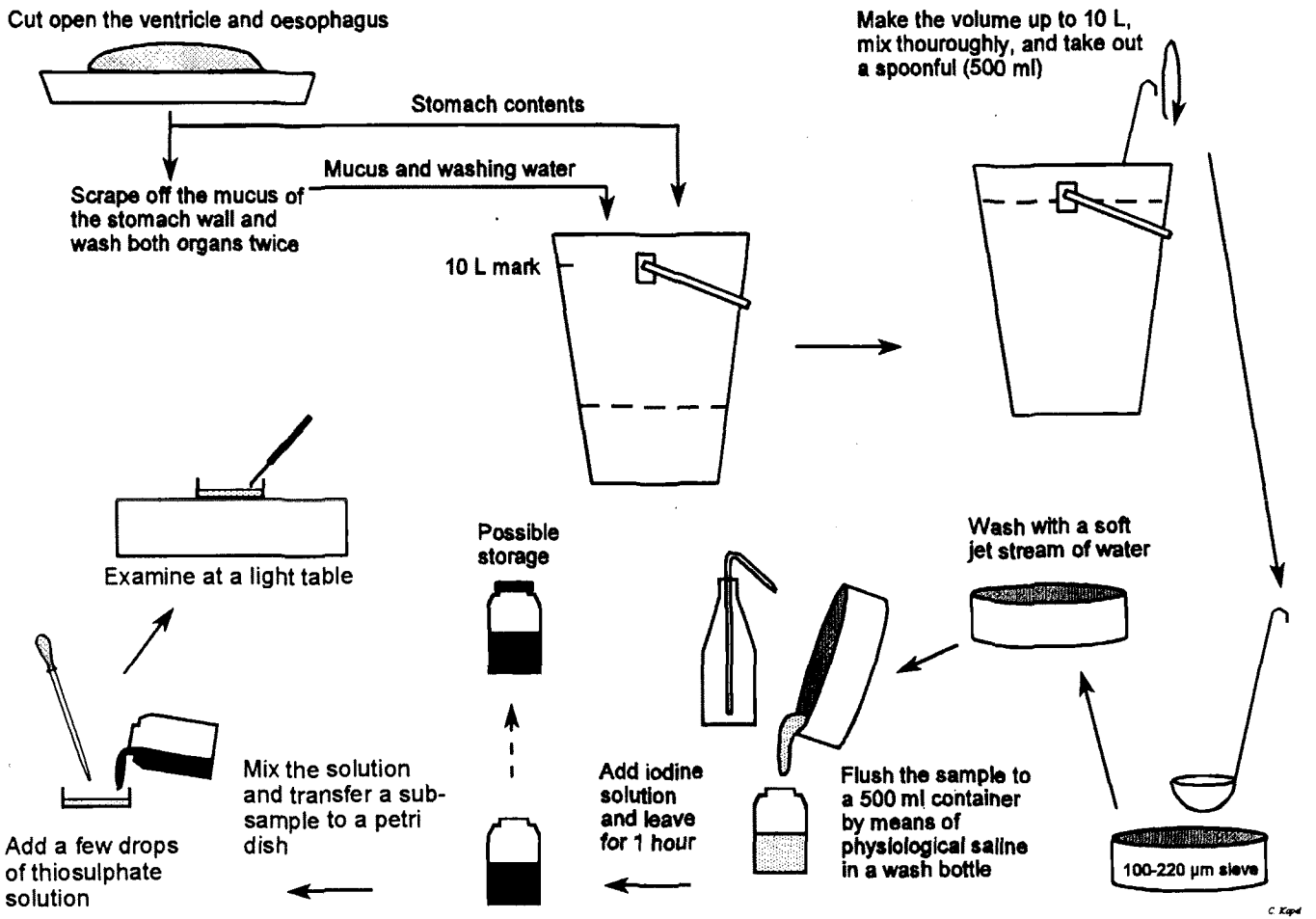


FIGURE 4.1 Handling of Stomach Contents

Wash the contents of the sieve well with a stream of water. It should not be a very hard jet stream, as some worms may be damaged.

Pour the sample from the sieve into a 500 ml container by means of physiological saline in the wash bottle. Be careful not to use instruments and not even fingers to remove the sample from the sieve, as the mesh is very susceptible to mechanical damage.

Repeat the last 3 steps one or more times if larger subsamples are needed. For example, 10% or 20% subsamples are obtained when 2 or 4 spoonfuls, respectively, are transferred to the sieve and washed.

Note: The best wash is obtained if the spoonfuls are sieved separately, and correctly washed samples are much easier to count than poorly washed samples.

Iodine solution is added to the sample in the container, and the stomach contents are stirred well in order to secure that all particles, including the worms, are stained adequately. The colour of the sample should be dark brown. For a limited period of time the iodine will work as a fixative. The sample should be allowed to stay in the iodine for at least one hour before proceeding to the next step. If the samples are stored for more than a few days, their colour should be checked and more iodine added, if necessary.

At counting, a little volume of the sample should be poured into a petri dish.

Add a few drops of sodium thiosulphate. This will decolourize the faecal particles, while all large worms and to a certain degree parasite larvae will remain dark. This decolouring process constitutes a delicate balance: If a large surplus of sodium thiosulphate is added and the parasites are small (many fourth-stage larvae), then the worms will decolourize rather quickly, and thus loose their contrast and 'disappear'. Therefore, the amount of sodium thiosulphate added should be just enough to decolourize the sample (i.e. no surplus), and

the sample should be counted immediately.

Count the number of each nematode species present in the petri dish placed on the light box, and repeat the two latter steps until the entire sample is counted. Needles or forceps are convenient tools for moving particles and worms around.

Note: The counting will be much safer if only small volumes of the sample are poured into the petri dish, and the counting will proceed faster, even if the number of petri dishes to be counted is increased.

In some cases it will be necessary to place the petri dish under a stereo microscope to identify the worms to species.

Finally, the total worm count of the sample should be multiplied by an appropriate factor to obtain the total worm burden in the stomach. For instance, when the water suspension of the stomach contents had a volume of 10 litres, and the subsample comprised 4 spoonfuls (each of 500 ml), then 2 litres out of 10 litres, equal to 20%, were counted, and thus the worm count of the subsample should be multiplied by 5 in order to obtain the total worm burden in the stomach.

4.3 THE STOMACH WALL

The isolation of helminth larvae from the stomach wall may be carried out by one of two alternative methods: *Incubation* of the stomach wall in physiological saline, or *Digestion* of the scraped-off mucosa in pepsin-HCl. If the appropriate laboratory facilities are available, the digestion method should be preferred. Each of these methods may be carried out in conjunction with the isolation of adult parasites from the stomach contents, and both can be used to determine the numbers of immatures and hence the ratio of immatures to adults. Furthermore, the number of inhibited larvae (*Hyostrongylus*) may be estimated precisely if the pig has been

isolated from reinfection for at least 3 weeks before slaughter, as this allows non-inhibited immatures to complete their development.

4.3.1 Incubation in physiological saline

Equipment

* One or two trays of about 40 x 60 x 15 cm. The precise size is not important. Suitable plastic trays are easily procurable. Rectangular trays are easier to pour from
* Sieve of 20-30 μm. A nylon net or another mesh of equivalent size may be mounted with appropriate sides
* Physiological saline, 0.9% (9 g of NaCl in 1 litre of water)
* Wash bottle
* Available jet stream of water. It may be a water pistol mounted on a hose
* Tight 500 ml containers, some with a calibrated scale on the outside
* Thermostat or a space with approx. 38°C
* Petri dishes, about 9 cm in diameter
* Microslides/coverslips
* Pasteur pipettes
* Aqueous solution of iodine to stain the samples. A strong solution may for example be: 80 g I_2 and 400 g KI dissolved in distilled water to a total volume of 1 litre
* Aqueous solution of sodium thiosulphate to decolourize the faecal matter, while the worms remain stained. The solution could for example be 30%, i.e. 300 g sodium thiosulphate crystals dissolved in water to a total volume of 1 litre

* Dissection microscope
* Microscope
* Needles or fine forceps to handle worms during counting
* 70% ethanol with 10% glycerol for storage of isolated worms, if necessary
* Small tubes (glass or plastic) with tight lids for eventual storage of isolated worms, if necessary

Procedure

The *Incubation of the Stomach Wall* is illustrated in Fig. 4.2.

The opened and washed stomach is placed with the mucosa downwards in a tray containing physiological saline. The saline should be lukewarm (38-40°C).

Incubate the stomach wall overnight at approx. 38°C.

Remove the stomach and wash it well with a jet stream of water.

Pour the incubation fluid and the washing water through a fine-meshed sieve or nylon net (20-30 μm), and wash the sieve/net with a jet stream of water.

Flush off the larvae from the sieve/nylon net into a container using the wash bottle, and total the volume up to 200 ml.

Add some iodine solution so that the whole sample is deeply stained, and allow the larvae to be coloured for at least one hour.

After thorough mixing, subsamples are transferred to petri dishes and decolourized with a few drops of sodium thiosulphate immediately before counting. To ensure that the larvae are easily visible, the sample in the petri dish should not be totally decoulorized, but a faint

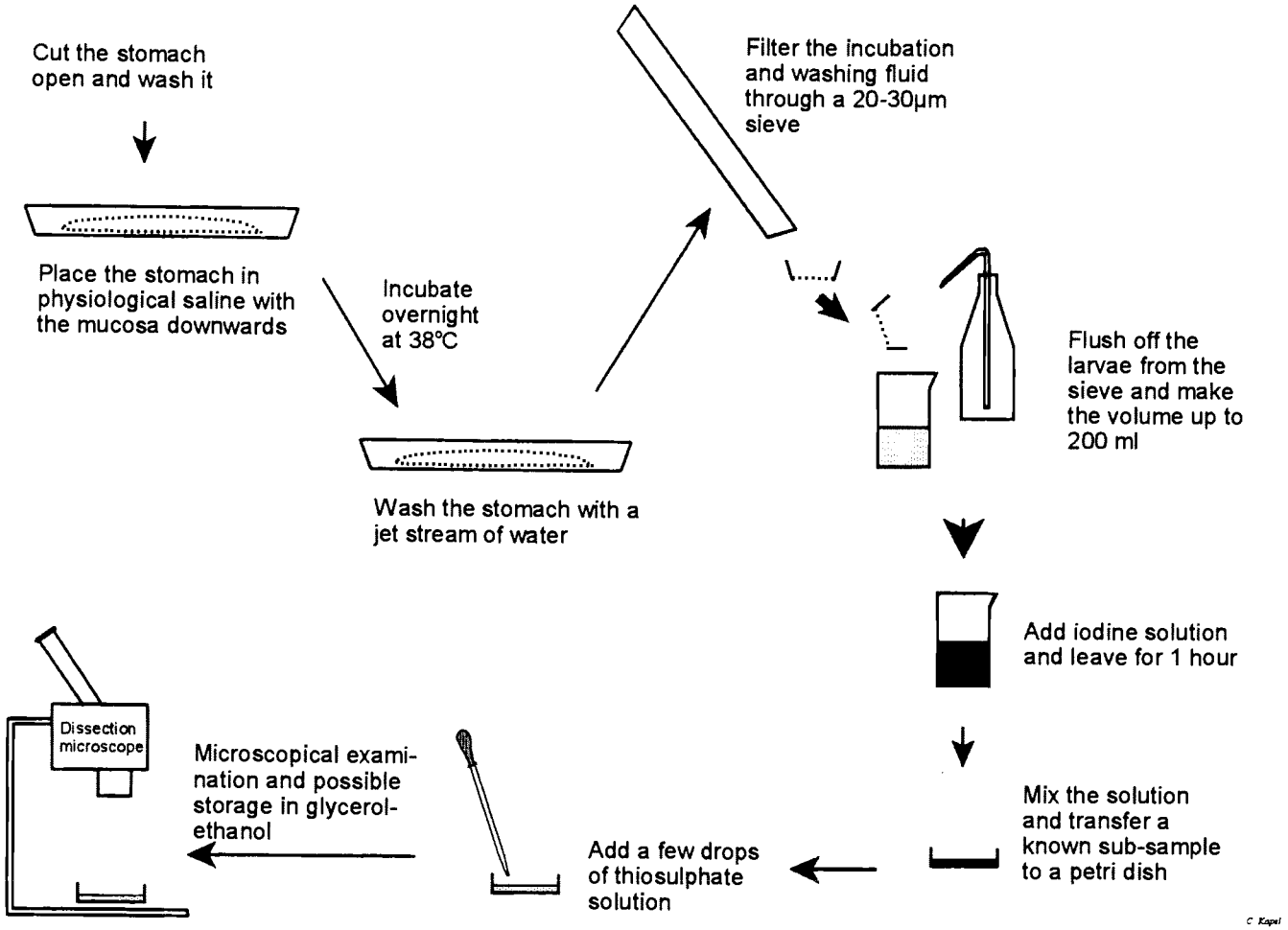


FIGURE 4.2 Incubation of the Stomach Wall

brown nuance should remain (see Section 4.13.2: *General comments on occupational hazards*).

It is necessary to place the petri dish under a stereo microscope during counting. Note again: The counting will be much safer if only small volumes of the sample are poured into the petri dishes.

To identify the parasite species, a certain number (e.g. 100-200) of randomly selected larvae should be transferred to microslides by means of a Pasteur pipette, and the larvae should be identified to species under the microscope.

Finally, the total worm count of the sample should be multiplied by an appropriate factor to obtain the total worm burden in the stomach.

If necessary, specimens may be stored in glycerol-ethanol for later observations.

4.3.2 Pepsin-HCl digestion

Equipment

* Blunt knife
* Pair of scissors
* Sieve of 20-30 μm. A nylon net or another mesh of equivalent size may be mounted with appropriate sides
* Freshly prepared Pepsin-HCl solution (10 g pepsin (3,000 i.u. per mg) dissolved in 10 ml concentrated hydrochloric acid and filled up to 1 litre with water (approx. pH = 2))
* Wash bottle
* Jet stream of water. It may be a water pistol mounted on a hose

* Tight 500 ml containers, some with a calibrated scale on the outside
* Stomacher apparatus with thermostat. Alternatively, regular mechanical stirring at approx. 38°C conditions
* Plastic bags for stomacher apparatus, if this equipment is available
* Thermostat or a space with approx. 38°C (not necessary if a heated stomacher is available)
* Petri dishes, about 9 cm in diameter
* Microslides/coverslips
* Pasteur pipettes
* Aqueous solution of iodine to stain the samples. A strong solution may for example be: 80 g I_2 and 400 g KI dissolved in distilled water to a total volume of 1 litre
* Aqueous solution of sodium thiosulphate to decolourize the faecal matter, while the worms remain stained. The solution could for example be 30%, i.e. 300 g sodium thiosulphate crystals dissolved in water to a total volume of 1 litre
* Dissection microscope
* Microscope
* Needles or fine forceps to handle worms during counting
* 70% ethanol with 10% glycerol for storage of isolated worms
* Small tubes (glass or plastic) with tight lids for storage of isolated worms

Procedure

The *Pepsin-HCl digestion* is illustrated in Fig. 4.3.

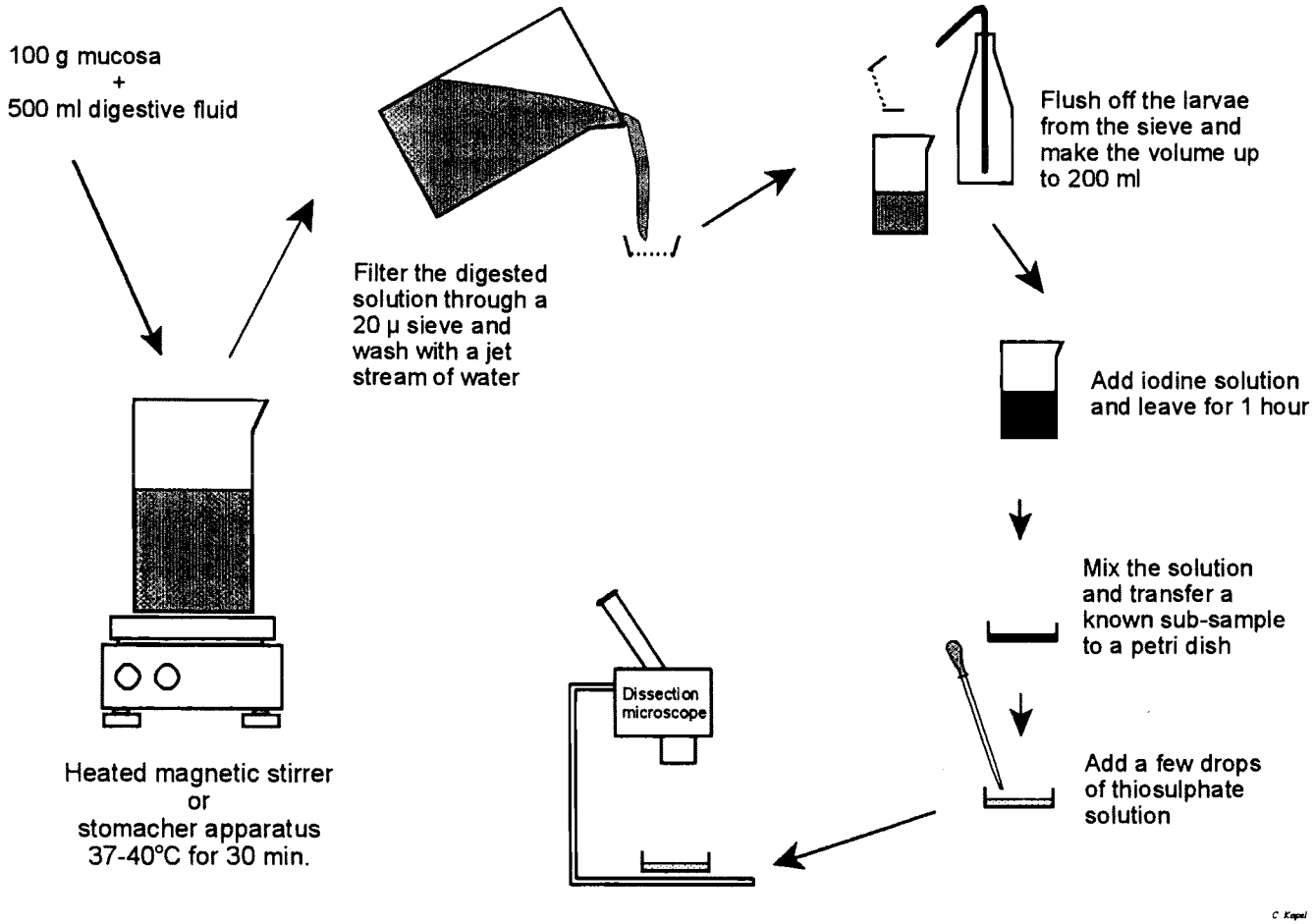


FIGURE 4.3 Pepsin-HCl digestion of the Stomach mucosa

The opened and washed stomach is divided into 4-6 pieces which are placed with the mucosa upwards, and the mucosa is then scraped off by means of a blunt knife.

The mucosa is transferred to a container/plastic bag containing pepsin-HCl (max. 100 g mucosa to 500 ml pepsin-HCl).

The mucosa is allowed to be digested at approx. 38°C until the digestion is completed. During digestion the suspension should be stirred regularly. This step may be carried out by means of a stomacher apparatus, if available, in which the sample is placed in a solid plastic bag which is kept warm and stirred during incubation. In a stomacher the mucosa will be completely digested in 30 minutes.

After total digestion of the mucosa, the digestion fluid is poured through a 20-30 μm sieve and washed with a jet stream of water.

Flush off the larvae from the sieve/nylon net into a container using the wash bottle, and total the volume up to 200 ml.

Add some iodine solution, so that the whole sample is deeply stained, and allow the larvae to be coloured for at least one hour.

After thorough mixing, subsamples are transferred to petri dishes and decolourized with a few drops of sodium thiosulphate immediately before counting. To ensure that the larvae are easily visible, the sample in the petri dish should not be totally decolourized, but a faint brown nuance should remain (see Section 4.13.2: *General comments on occupational hazards*).

Finally, the larvae should be counted under a stereo microscope, and identified to species under a microscope. The total worm burden should be calculated.

4.4 THE CONTENTS OF THE SMALL INTESTINE

The principles and application of the methods for differential parasite counts of the small intestine are largely the same as those for parasite counts of the contents of the stomach. There is no strong need for incubation or digestion of the intestinal wall. The parasites of the small intestine vary considerably, from *Macracanthorhynchus hirudinaceus* and *Ascaris suum*, measuring up to 65 cm and 40 cm, respectively, to small, very slender parasites such as *Strongyloides ransomi* and adult *Trichinella spp.*, measuring 10 mm and 1-3 mm, respectively.

Equipment

* The same equipment as mentioned under 'contents of the stomach'
* Mucosa scraper, i.e. two smooth pieces of wood/bamboo (approx. 10 cm long) held together at one end by a strong rubber band
* Large-meshed sieve (500-1000 μm), a common household sieve may be used

Procedure

The handling of the *Small Intestinal Contents* is illustrated in Fig. 4.4.

Ligate the small intestine at pylorus and close to caecum if necessary, and cut the small intestine free. Remove fatty tissue from the outer surface of the small intestine (this will facilitate mucosa scraping, see below).

Open the small intestine so that all its contents fall into a tray.

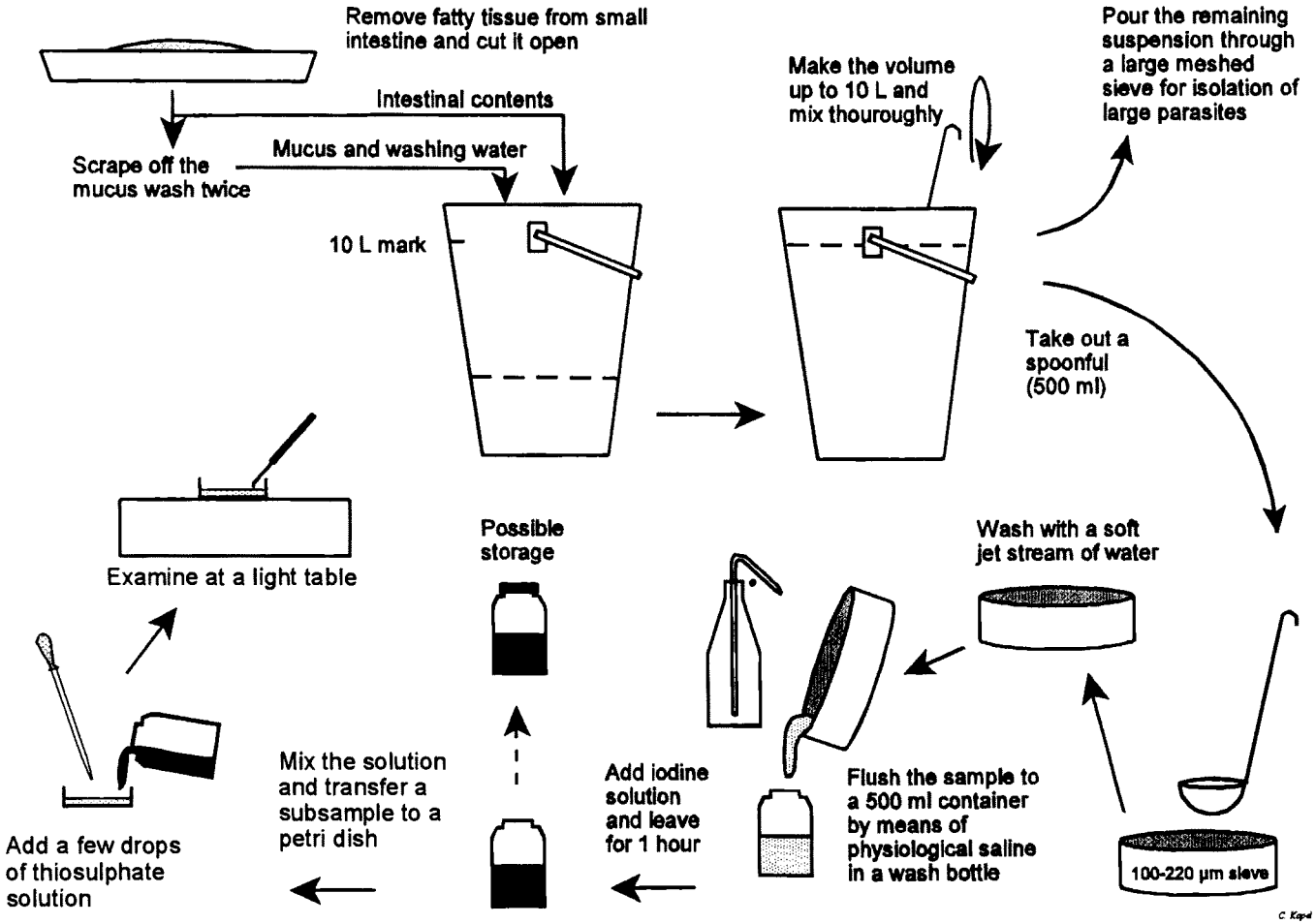


FIGURE 4.4 Handling of the Small Intestinal Contents

It is important to scrape off the mucous membrane in some manner in order to recover the smallest parasites, e.g. *Strongyloides* and adult *Trichinella*. This may be done by pulling the opened intestinal wall through the two pieces of wood of the mucosa scraper. This procedure should be repeated once.

The procedure for washing, subsampling, staining and counting is just like that previously described for parasites in the stomach contents, with the exception of counts for *Ascaris* and *Macracanthorhynchus*.

All contents, and not merely a subsample, should be examined for *Ascaris, Macracanthorhynchus*, and *Fasciolopsis,* as these large parasites are usually present in rather low numbers. Therefore, after subsampling from the bucket, a special 'large parasite examination' may easily be carried out by washing the remainder of the sample in the bucket through a large-meshed sieve by means of a strong jet stream of water.

Pour the contents of the sieve into a tray with some water. The large worms are then easily recognized in the water.

4.5 THE CONTENTS OF THE LARGE INTESTINE

The principles and application of the methods for differential parasite counts of the large intestine are largely the same as those for parasite counts of the contents of the stomach and incubation of the stomach wall. The large intestinal walls should not be digested with pepsin-HCl, as *Oesophagostomum* larvae will also be digested, and therefore cannot be found. Lumen-dwelling parasites of the large intestine vary in size from 3-4 mm (immature *Oesophagostomum* sp.) to 4-5 cm (adult *Trichuris suis*).

Equipment

* The same equipment as previously mentioned under *Contents of the Stomach* (Section 4.2)

Procedure

The same procedure as previously mentioned under *Contents of the Stomach* (Section 4.2 and Fig. 4.1).

4.6 THE LARGE INTESTINAL WALL

The large intestinal wall may be infected with tissue stages of *Oesophagostomum* spp. (L_3 and L_4). Furthermore, mature and especially immature *Trichuris suis* may be so firmly attached to the mucosa that they are difficult to wash off the mucosa with water. These worms may be isolated by incubation of the large intestinal wall in physiological saline at 38°C overnight, as mentioned for the stomach wall.

Note: the pepsin-HCl digestion procedure is not applicable to the large intestinal wall, as many of the worms will be digested together with the mucosa.

Equipment

* The same equipment as previously mentioned under *Incubation of the Stomach Wall*
* An extra bucket for each intestine
* Small lengths of s-shaped non-corrosive steel wire

Procedure

The *Incubation of the Large Intestinal Wall* is illustrated in Fig. 4.5.

The same procedure as previously mentioned under *Incubation of the Stomach Wall* is used, except that the intestinal wall may most easily be incubated in a bucket in which the intestinal wall is held in position by small lengths of steel wire.

4.7 THE LIVER AND OMENTUM

Many different parasitic infections may cause similar reactions in the liver. Thus, migrating larvae of *Ascaris suum* and other ascarids (e.g. *Toxocara* sp.), immature *Fasciola hepatica,* and eggs of *Schistosoma japonicum* may all cause accumulation of lymphocytes and increased amount of connective tissue, i.e. 'white spots' or 'milk spots'. When 'white spots' are found in the liver, they indicate a recent passage or entrapment of parasites in the liver. Enlarged, thick-walled bile ducts may indicate presence of *Fasciola hepatica* or *Dicrocoelium dendriticum*.

The liver and the omentum should be examined for the presence of metacestodes (e.g. *Taenia hydatigena* up to 8 cm, *Echinococcus granulosus* up to 20 cm or more), which may be clearly visible as bladders or pronounced cysts on the surface of the liver, embedded in the liver tissue, or in the omentum or in other internal organs. Care should be taken to discriminate between white spots of the lymphonodular type in the liver and small *Taenia hydatigena* cysts, and in cases of doubt, microscopical examination may be necessary to reveal the scolex.

When examining for the presence of liver flukes (*Fasciola hepatica* up to 35 mm, *Dicrocoelium dendriticum* up to 10 mm), the following method can be followed.

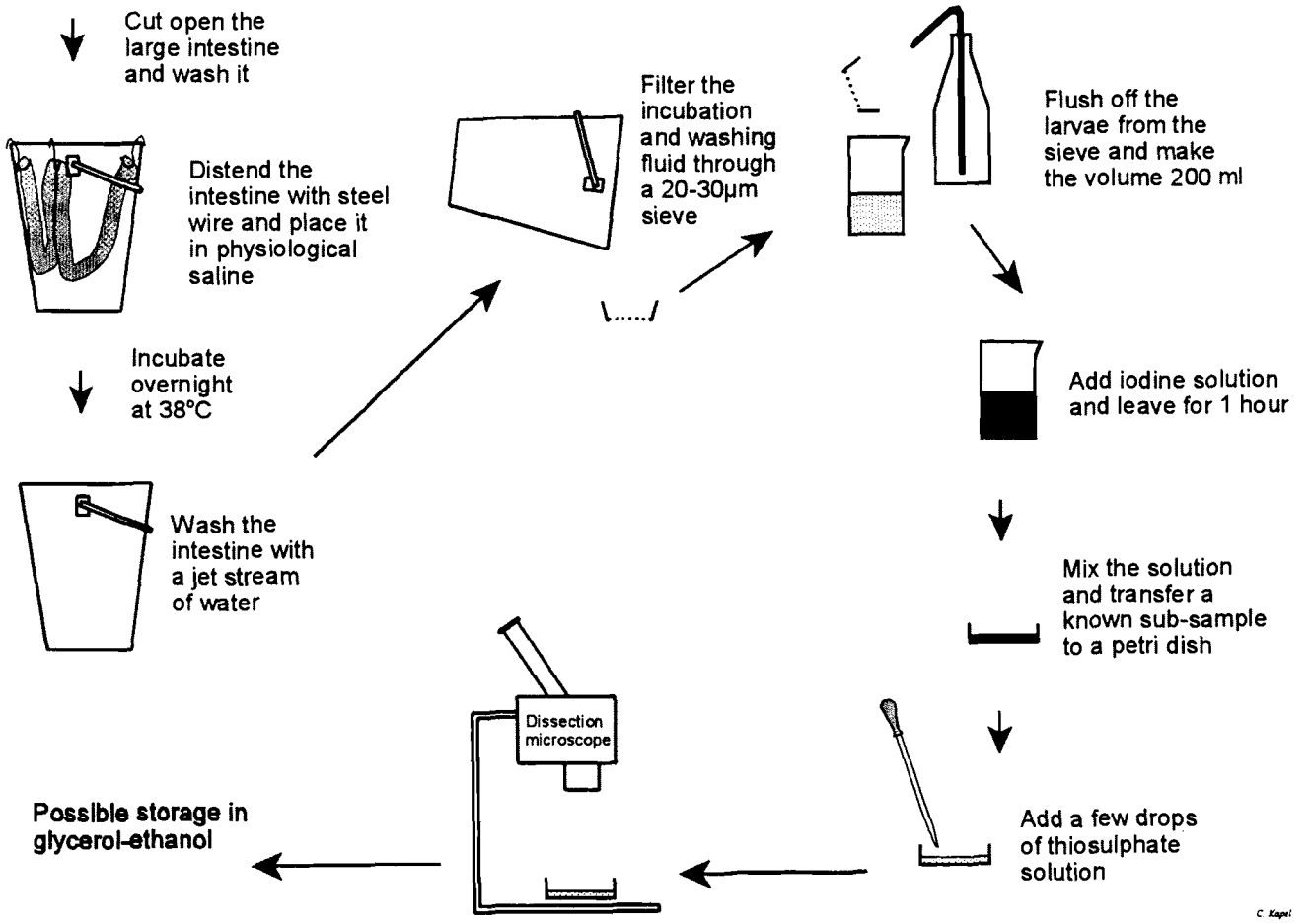


FIGURE 4.5 Incubation of the Large Intestinal Wall

Equipment

* Sharp knife
* Tray of about 40 x 60 x 15 cm or smaller. The precise size is not important. Suitable plastic trays are easily procurable. Rectangular trays are easier to pour from
* Physiological saline, 0.9% (9 g of NaCl in 1 litre water)
* Sieve of 100-220 μm. A nylon net or another mesh of equivalent size may be mounted with appropriate sides
* Shallow tray or a few large petri dishes
* Petri dishes, about 9 cm in diameter
* Dissection microscope
* 70% ethanol with 10% glycerol for storage of isolated worms

Procedure

The handling of *the Liver* is illustrated in Fig. 4.6.

The liver and omentum are examined macroscopically for metacestodes (see Fig. 4.7).

The liver is examined for superficially visible white spots (see Fig. 4.7).

Make three cuts in the liver (see illustration) and examine the exposed tissue and bile ducts.

Transfer the liver into a tray with some litres of physiological saline and squeeze and tear the liver manually (press the soft liver tissue

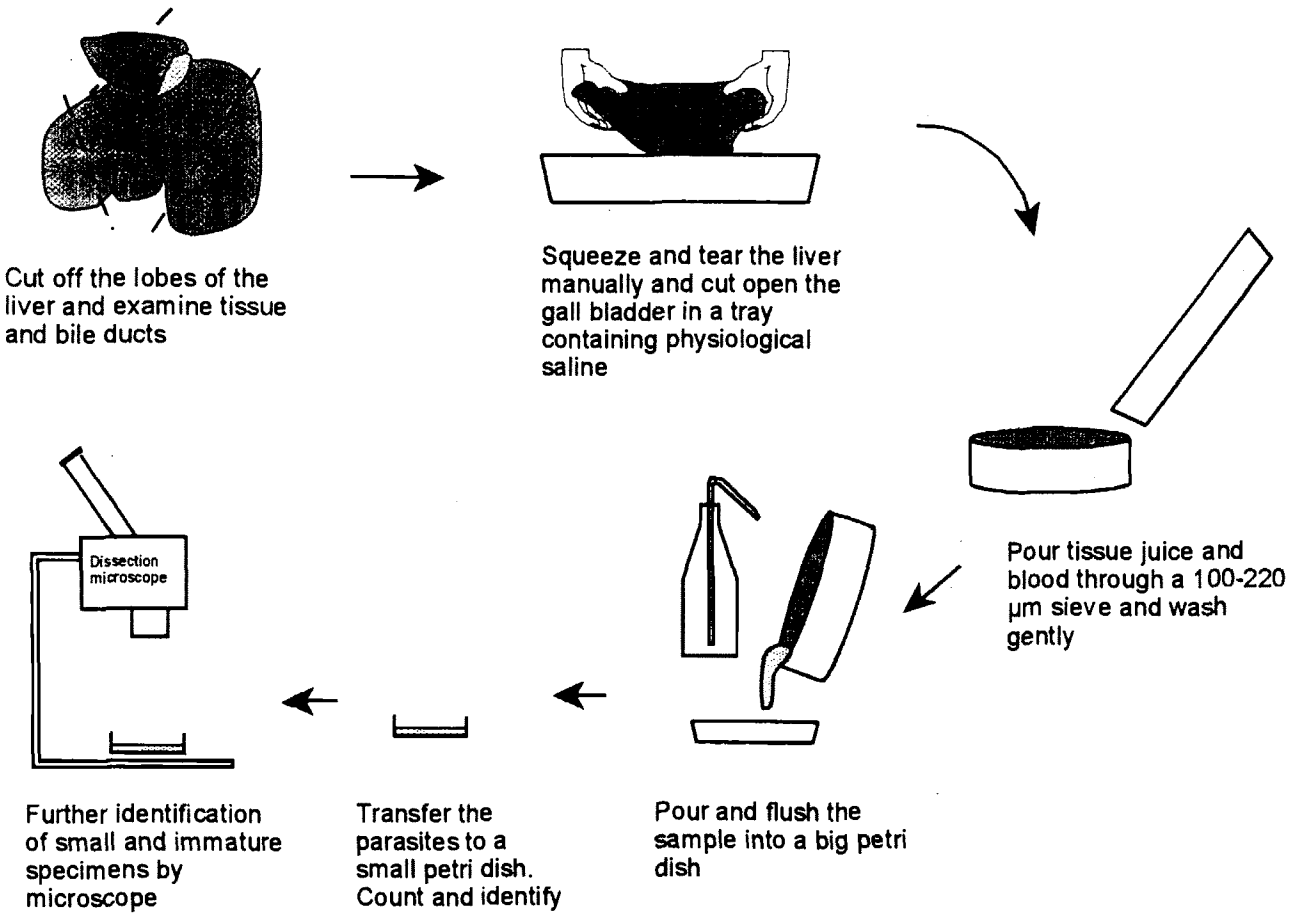


FIGURE 4.6 Handling the Liver

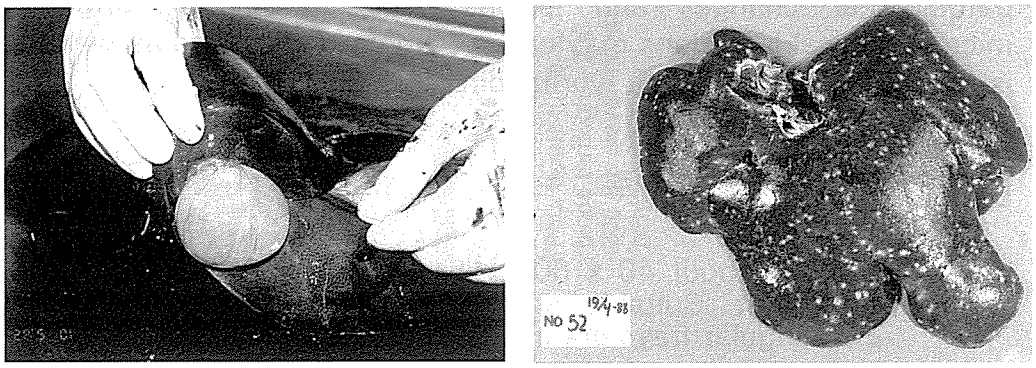


FIGURE 4.7 Liver with a big cyst of *Taenia hydatigena* (~*Cysticercus tenuicollis*, left) and liver with numerous 'white spots' or 'milk spots', caused by recently migrating *Ascaris suum* larvae (right)

between the fingers until all pieces are max. 10 mm in size) in order to get the flukes out of the bile ducts. Cut the gall bladder open.

Tissue juice and blood are poured through a 100-220 μm sieve and washed with a soft stream of water. The contents are poured into a tray and examined directly.

The washed sample is poured into a tray or a large petri dish, and parasites are transferred to a small petri dish, identified and counted. Microscopical examination may be necessary when immature parasites are to be found.

4.8 THE LUNGS

The lungs may be infected with several species of long slender lungworms, *Metastrongylus* spp. (up to 6 cm long), located in the

bronchi and the bronchioles. These worms may cause gross pathological changes and lesions, but as several different microbiological infections may cause pathological lesions as well, it is necessary to isolate the worms directly.

Equipment

* Tray of about 40 x 60 x 15 cm. The precise size is not important. Suitable plastic trays are easily procurable. Rectangular trays are easier to pour from
* Pair of scissors, which should be pointed and sharp
* Physiological saline, 0.9% (9 g of NaCl in 1 litre of water)
* Wash bottle
* Needles and fine forceps to handle worms during preparation and counting
* Sieve of 200-300 μm. A nylon net or another mesh of equivalent size may be mounted with appropriate sides
* Petri dishes about 9 cm in diameter
* Slides and coverslips
* Microscope

Procedure

The handling of *The Lungs* is illustrated in Fig. 4.8.

The bronchi and the bronchioles are opened carefully with a pair of fine scissors, and the lumen is washed with saline (washing water is collected in the tray). Special observations are made on the presence of mucus, fluid, bubbles and other signs of infection.

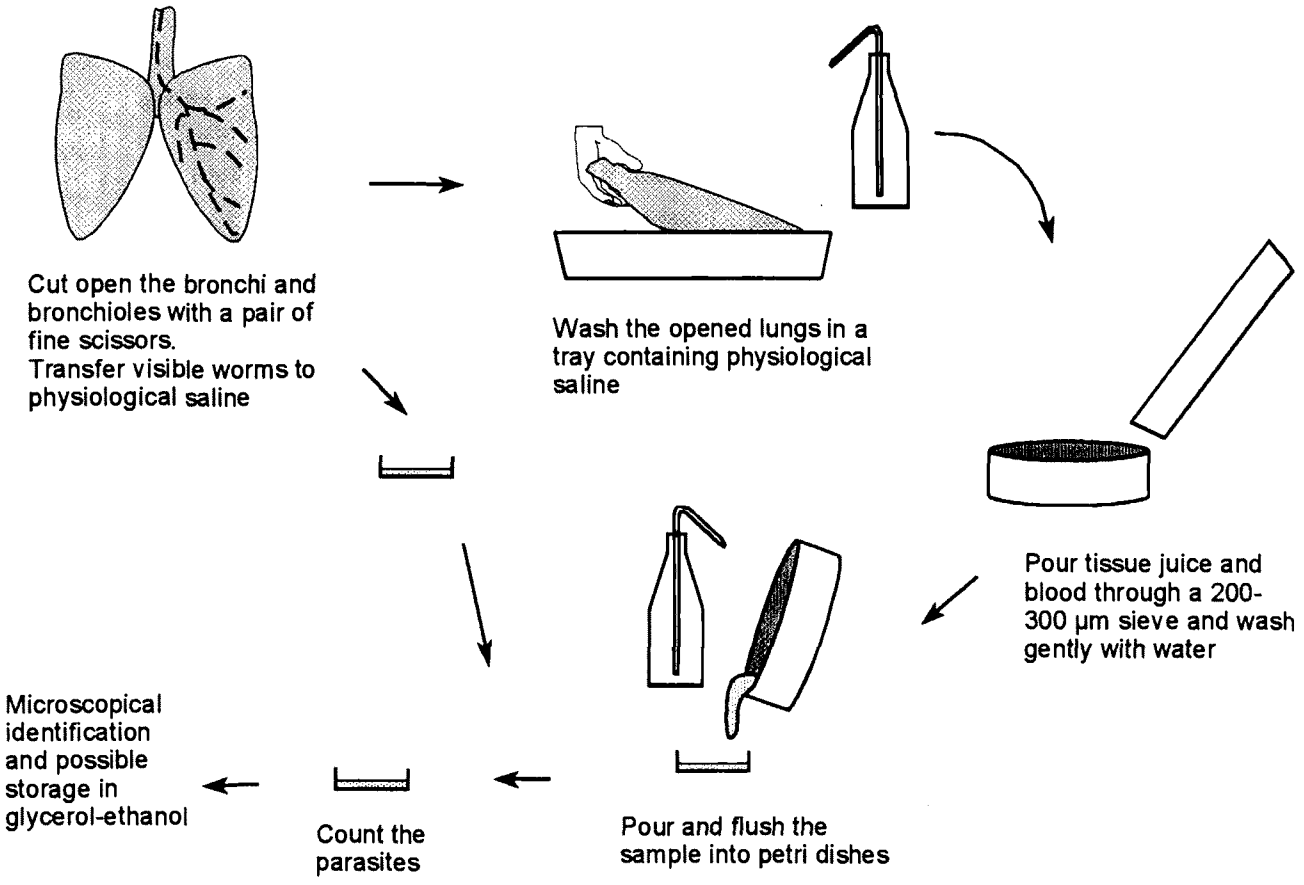


FIGURE 4.8 Handling of the Lungs

Macroscopically visible lungworms are transferred directly to a petri dish with physiological saline.

The lungs, with opened bronchi and bronchioles, are washed with physiological saline.

Tissue juice, blood and saline are poured through a 200-300 μm sieve and washed with water (as the worms are very susceptible to mechanical damage, the procedure should be rather gentle).

The contents of the sieve are flushed off with physiological saline into petri dishes, and the parasites are counted.

Identification to species level is carried out by placing male specimens on some slides, with coverslips on top, and examining the spicules under a microscope.

Isolated specimens may be preserved in 70% ethanol (9 parts) with glycerol (1 part).

4.9 THE KIDNEYS

The kidneys and the perirenal fatty tissue may be infected with *Stephanurus dentatus*, which is a stout worm, approx. 45 mm long. The examination includes general observations on macroscopic pathological changes, followed by direct search for macroscopically visible worms.

Equipment

* Sharp knife
* Physiological saline, 0.9% (9 g of NaCl in 1 litre of water)
* Wash bottle

* Needles and fine forceps to handle worms during preparation and counting

* Petri dishes, about 9 cm in diameter

* Dissection microscope

Procedure

The perirenal tissue is examined, and the kidneys are opened with a knife. Special observations are made on the presence of pathological lesions and worms embedded in capsules which may contain greenish pus.

Any worms are freed with saline water in a wash bottle and fine forceps.

The parasites are transferred to a petri dish with physiological saline, identified and counted.

4.10 THE MUSCLES

Muscle-dwelling larvae of *Trichinella* may be found in many striated muscles all over the body, but the number of larvae per gram, and thus the possibility of finding the larvae, varies considerably from muscle to muscle. The predilection sites of the larvae, i.e. the most heavily infected muscles in the pig, are *musculus masseter* and *diaphragm*, which is rather convenient, as the commercial value of these muscles is low.

The traditional way of analyzing meat for trichinae is to use a compressorium. This method is easy to perform, as well as inexpensive, but it is commonly replaced by a pepsin-HCl digestion method, which has a much higher sensitivity. Both methods will be mentioned below, while the ELISA method for detecting circulating

antibodies is omitted, as it is more expensive and has higher technological demands.

4.10.1 The compression method

This method is very simple and has been used for many years for routine screening of pork. It is also a very inexpensive method, because the principle is to firmly press a little piece of meat between two thick pieces of glass mounted with screws (a compressorium), and then to examine for muscle larvae directly under a dissection microscope. Unfortunately, this method has a rather low sensitivity (the detection limit is approx. 3-5 larvae per gram), and the recovery rate is to a large degree dependent on highly skilled technicians.

Equipment

* Pair of scissors for cutting the meat into small pieces
* Compressorium
* Dissection microscope

Procedure

The *Compression Method* is illustrated in Fig. 4.9.

One gram of *diaphragm* or *m. masseter* is cut into small pieces.

After being placed between the glass plates of the compressorium, the plates are screwed tightly together, so the meat is pressed into a thin, transparent layer.

The compressorium is examined directly in a dissection microscope.

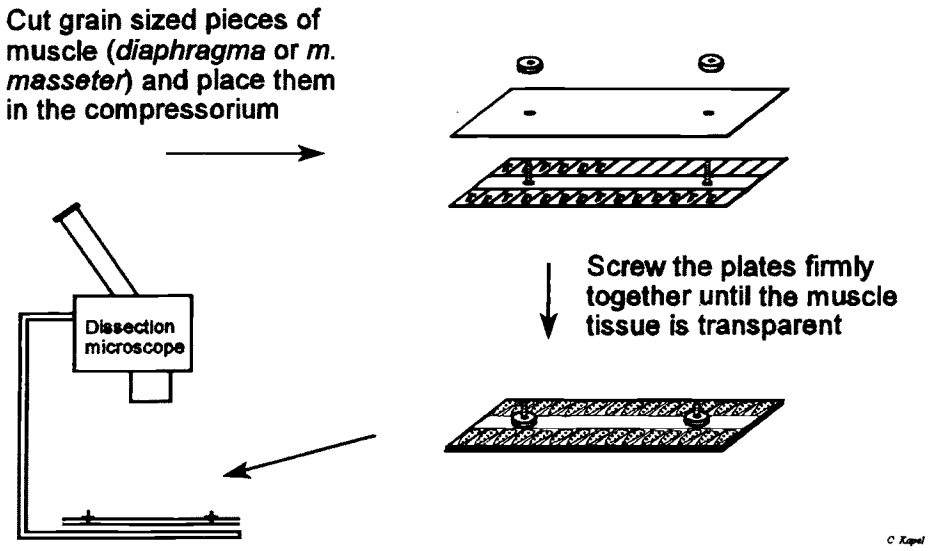


FIGURE 4.9 The Compression Method

4.10.2 The pepsin-HCl digestion method

Muscle larvae of trichinae are easy to isolate by removing the surrounding host tissue by means of pepsin-HCl. When digestion succeeds perfectly, only very few muscle fibres are left, and the stained larvae are clearly visible and easy to enumerate. Therefore, this method has a rather high sensitivity. As trichinae may cause serious zoonotic infections and therefore have impact on human health, it is often recommended to use a detection limit of 0.1 larvae per gram of meat, i.e. 10 grams of muscle tissue is examined.

Equipment

* Pair of scissors for cutting the meat into small pieces

* Freshly prepared Pepsin-HCl solution (30 g pepsin (3,000 i.u. per mg) dissolved in 10 ml concentrated hydrochloric acid and filled up to 1 litre with water (approx. pH = 2))

* Stomacher apparatus with thermostat. Alternatively, regular mechanical stirring at approx. 38°C conditions

* Plastic bags for stomacher apparatus, if this equipment is available

* Sieve of 400-500 μm and one sieve of 20-30 μm. Nylon nets or other meshes of equivalent sizes may be mounted with appropriate sides

* Jet stream of water. It may be a water pistol mounted on a hose. Alternatively, a vacuum pump and vacuum devices may be used to get the digested fluid through the sieves (see Fig. 4.10)

* 2000 ml conical container

* Aqueous solution of iodine to stain the samples. A strong solution may for example be: 20 g I_2 and 40 g KI dissolved in distilled water to a total volume of 1 litre

* Aqueous solution of sodium thiosulphate to decolourize the other matter, while the larvae remain stained. The solution could for example be 30%, i.e. 300 g sodium thiosulphate crystals dissolved in water to a total volume of 1 litre

* Petri dishes, about 9 cm in diameter

* Dissection microscope

Procedure

The *Pepsin-HCl digestion* is illustrated in Fig. 4.10.

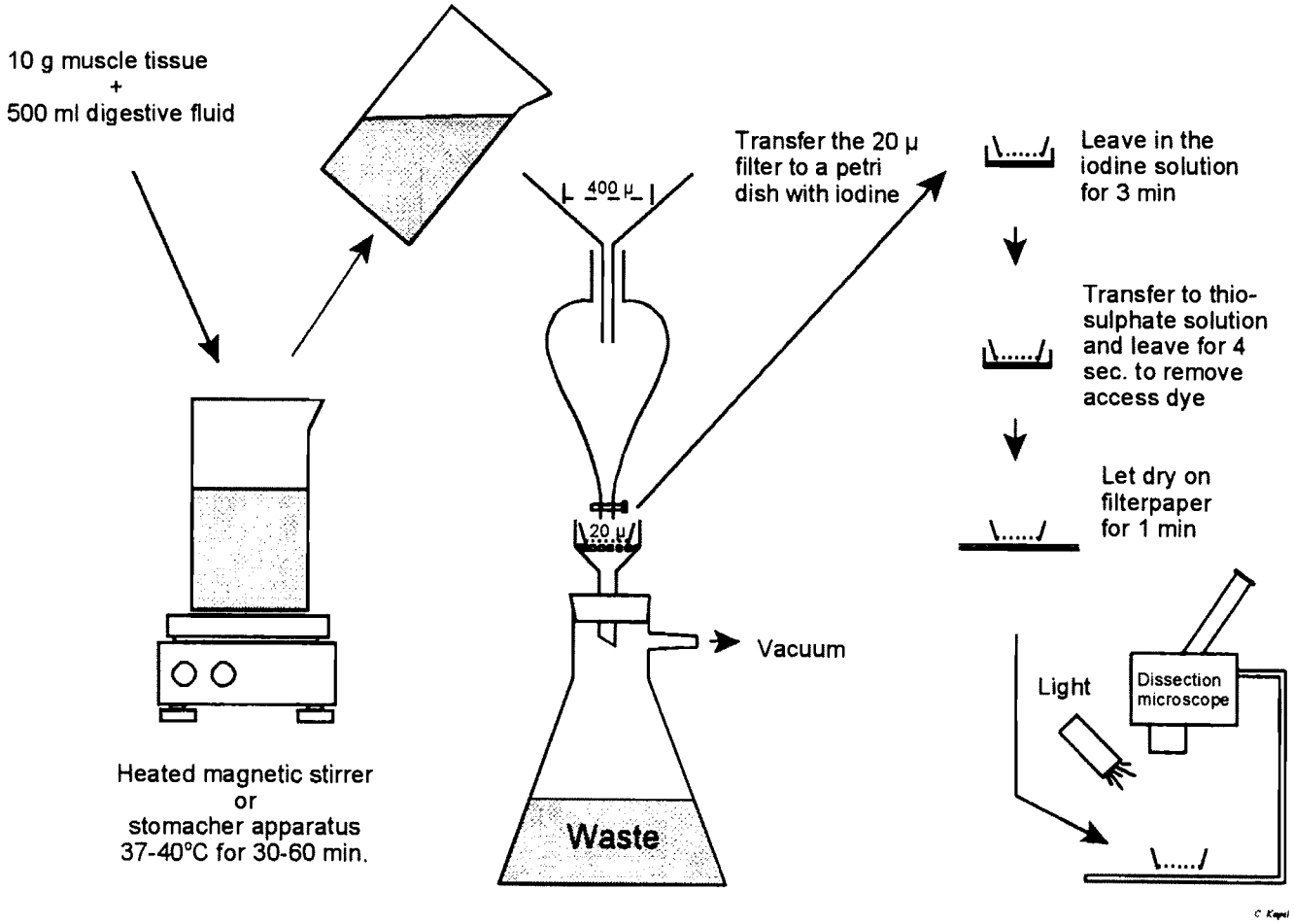


FIGURE 4.10 Pepsin HCl-digestion of muscle

Ten grams of *diaphragma* or *m. masseter* are cut into small pieces.

The muscle tissue is transferred to a beaker/plastic bag containing pepsin-HCl (10 g muscle to 500 ml pepsin-HCl solution).

The meat is allowed to be digested at 37°C until the digestion is completed (approx. ½ hour). During digestion, the suspension should be stirred regularly. This step may be carried out by means of a stomacher apparatus, if available, in which the sample is placed in a solid plastic bag which is continuously heated and stirred during incubation.

After total digestion of the muscle tissue, the digestion fluid is poured through a 400-500 μm sieve and then through a 20-30 μm sieve. The digestion fluid is forced through the fine sieve by vacuum.

Place the fine sieve in the iodine solution, so that the whole sample is deeply stained, and allow the larvae to be coloured for 3 minutes (4 min. for frozen muscle tissue).

Next, place the fine sieve in sodium thiosulphate for 4 sec. to decolourize any other tissue than the larvae.

Finally, the larvae are counted under a stereo microscope.

Note: Care should be taken to count only trichinae. Larvae of migrating nematodes may occasionally be found in muscle tissue, and these larvae should not be counted as trichinae. Therefore, the larvae should be viewed at a higher magnification if identification is doubtful.

4.11 IDENTIFICATION OF HELMINTHS

Table 4.1 provides simple descriptions of the most common gastro-intestinal helminths of pigs, and Table 4.2 describes the helminths

found outside the gastro-intestinal tract. These tables do not represent any elaborate identification key for porcine helminth species and not even genera, as this is beyond the scope of the present guidelines, but the tables are intended merely as an overview of the most commonly found genera and species. For exact identification of nematodes to genus, the reader may consult the *CIH Keys to the Nematode Parasites of Vertebrates* (Anderson, Chabaud and Willmott, 1974-1983).

4.12 INTERPRETATION OF WORM COUNTS

It is difficult to present a guideline for the interpretation of worm counts, as a complexity of factors concerning the intrinsic properties of the helminth species (pathogenicity to pigs as well as man), the specific host-parasite relationship (transmission route; acquired immunity - and thus age group of pigs), and the management system (e.g. risk of transmission; use of anthelmintics) should be considered before classifying a worm burden as being light or heavy.

4.12.1 The helminth species

For some highly pathogenic helminth species, a single specimen is one too many, as they may constitute a hazard to public health. This applies to the most deleterious zoonotic species, such as *Taenia solium* and *Echinococcus* sp., and *Trichinella spiralis*.

For non-zoonotic helminths, the primary effect is exaggerated in the pig, and here a fixed number of e.g. 50-100 specimens of liver flukes (*Fasciola hepatica*, *Dicrocoelium dendriticum*), lungworms (*Metastrongylus* spp.), or adult *Ascaris suum* or *Macracanthorhynchus hirudinaceus* may cause so much harm that they constitute an unacceptably high infection, whereas a similar number of less pathogenic species (e.g. *Oesophagostomum* spp., *Hyostrongylus rubidus* and the spiruroids) is normally regarded as a light infection.

But even within the same species, one should distinguish between stages; e.g. 200 small immatures of *Ascaris suum* in the small intestine may not be alarming, as the large majority of these are expected to be expelled rapidly, while the same number of fully grown adults may severely affect the growth rate and may indicate that the regulation of the parasite population is out of function.

TABLE 4.1 Characteristics of gastro-intestinal helminth species in pigs (to be continued)

Organ	Helminth	Max. Length	Appearance	Characteristics of the parasite
Oeso-phagus	*Gongylonema pulchrum*	15 cm	long, slender worms (embedded in the mucosa)	longitudinal rows of cuticular plaques in the anterior body region
Stomach	*Trichostrongylus axei*	7 mm	hairlike	no buccal capsule, distinct excretory notch in oesophageal region
	Hyostrongylus rubidus	8 mm	small, slender worms	reddish, small cephalic vesicle
	Ollulanus tricuspis	1 mm	very tiny worms	the head is spiral coiled (microscopical examination)
	Ascarops strongylina	22 mm	small, slender worms	single cervical ala on the left side, pharynx with 2-3 spiral chitinous thickenings
	Physocephalus sexalatus	20 mm	small, slender worms	3 pairs of cervical alae, pharynx with 1 spiral chitinous thickening
	Simondsia paradoxa		small, slender ♂ (free in lumen). Gravid ♀ with sack-formed posterior end (in mucosa crypts)	2 cervical alae, a dorsal and a ventral tooth in buccal cavity
	Gnathostoma hispidum *G .doloresi*	4 cm	thick-bodied worms in gastric nodules	swollen anterior ends with rows of small hooks
	Gongylonema pulchrum	9 cm	long, slender worms (embedded in the mucosa)	longitudinal rows of cuticular plaques in the anterior body region

TABLE 4.1 Continued

Organ	Helminth	Max. Length	Appearance	Characteristics of the parasite
Small Intestine	*Trichostrongylus colubriformis* *T.vitrinus*	7 mm	hairlike	no buccal capsule, distinct excretory notch in oesophageal region
	Globocephalus urosubulatus *G.longemucronatus*	9 mm	small, robust worms	spherical buccal capsule without leaf crown, ♀-tail with terminal spine
	Strongyloides ransomi	10 mm	hairlike	only ♀♀, blunt tail, long oesophagus
	Ascaris suum	40 cm	long, stout, white worms	may be confused only with *Macracanthorhynchus* (see below)
	Trichinella spiralis	3 mm	tiny worms, rarely found as they are short-lived	long oesophagus, ♂: 2 cloacal flaps, but no spicule, ♀: larvae in uterus
	Fasciolopsis buski	40 mm	large, thick bodied flukes 10 mm width	intestinal caeca without side branching
	Macracanthorhynchus hirudinaceus	65 cm	large, stout, white worms	the spiny proboscis is protruded when placed in water
Large Intestine	*Oesophagostomum dentatum*[1] *O.quadrispinulatum*[2] *O.brevicaudum*	15 mm	small, stout, white worms	small buccal capsule, inflated cuticular cephalic vesicle
	Trichuris suis	6 cm	whip-like, white worms (the anterior end embedded in the mucosa)	long, filamentous anterior end, thick posterior end

1: *O.granatensis* and *O.georgianum* are regarded as variant forms of *O.dentatum*

2: *O.longicaudum* is synonymous with *O.quadrispinulatum*

TABLE 4.2 Characteristics of non-gastrointestinal helminths in pigs (to be continued)

Organ	Helminth	Max. Length	Appearance	Characteristics of the parasite
Liver	*Taenia hydatigena* (cysticercs)[1]	8 cm	small, thin walled, white cysts → large, fluid-filled bladders	the single scolex may be recognized after dissection
	Echinococcus granulosus E.multilocularis (hydatids)	20 cm	large, thick walled, white hydatids	numerous scolices in each hydatid
	Dicrocoelium dendriticum	10 mm	lanceolate, semi-transparent flukes	uterus filled with dark brown eggs, testes in the anterior part of the body
	Fasciola hepatica	35 mm	grey-brownish, 10 mm width	immatures: lanceolated, in parenchyma adults: leaf-shaped, conical anterior end with distinct shoulders, intestinal caeca with numerous branches
	Opisthorchis noverca	8 mm	small, lanceolate flukes	grossly resembling *D. dendriticum*, the suckers are smaller, the eggs are light brown, and testes are located in the posteriorly part of the body
	Ascarid migrating larvae	1 mm	small, robust larvae milk spots of varying appearance	Larvae: no characteristics Milk spots: non-specific host response
	Schistosoma spp. (eggs)		milk spots of varying appearance	non-specific host response

TABLE 4.2 Continued

Organ	Helminth	Max. Length	Appearance	Characteristics of the parasite
Lungs	*Metastrongylus apri*[2] *M.salmi* *M.pudendotectus*	6 cm	long, slender, white worms	the host, the site and the long slender form are sufficient for generic identification
Blood system	*Schistosoma japonicum* *S.mansoni* *S.incognitum*	2 cm	males and females in permanent copulation	♂: broad, flat with a gynaecophoric canal, carrying the slender ♀
Kidneys	*Stephanurus dentatus*	45 mm	large, stout worms	prominent buccal capsule, transparent cuticle
Connect. tissue	*Taenia hydatigena* (cysticercs)[1]	8 cm	small, white cysts – large, fluid-filled bladders	the single scolex may be recognized after dissection
Muscles	*Trichinella spiralis* (larvae)	1 mm	L1 encapsulated in striated muscles, old capsules may be calcified	the coiled, encapsulated larvae within striated muscles are sufficient for generic identification
	Taenia solium (cysticercs)[3]	10 mm	elongated, white cysts	within the cyst is a scolex with 4 suckers and a rostellum bearing 2 concentric rows of hooks

1: The cysticerc of *T.hydatigena* may be called *Cysticercus tenuicollis*
2: *M.elongatus* is synonymous with *M.apri*
3: The cysticerc of *T.solium* may be called *Cysticercus cellulosae*

4.12.2 Specific host-parasite relationships

Most helminths have a characteristic infection pattern related to the age of their hosts. This pattern may be influenced by the risk of being infected, but more often the immunogenicity is the most crucial factor. Thus, for example the highly immunogenic *Strongyloides ransomi*, which is transmitted to newborn piglets via colostrum, normally has the highest intensity of infection in very young pigs. Growing pigs, 3-6 months of age, may be the age group most heavily infected with *Ascaris suum* and *Trichuris suis*, as these parasites are highly immunogenic, while they are not transmitted so effectively to the piglets. Finally, helminths such as *Oesophagostomum* spp. and *Hyostrongylus rubidus* seem to have relatively lower immunogenicity and they are gradually accumulated with age and thus dominate in adult pigs.

Therefore, the age of the infected pigs should be considered when evaluating a recorded worm burden. If, for example, young pigs harbour many *Oesophagostomum* spp. or *Hyostrongylus rubidus*, this indicates an unusually high transmission, while the same worm burdens may be acceptable in sows. On the other hand, many *Ascaris suum* or *Trichuris suis,* or even low numbers of *Strongyloides ransomi* in sows may indicate that these hosts have not had substantial experience with these infections during their growth, and therefore the herd's overall transmission level with these helminths may be low.

4.12.3 Management systems

The management system has been shown to have a strong influence on helminth prevalences. If pigs are reared in an extensive outdoor production system without any helminth control, it is not surprising if the worm burdens are heavy. On the other hand, only few helminth species (perhaps only *Ascaris suum*) are expected to be found in a highly intensive production system, and therefore even moderate worm burdens with unexpected helminths in such a system should

call for attention. If, for example, moderate numbers of *Taenia hydatigena* or *Oesophagostomum* spp. are found in a highly industrialized herd with routine anthelmintic prophylaxis, the veterinary advisers should look for either control with *Taenia*-infected dogs, or for particular niches in which *Oesophagostomum* larvae are able to develop. In the latter case, one should also be aware of anthelmintic resistance.

4.13 GENERAL COMMENTS

4.13.1 The subsample technique

The principle of subsampling is easily understandable, and subsampling is a convenient way to reduce the workload. But in practice subsampling is not so easy to carry out in a correct way. If the mixing of the total volume is not appropriate, the counts of the subsamples may either overestimate or underestimate the total worm burden. Therefore, the total sample should be mixed very well either until *immediately before* each subsample is taken or *while* it is being taken.

For small samples in a container (e.g. a container with a total of 200 ml), a commercially available air pump used for non-professional aquaria may be a good solution. The air may be led through a plastic tube mounted with a glass Pasteur pipette to the bottom of the sample, and the mixing effect of the bubbles should be allowed to continue during the subsampling.

Subsampling techniques may never be totally adequate. One way to overcome the inaccuracy is to use exactly the same procedure every time, in order to make the error systematical and thus to reduce the between-samples variation. Furthermore, this variation should be checked by counting a series of subsamples from the same source.

4.13.2 Occupational hazards

When using iodine or other chemicals which may be harmful to human health, one should be aware of the risks and know some ways to reduce them. Iodine will evaporate from stained samples when they are counted, i.e. when people are bending over the petri dish. The risk may be reduced by adding sodium thiosulphate, which reduces the amount of free iodine, and by sitting in a well-ventilated place or outdoors. If iodine samples are counted routinely in the laboratory, a local ventilation outlet located close to the petri dish may be a good permanent solution, and it may for example be constructed of a commercial vacuum cleaner with an outlet through the window.

CHAPTER 5

EXAMINATIONS FOR INFECTIVE LARVAE AND EGGS IN HERBAGE AND SOIL

5.1 INTRODUCTION

This chapter deals with supplementary diagnostic procedures for isolation of infective stages of parasites in the environment of the pigs. In contrast to most other domestic animals, the pig will eat both herbage and contaminated soil. Infective third-stage larvae (*Oesophagostomum* spp. and *Hyostrongylus rubidus*) may be found both in grass and soil, while infective eggs (e.g. of *Ascaris suum* and *Trichuris suis*) may primarily be found in soil, and it may be relevant to make estimates for both types of infective stages. However, even if herbage and soil have been examined thoroughly, one should be aware that the results make sense only for helminth species with an uncomplicated direct life cycle, such as those mentioned above.

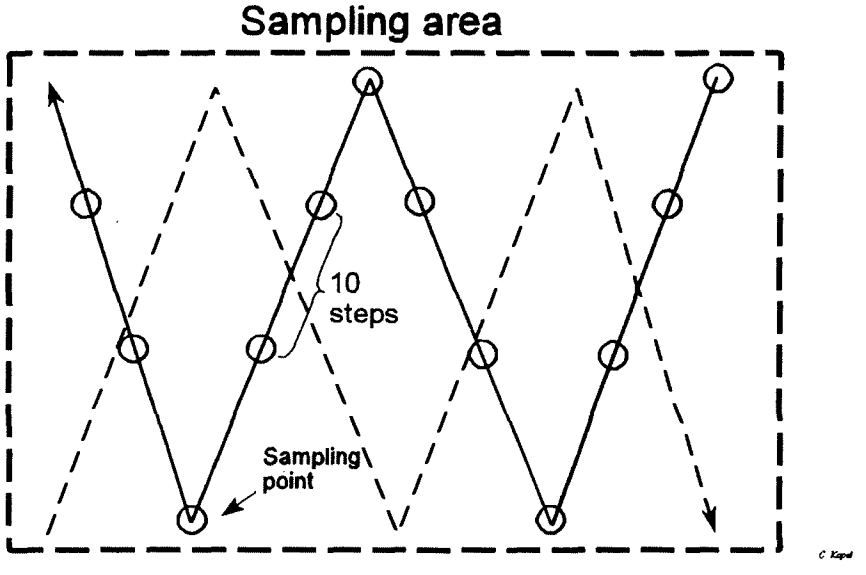
The techniques all have variable efficiencies, and the numbers of eggs/larvae within the samples may vary considerably; therefore the procedures described below should all be regarded as qualitative and semiquantitative.

5.2 COLLECTION OF HERBAGE AND SOIL SAMPLES

The principles for collection of herbage and soil are similar. The samples should be as representative as possible, and therefore one sample should be collected as many small subsamples, picked up from all over the area concerned, and this sampling should be repeated, so two identical samples are examined (i.e. duplicate sampling). This precaution is important, because the eggs/larvae are expected to be unevenly distributed with 'hot spots' of high numbers of infective stages scattered in otherwise low-infective areas.

The collection of herbage samples may in practice be performed by walking along two W-routes covering the whole area (Fig. 5.1), and for every 10 steps picking up a little amount of herbage (without soil, it may be necessary to use scissors) and placing it in a plastic bag (total amount for the whole area: 300-600 g). If the pasture is small, herbage should be sampled more often than for every 10 steps to get at least 30 subsamples. As pigs may destroy the vegetation by rooting, only limited areas with vegetation may be available for sampling. Herbage contaminated with faeces should be avoided.

Similarly, the collection of soil samples may be performed by following two W-routes covering the entire area, picking up a little amount of soil between 3 fingers (without vegetation and roots), and then placing it in a plastic bag (total amount for the whole area: 200-300 g). Soil samples cannot be collected in areas with very dense vegetation. Direct sampling of faeces should be avoided.



FIGUR 5.1 Two schematic W-routes for collection of herbage (or soil) samples. At least 30 subsamples should be collected from each W-route.

5.3 ISOLATION OF INFECTIVE LARVAE

Infective third-stage larvae may be found both on the vegetation and in the soil. Unlike e.g. the ruminants, pigs both ingest herbage and pick up significant amounts of soil when rooting for earthworms, insects, roots etc. Furthermore, *Oesophagostomum* larvae seem to be much less motile than *Hyostrongylus* larvae, and they do not migrate upwards on the herbage to the same extent. Therefore it may be relevant to examine herbage as well as soil for infective parasite larvae.

5.3.1 Isolation of infective larvae from herbage

The procedure is a simple Baermann technique.

Equipment

* 1-2 buckets
* Double-layer cotton gauze
* Balance
* Domestic detergent
* Conical sedimentation beaker
* Measuring cylinder (1 litre) and some kind of vacuum device may be useful
* Pipette (e.g. 5 ml)
* Test tube
* Test tube rack
* Aqueous iodine solution (IIK). Either Lugols solution (10 g I_2 and 30 g KI dissolved in distilled water to a total volume of 1 litre) or

a stronger solution (e.g. 80 g I_2 and 400 g KI dissolved in distilled water to a total volume of 1 litre)

* Sodium thiosulphate solution

* Pasteur pipette

* Microscope slides, preferably large, with a paraffin bank surrounding the sample area.

* Microscope with 10-40x magnification

Procedure

The method for *Isolation of infective larvae from herbage* is illustrated in Fig. 5.2.

Place the collected grass sample on a large piece of double-layer cotton gauze.

Form the gauze into a bag and immerse the bag in a bucket filled with tap water to which a few drops of detergent have been added.

During the first 3-4 hours, move the bag gently up and down the bucket several times to agitate the sample.

Leave the bag in the water at room temperature overnight.

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 at least 1 hour.

The bag of grass should be dried (sun/oven/incubator), and the net dry weight (gross dry weight minus dry weight of the gauze) is measured when the sample is completely dry.

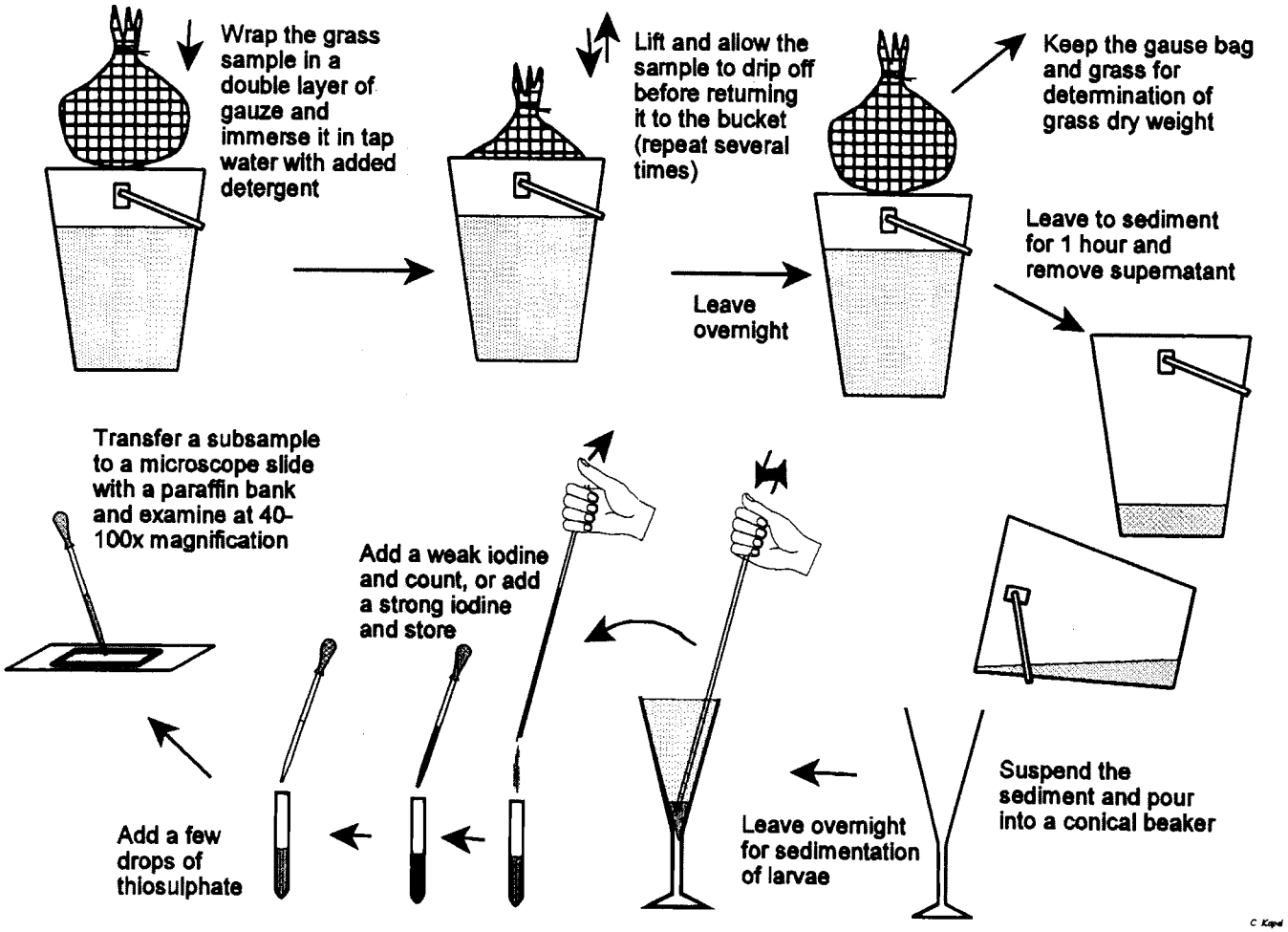


FIGURE 5.2 Isolation of Infective Larvae from Herbage

Remove the supernatant carefully (decantation, vacuum), avoiding resuspension of the sediment. Leave ½-1 litre containing all sediment.

If the volume is too large, the sedimentation may be repeated in a high, narrow glass (e.g. a 1-litre measuring cylinder), whereafter the supernatant again is discarded after 1 hour.

Suspend all the sediment, pour it into a conical sedimentation beaker, and leave it at room temperature overnight. Parasite larvae will then accumulate at the bottom.

Harvest the larvae with a 5 ml pipette, by placing your fingertip at the end of the pipette, move the tip of the pipette to the bottom of the beaker, let the larvae be sucked up into the pipette, and transfer the larvae to a test tube. This step may be repeated if some larvae remain in the beaker.

If any large particles clog the pipette opening, the pipette may simply be turned around, so the larvae are sucked into the wide end. Do not use mouth suction, as larvae may then be accidentally sucked into your mouth.

An alternative to the conical sedimentation beaker is a large glass funnel (20 cm diameter), fixed in a stand, and fitted with a flexible, transparent tubing. The tubing should carry 2 screw clamps placed 10 cm apart. The lower clamp is fastened, while the upper clamp is open, allowing the larvae to accumulate just above the lower clamp overnight. Harvest the larvae by closing the top clamp and collecting the trapped sediment with about 10 ml of fluid in a test tube by opening the bottom clamp.

The samples may be stored for max. 7 days in a refrigerator (4-5°C) before examination.

The samples may now be stained either with a weak iodine solution (Lugols solution) or with a strong iodine solution followed by

decolouring with sodium thiosulphate.

When using Lugols solution, some drops of the samples are transfered to the microscope slide with a paraffin bank/ring, a drop of Lugols solution is added, and the larvae should be counted immediately (within 15 minutes) under a microscope (10-40x magnification). There will usually be many more non-parasitic nematodes present than parasite larvae, and the counting may therefore be rather laborious. But the parasitic larvae will tend to obtain the brown iodine colour rather slowly, while free-living nematodes will obtain the brown colour immediately. As the parasitic larvae are uncoloured when counted, their morphological characteristics are clearly visible.

If iodine is to be used for preservation, some drops of the strong iodine solution should be added to the samples, which then may be stored for a considerable period of time before counting. After transfer of some drops of the sample to the microscope slide with a paraffin bank/ring, 1-2 drops of sodium thiosulphate are added (as little volume as possible, but the aqueous solution should loose its iodine colour) and the larvae are immediately identified and counted under a microscope. When using this counter-staining, the parasitic nematodes will tend to retain the brown iodine colour for 15 minutes after the decolouring with sodium thiosulphate, while the free-living nematodes will loose the brown colour immediately. As the parasitic larvae are brown when counted, their morphological characteristics are difficult to observe.

Both alternative methods help to distinguish between free-living and parasitic nematodes. Nevertheless, the cuticle sheath with the prolonged tail is the most important key character, and the colour should be used only as a help.

As the net dry weight of the sample is known, the results may now be expressed as numbers of larvae per kg dry weight, although it should be emphasized that this method is only semiquantitative.

5.3.2 Isolation of infective larvae from soil

The procedure is a simple Baermann technique. The first steps are different from the handling of grass samples, as the soil contains a lot of small particles which should be retained when the baermannization occurs.

Equipment

* Beaker (e.g. 500 ml)
* Balance
* Tray. The precise size is not important, but it should be bigger than the toy sieve (see below). Suitable plastic trays are easily procurable. A rectangular shape facilitates pouring from it
* Plastic sieve with flat bottom. A cheap toy sieve is suitable, but a sieve like a letter tray may have a larger surface
* 4 small rubber stoppers (for test tubes), or other objects of similar size
* Sheets of fine, loosely woven laboratory paper ('kleenex')
* Conical sedimentation beaker
* Pipette (e.g. 5 ml)
* Test tube
* Test tube rack
* Aqueous iodine solution (IIK). Either Lugols solution (10 g I_2 and 30 g KI dissolved in distilled water to a total volume of 1 litre) or

a stronger solution (e.g. 80 g I_2 and 400 g KI dissolved in distilled water to a total volume of 1 litre)

* Sodium thiosulphate solution

* Pasteur pipette

* Microscope slides, preferably large, with a paraffin bank surrounding the sample area

* Microscope with 10-40x magnification

Procedure

The *Isolation of infective larvae from soil* is illustrated in Fig. 5.3.

Mix the soil sample thoroughly in a beaker and take a subsample of 50 g by means of the balance. The subsample should be collected from several different places in the soil mixture.

Place the toy sieve or another large-meshed plastic device on the 4 stoppers in a tray.

Place a single layer of kleenex inside the sieve, and add tap water until the water level just touches the kleenex; if too much water is added, the sample tends to be more dirty. Ensure that the kleenex is not torn, as any holes will allow soil particles to pass.

Distribute the 50 g soil sample in the sieve. Continue to be careful not to tear the kleenex.

Leave the sample in the sieve for one or two nights at room temperature, or 37°C if an incubator is available. Now the larvae will migrate through the loosely woven kleenex, while soil particles are retained. Carried out correctly, the resulting larval suspension is rather clean.

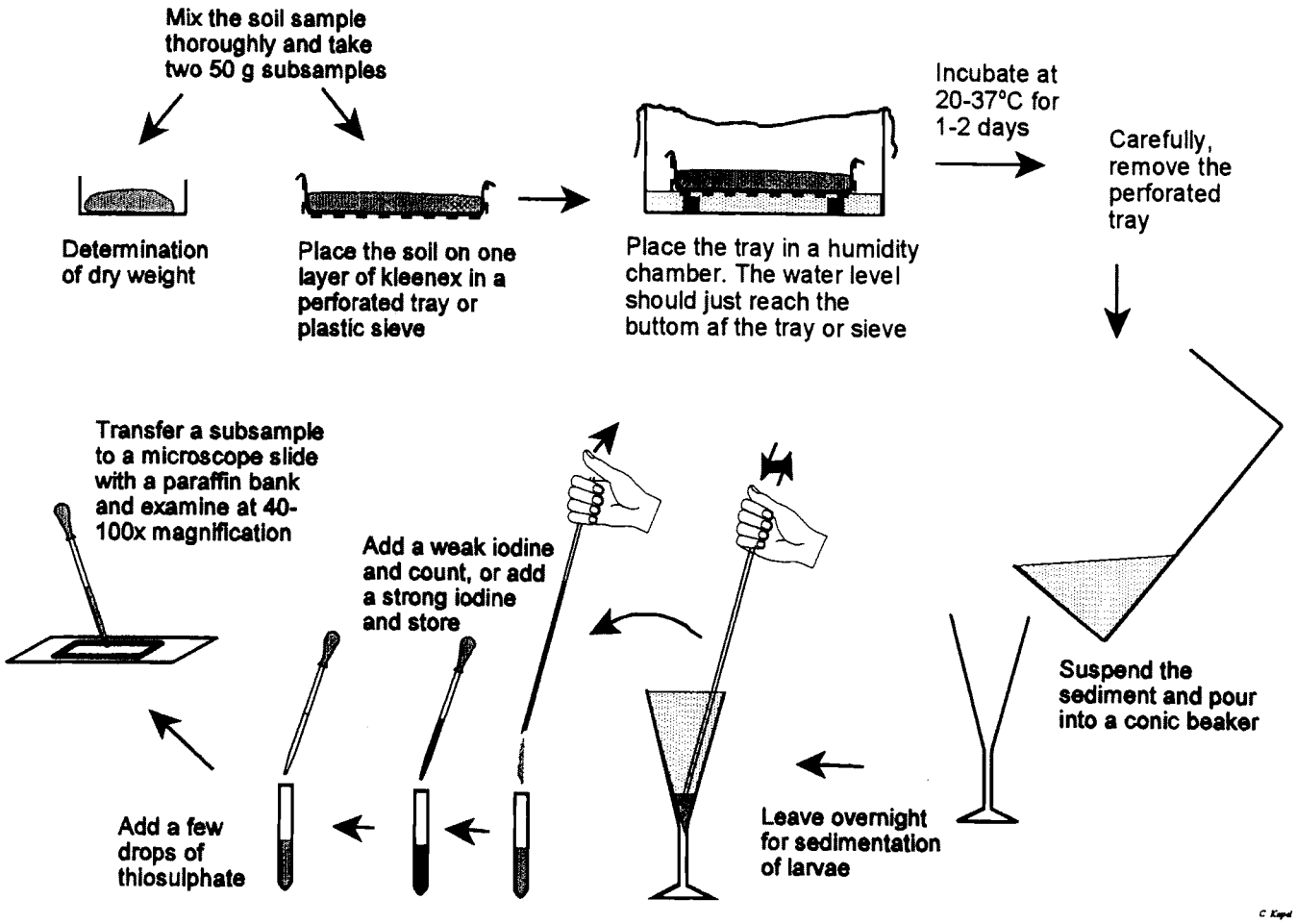


FIGURE 5.3 Isolation of Infective Larvae from Soil

When harvesting, remove the sieve carefully, pour the contents of the tray directly into a conical sedimentation beaker, and leave it at room temperature overnight.

Parasite larvae will now accumulate at the bottom of the sedimentation beaker. If the sample volume is too large for the sedimentation beaker, sedimentation for 1 hour in a high, narrow glass (e.g. a 1-litre measuring cylinder) may be inserted. Again, an alternative to the conical sedimentation beaker is a large glass funnel, as described in Section *5.3.1*.

Now the remaining part of the procedure is identical to the procedure previously described in Section 5.3.1: *Isolation of infective larvae from herbage*. The results may be recalculated as numbers of larvae per kg soil, although it must be emphasized that this method is only semiquantitative.

5.4 ISOLATION OF INFECTIVE EGGS FROM SOIL

The helminth eggs, which do not hatch outside a host, will remain in the faeces until they are spread to the soil by rain, earthworms, insects etc. Thus, it is relevant to look for infective eggs in soil samples and not in herbage. This method may be useful for common helminths like *Ascaris suum* and *Trichuris suis*, but it may also be used for detection of *Metastrongylus* spp., even though infected earthworms may complicate the interpretation of the results.

Equipment

* Beaker (e.g. 500 ml)
* Pair of scissors
* Stirring device (fork, tongue depressor)

* Balance
* Test tube, 50 ml (with a 50 ml mark)
* Test tube stopper/lid
* Test tube rack (for 50 ml tubes)
* 0.5 Molar NaOH
* Centrifuge with holders for 50 ml tubes
* Pasteur pipettes and rubber teats
* Vacuum device, if available
* Flotation fluid: Saturated NaCl with 500 g glucose per litre
* Laboratory sticks
* Erhlenmeyer flask (min. 250 ml), preferably having a cork with two holes, mounted with small glass tubes and rubber tubes, if a vacuum device is available
* Sieve, 200-300 μm (e.g. 212 μm)
* Sieve, 20 μm. The mesh size is critical, as the small eggs of *Trichuris*, having a width of 21-31 μm, must not be able to pass. On the other hand, the mesh size should be as large as possible to avoid blocking
* Jet stream of water. It may be a water pistol mounted on a hose
* Wash bottle
* McMaster counting chamber
* Filtering paper cut into approx. 1 cm wide strips
* Microscope with 40-100x magnification

Procedure

The *Isolation of infective eggs from soil* is illustrated in Fig. 5.4.

Cut any roots into small pieces with a pair of scissors, and mix the soil sample thoroughly in a beaker, using a stirring device.

Weigh out a subsample of 10 g in a 50 ml test tube on a balance. The subsample should be collected from several different places in the soil mixture.

Weigh out a similar subsample of 10 g on a petri dish. This sample should be dried (sun/oven/incubator), and the net dry weight (gross dry weight minus the weight of the petri dish) is measured when the sample is completely dry.

Add 0.5 M NaOH to the 50 ml mark on the test tube and mix the soil suspension by means of a small applicator stick. Close the test tube with a stopper/lid, and let the soil sample soak overnight at room temperature or in a refrigerator.

The NaOH makes the eggs less sticky and breaks down the texture of the soil particles.

Next day, centrifuge the sample at 1200 RPM (revolutions per minute) for 7 minutes.

Remove the supernatant with a pipette or another vacuum device, and discard the supernatant. Be careful not to resuspend the sediment.

Add 20-30 ml flotation fluid (saturated NaCl with glucose) and resuspend the sediment by stirring with a small applicator stick. After thorough mixing, flotation fluid is added up to the 50 ml mark.

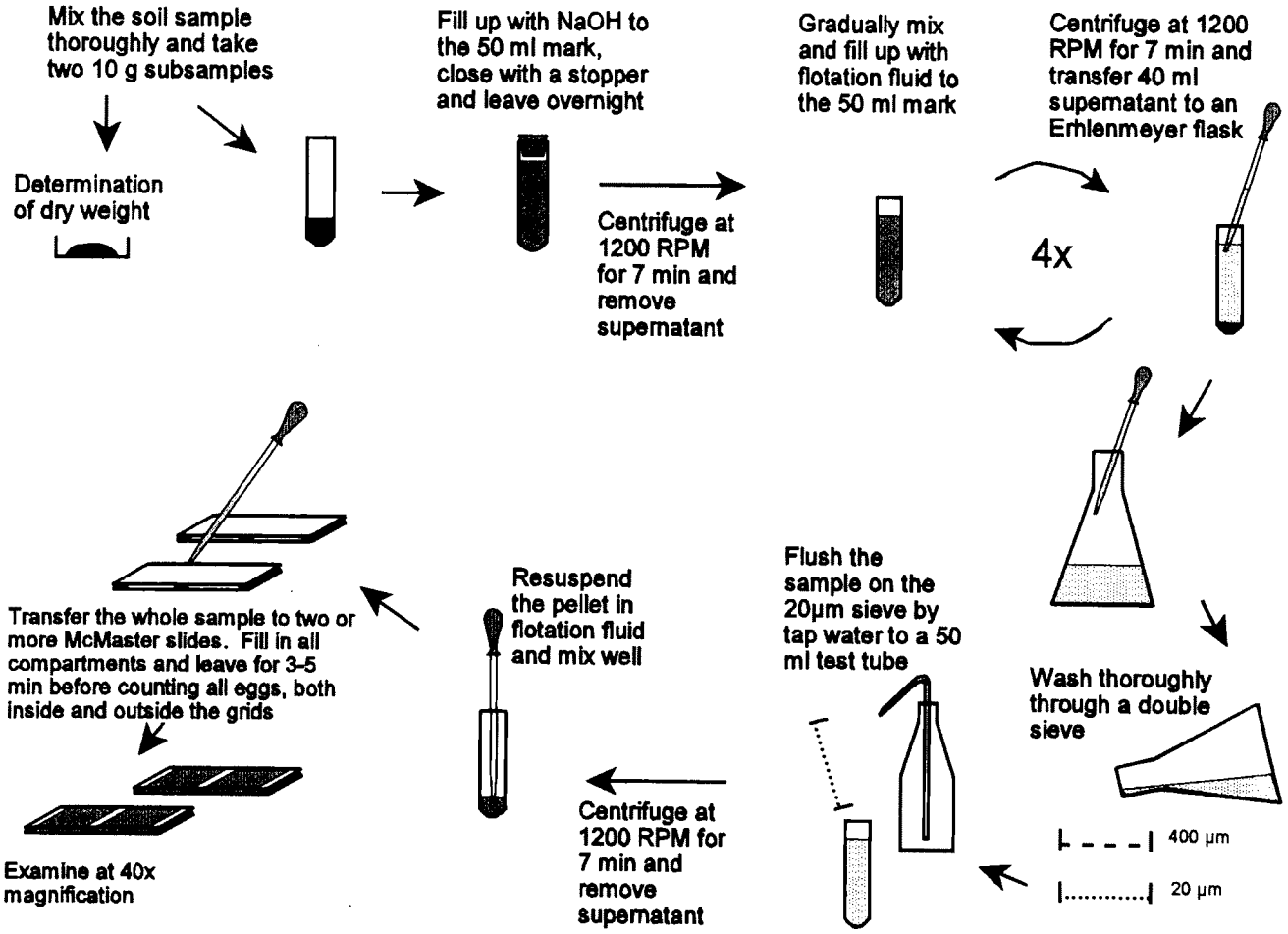


FIGURE 5.4 Isolation of Infective Eggs from Soil

Centrifuge the sample for 7 minutes at 1200 RPM. Many helminth eggs will now flotate.

Suck approx. 40 ml supernatant into a 250 ml Erhlenmeyer flask. If a vacuum device is available, this may be most easily done by means of a cork with two holes, one for inlet of the supernatant and one for outlet of air. It is advisable to suck a small amount of water through the system after the flotation fluid in order to avoid crystallization of salt and glucose.

Be careful not to suck sediment from the test tube, as any sediment particles will easily stop the fine sieve later in the procedure (see below).

Repeat the last 3 steps, i.e. add flotation fluid, centrifuge and transfer the supernatant to the Erhlenmeyer flask, three times. Each time the supernatant should be collected in the same Erhlenmeyer flask. The repetitions are necessary to increase the recovery of helminth eggs.

Discard the washed sediment in the test tube, and wash the test tube with water. The test tube may now be reused after the supernatant has been sieved.

Pour the supernatant from the Erhlenmeyer flask into a double sieve (a large-meshed sieve, 200-300 μm, placed over a fine-meshed sieve, 20 μm).

Wash the sample thoroughly with a jet stream of water. Now the coarse particles will be trapped in the large-meshed sieve, while fine particles, including any helminth eggs, will accumulate on the fine sieve.

Separate the two sieves and wash the fine sieve once more with water, while accumulating the particles in one side of the sieve. Use a washing bottle with tap water for the final accumulation of particles and for their transfer to the 50 ml test tube.

Centrifuge the test tube for 7 minutes at 1200 RPM. As the eggs are suspended in water, they will now be spun down.

Suck off and discard the supernatant.

Add a volume of flotation fluid (saturated NaCl with glucose), corresponding to at least 4-5 times the volume of the sediment. Very often the total volume of flotation fluid and sediment may be less than 2.0 ml.

Resuspend the sediment by sucking slowly up and down in a pasteur pipette, and transfer the total volume to 1-3 McMaster counting chambers, the number of counting slides depending on the sample volume.

Add a little more flotation fluid to the test tube and transfer this to the counting chambers to wash out the test tube and the pipette.

As the entire sample is to be counted, the McMaster chambers should be filled restrictively, with no surplus of sample along the edges of the chambers, i.e. the total amount of flotation fluid should be placed underneath the upper glass, allowing all helminth eggs present to accumulate under this glass.

Count all helminth eggs in the counting chambers, not only inside the normal counting area, but also outside the counting area. Count all McMaster chambers. A magnification of approx. 40x is suitable.

Any helminth eggs should be identified to species, and at a higher magnification it is clearly visible whether they contain developed embryos (see Section 5.5).

After counting, the McMaster chamber should be washed under a stream of tap water, shaken to remove most of the water, and dried with a cotton cloth on the outside and with a strip of filter paper inside the chamber.

As the total sample represents 10 g soil, the counts may be expressed as number of helminth eggs per gram of soil by multiplying by a factor of 0.1.

Example: 15 *Ascaris suum* eggs are found in the entire sample. Then the number of eggs per gram of soil is 15x0.1 = 1.5 eggs per gram.

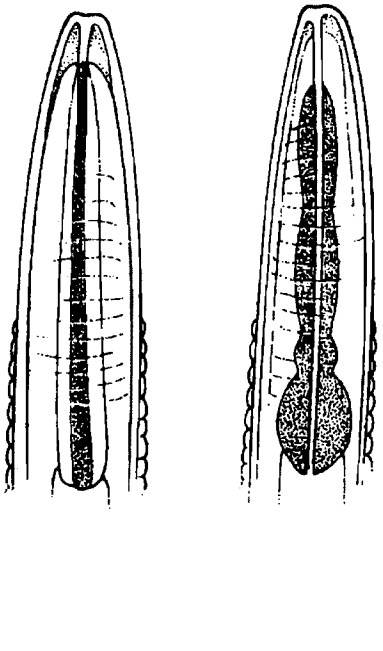


FIGURE 5.5 A filariform oesophagus of a infective L_3-larva (left) and a rhabditiform oesophagus of a free-living nematode (right)

5.5 IDENTIFICATION OF LARVAE AND EGGS

The infective third-stage larvae may be distinguished from free-living nematodes, being located inside a cuticular sheath, i.e. the cuticle of the L_2 larvae. Normally, this sheath is clearly visible at good magnification. Furthermore, the L_3 larvae have a filariform oesophagus, while soil nematodes (and L_1 and L_2 larvae of parasitic nematodes) have a bulbous rhabditiform oesophagus (Fig. 5.5). Very often herbage samples contain a number of infective larvae of non-porcine helminths, e.g. ruminant genera such as *Ostertagia*, *Cooperia*, *Trichostrongylus* etc., and these larvae should be omitted when making research on pig helminths.

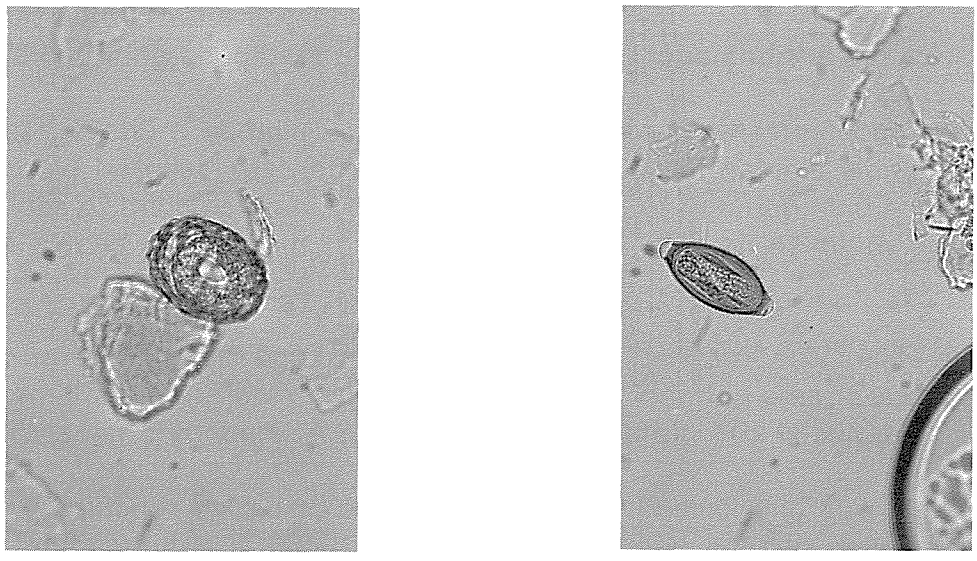


FIGURE 5.6 Infective eggs of *Ascaris suum* (left, the thickshelled egg contains a slender infective L3-larva) and *Trichuris suis* (right, the thickshelled egg contains a slender infective L1-larva).

The characteristics of *Oesophagostomum* spp. and *Hyostrongylus rubidus* are presented in Section *3.6, Identification of eggs and larvae*, while some characteristics of L_3 larvae of non-porcine helminths may be seen in the *FAO guidelines on Helminth Parasites in Ruminants* (Hansen & Perry, 1994).

The infective helminth eggs which may be found in soil samples, are identical to the corresponding eggs found in freshly deposited faeces (see Section *3.6, Identification of eggs and larvae*), except that they may contain larvae, which may be clearly visible in a good microscope (Fig. 5.6).

CHAPTER 6

INVESTIGATING HELMINTH OCCURRENCE AND EPIDEMIOLOGY IN A PIG POPULATION

6.1 INTRODUCTION

This chapter deals with strategies for investigating helminth occurrence in a pig herd or flock and helminth epidemiology, using the methods described in Chapters 3-5. Investigations may be divided into 3 parts:

a) Estimation of the helminth occurrence in a herd/flock

b) Long-term monitoring of helminth status in a herd/flock or of a control programme

c) Plot experiments

While a) in general is the first step of an investigation, b) and c) are two different ways of throwing light on the more dynamic epidemiology of the helminth species. b) may give information on the changes in prevalence rates and intensities of the infection in the pig population over time, and c) may provide knowledge about the development, dissemination and survival of the infective free-living stages.

The unit of investigation is the herd or flock. Where groups of herds are grazed communally or have close contact with each other, the whole group should ideally be the unit of investigation. Helminth infections generally involve an entire herd or flock, and to be effective, diagnosis, treatment and control measures should be directed at the entire unit.

When investigating a herd of pigs, one should be aware that many

factors may exert an influence on the helminth infections (number of species, prevalence rates, infection intensities). One of the most important is the age of the pigs combined with the immunogenicity of the helminth species concerned (see Chapter 4.12). Other important factors are management (housing facilities, hygiene) and local seasonal climatic variations, which may have immense influence on the transmission rates. Also, the reproductive status of the host (dry or lactating sows), the nutritional status of the hosts, and any control measures (management and anthelmintic routines, especially the time span since the latest anthelmintic treatment) may closely determine to the infection rates.

6.2 HELMINTH OCCURRENCE IN A HERD/FLOCK

To reveal the significance of the helminth infections within a population of pigs, faecal samples (see Chapter 3) should be taken from a representative number of live animals, preferably belonging to selected age groups. Furthermore, as many dead (moribund or sacrificed) animals as possible should be examined using the *post-mortem* procedures (see Chapter 4).

Live animals

It is necessary to collect faecal samples from at least the following three groups of pigs, to increase the chance for diagnosing the parasite species present in the population, and in order to measure the infection levels in those age groups in which the parasite species are prevailing and potentially causing problems (Table 6.1, where only the most common parasites are mentioned).

Any animals with obvious clinical disease suggestive of parasitism should be sampled. However, most helminth infections in pigs are subclinical, and samples from diseased animals should never replace the sampling from a representative number of randomly selected ani-

Table 6.1 Suggested age groups of pigs to sample, together with some of their most prevalent parasites.

Age group of pigs to sample	Most prevalent parasite species
piglets (2-3 weeks of age)	*Strongyloides ransomi*, *Isospora suis*
fatteners (5-6 months of age)	*Ascaris suum*, *Trichuris suis*
sows	*Oesophagostomum* spp., *Hyostrongylus rubidus*, *Stephanurus dentatus*, *Eimeria* spp.

mals. The latter sampling is necessary for a full understanding of the herd/flock problem.

There is no magic number of samples; but in general, the more animals sampled, the greater the validity of the results and the better the understanding of the infection patterns. The following suggested numbers are based on both general principles and practical/logistical constraints (Table 6.2), however, factors like expected prevalence rates, variability in the egg counts, and accuracy of the estimations should also be taken into account.

Table 6.2 Suggested sample sizes for given total numbers of pigs in an age group.

Number of animals in the age group	Number (percentage) of animals to sample	
1-10	all animals	(100%)
11-25	10→15 animals	(90%→60%)
26-100	15→30 animals	(58%→30%)
101-200	30→40 animals	(30%→20%)
201-500	40→50 animals	(20%→10%)
>500	50→ .. animals	(10%)

Dead (moribund or sacrificed) animals

As many as possible of the animals that die should be examined (see Chapter 4 for *post-mortem* examinations). If a considerable number within the flock is affected, one or two very ill or moribund animals should be sacrificed for examination. However, care should be taken with respect to the interpretation of results originating from only a few animals, as some helminths (e.g. *Ascaris suum*) are heavily overdispersed within the host population, and therefore a few negative *post-mortem* examinations do not exclude presence of parasites.

6.3 LONG-TERM MONITORING OF A HERD/FLOCK

The initial estimation of helminth occurrence in a herd/flock is important, but it does not provide full understanding of the epidemiology. Therefore, a long-term monitoring of parasites in the herds/flocks should be carried out, if possible.

Faecal samples (see Chapter 3) from living animals will provide the most important information, but they may be supplemented with *post-mortem* examinations (Chapter 4) on dead (moribund or sacrificed) pigs or tracer (sentinel) pigs, and examination of soil/grass (Chapter 5) may also be valuable. Furthermore, climatic data should be recorded.

Live animals

If the flock/herd is large, and the sows are farrowing all the year round, it may be possible to sample only the age groups specified in Table 6.1. However, in most cases cohorts of piglets must be selected (preferably several cohorts, starting at different times during the year in order to reveal seasonal variation), and then identified

animals should be sampled repeatedly at specified intervals (see below). With respect to the sows, a number of individuals should be randomly selected at the beginning of the monitoring period, and these individuals may then be sampled at the same specified intervals.

Again, there is no magic number of samples, but the above mentioned sample sizes (Table 6.2) is suggested.

Ideally, the sampling should be carried out every 2 weeks during periods with suspected high transmission rates (rainy season, summer season), continuing for approx. every month into the more unfavourable season (dry season, winter season). For the remaining dry/winter season, samplings every 4-6 weeks will be appropriate. The samplings should optimally cover 3 calendar years to ensure the recording of an average situation and provide an impression of the year-to-year variation.

In practice, this ideal sampling may not be feasible. However, it is acceptable to sample once a month during the rainy/summer season (including the first month of the dry/winter season), and once every 2 months during the remaining part of the dry season/winter.

Dead (slaughtered, moribund or sacrificed) animals

Every opportunity should be taken to sample animals that die from whatever reason, but sacrificing animals are not advocated for long-term monitoring programmes unless special parasite problems are suspected. Instead, pigs are slaughtered regularly (slaughter weight depends on local traditions) and representative numbers of randomly selected fatteners and sows should be examined at the various seasons to monitor the worm burden.

Tracer (sentinel) animals

Tracer pigs are intended to provide direct information on the bioavailability of infective larvae/eggs in the environment, i.e. not only a number of infective stages in a handful of grass or soil selected by the researcher, but the number of parasites which in practice are established in a pig grazing and rooting area.

Tracers should be parasite-naïve. The principle is to place these animals on the pasture for a predetermined short period of time (e.g. 2 weeks), during which they will pick up infective parasite stages. The tracers are then moved to a completely helminth-free environment for the following 4 weeks, during which the young parasites will grow up so that they will be easy to identify *post-mortem*. The pigs are then slaughtered and subjected to *post-mortem* examinations. Ideally, 2 or more tracer pigs should be introduced to the pasture once a month over at least one calendar year.

The suggested tracer protocol will work well for many helminths, however it should be noticed that the large majority of worms of very immunogenic species, like *Ascaris suum*, may already have been expelled by the tracer pigs, when these are slaughtered 4 weeks after the last exposure.

The tracer principle has proven to be very valuable in epidemiological research on ruminant helminths. However, there are several practical problems associated with this principle in pig herds. Firstly, it may be very difficult or impossible to rear helminth-free and non-immune animals unless very intensively managed indoor herds are available. Secondly, good animal housing facilities are required for the tracer pigs after they have picked up the infections, as they should not be further infected in these pens, and they should not be able to re-infect themselves. Finally, if the tracer pigs are introduced into a population of pigs with an established hierarchy, they will be regarded as strangers in the flock and be more or less suppressed and stressed, and as a consequence they may not graze and root

normally. The latter situation may, however, be eliminated either at very low stocking rates, or by having the tracer pigs fenced off in a separate area.

Sampling of grass/soil

When carrying out a long-term monitoring programme, it may be very valuable to obtain measures on the numbers of infective stages in soil and grass, even though the measures are not identical to the bioavailability measured by tracer pigs (see above).

In practice, the sampling of soil and grass should follow the same intervals as the animal sampling mentioned above (see *Live animals* in this section). The samples should be taken at the same time of the day on each occasion. Furthermore, every sampling should cover the same area of the pasture. This should at least be the 'general' grazing area, but very often it will be a good idea to include separate samplings from suspected high-risk areas (e.g. muddy pools or other wet areas).

6.4 PLOT EXPERIMENTS

Useful epidemiological information can be obtained by studying the seasonal development, dissemination and survival of helminth stages in or around pig faeces. Faecal material, containing a known number of eggs, should be deposited on a pasture typical for the geographic area, and the deposition should be carried out at regular intervals throughout a minimum period of one year.

The following is a simple experimental design, which will still provide good information on transmission characteristics of helminths with a direct life cycle.

Identify a parasite-free grass-covered area representative of pastures with pigs. As especially *Ascaris* and *Trichuris* have eggs with a very long potential survival time, it may be difficult to find a completely helminth-free area, but then the background contamination should be monitored before the start of the experiment.

Select at least 12 plots of approx. 1 m^2 each. Fence these plots to prevent grazing, and cover the plots with wide-meshed nets to keep birds away. Care should also be taken to prevent mice, voles etc. from disturbing the plots.

Cut the grass to a height similar to that of grazed paddocks, and keep the grass at this level throughout the experiment, but without removing the cut-off.

Identify a source of infected animals for continuous supply of faecal material. If the origin of faeces is a commercial farm, fixed arrangements should be made to exclude anthelmintic treatment of the donor animals. If no naturally infected pigs are available, a group of pigs should be experimentally infected.

Collect as much faeces as possible from the donor animals, but keep the faecal clumps separate until faecal examinations have revealed which of them contain the highest concentrations of eggs (or any eggs at all).

Mix approx. 6 kg faeces so that all helminth species are present with as high numbers of eggs as possible. If the faeces is too dry, water may be added. Care should be taken to mix the faeces thoroughly.

Determine the egg concentrations in the final faecal mixture by running 10 McMaster tests (preferably the concentration McMaster technique; see Chapter 3) on 10 samples collected at different sites in the material (if the variation between egg counts is too great, the mixing and the McMasters should be repeated), and calculate the mean EPGs.

Deposit 5 kg faeces in small clumps of 10-50 g evenly on one plot.

Collect samples (small amounts of grass, soil beneath the faeces clumps, and faecal material, as long as the faeces is not totally decomposed) by taking a number of small subsamples at different places on the plot, and combine these subsamples into 3 large samples (i.e. grass, soil and faeces, respectively).

Analyze for eggs in faeces and in soil (EPG, determine species and developmental stage), and for larvae in faeces, soil and grass (larvae per gram, determine species). All methods are described in Chapter 5. All quantitative measures should be related to the total number of eggs of each species deposited on the plot.

Samples should be collected frequently (e.g. 1 week intervals) shortly after the deposition and with larger intervals later on. The exact sampling protocol may, however, depend on the helminth species present (slow or fast embryonation), the climate and the region. It is recommended that the sampling continues for at least one year.

Repeat the deposition and thus the sampling schedule every month for at least one year.

Monitor at least ambient temperature and rainfall daily, during the entire period.

CHAPTER 7

CONTROL OF HELMINTHS IN PIGS

7.1 GENERAL PRINCIPLES OF CONTROL

The purpose of a helminth control strategy is to keep the parasitic challenge (especially to young pigs and lactating sows) at a minimum rate to avoid clinical symptoms and production losses. Total eradication from a geographical region is unlikely for most parasites, due to the immense numbers of eggs shed and the high persistence of the infective stages in the environment.

Before choosing and starting any control programme, it is necessary to have a detailed knowledge about the helminth infections in the pig population(s), i.e. helminth species present, prevalence rates and transmission patterns. These characteristics may differ between geographical regions, local herd management traditions etc. If this knowledge is missing or is only scarce, any control programme should, therefore, start with an investigation of the helminth occurrence and epidemiology, as described in detail in Chapter 6.

The success of helminth transmission is to a large extent depending on herd factors. This is visualized in Table 7.1, where a rough overview of helminth occurrence in different production systems is presented for a number of the most important helminths.

It is clearly seen that while extensively outdoor-reared pigs may theoretically harbour all existing helminth species, intensive outdoor management may eliminate those helminths whose transmission depends on contact with human faeces, dog faeces or fresh water. Furthermore, helminths which depend on intermediate hosts may be more or less eliminated if the outdoor-reared pigs are confined to concrete yards. All helminths with an indirect life cycle are eradicated in indoor production systems, perhaps with the exception of *Trichi-*

Table 7.1 An overview of anticipated helminth occurrence in different production systems.

Helminth	Summary of transmission characteristics	Management system			
		outdoor extensive	outdoor intensive	indoor extensive	indoor intensive
Ascaris	eggs	+	+	+	+
Oesophagostomum	larvae	+	+	+	+
Trichuris	eggs	+	+	+	+
Strongyloides	milk/larvae	+	+	+	(+)
Hyostrongylus	larvae	+	+	(+)	
Stephanurus	larvae	+	+	(+)	
Trichinella.	muscles	+	(+)	(+)	
Gongylonema	dung beetles	+	(+)		
Ascarops	dung beetles	+	(+)		
Physocephalus	dung beetles	+	(+)		
Metastrongylus	earthworms	+	(+)		
Echinococcus	dog faeces	+			
Fasciola	aquatic snails	+			
Taenia solium	human faeces	+			

nella sp.. When indoor pig rearing develops from extensive to intensive management, the number of helminths with a direct life cycle will gradually decrease, *Ascaris suum,* and to a lesser degree *Oesophagostomum* sp., being the most persistent of them all.

From this simple table it is evident that the most efficient way to control porcine helminths is to improve the management and hygiene of the herd. First of all, such improvement will eliminate some helminth species, but additionally the worm burdens of the remaining species may be reduced to more acceptable levels. Eradication of helminth species is more or less impossible to obtain merely by routine anthelmintic treatment programmes, which is the other major control principle. Yet, it is often practically impossible to improve management sufficiently, and therefore helminth control programmes normally include both management and the use of anthelmintics.

7.2 CONTROL OF NEMATODES

Apart from the beneficial results of improving management (see above), control of nematodes may be achieved as described below.

7.2.1 Stocking rate

The density of pigs (stocking rate) in extensive outdoor pig rearing should not be too high. Overstocking will force the pigs to a closer contact with faecal material and may result in the consumption of a higher number of infective nematode stages.

7.2.2 Grazing management

Grazing management may be used to minimize the uptake of infective eggs/larvae and to create safe pastures. Alternating plant crops with pig rearing will reduce the contamination on a pasture considerably, although it should be recognized that infective eggs of especially *Ascaris* and *Trichuris* may survive for years under favourable conditions. The development of such grazing programmes requires a thorough knowledge of the parasites seasonal development and survival in the particular area. As an example, in the temperate regions, the eggs of *Oesophagostomum*, *Ascaris*, and *Trichuris* cannot embryonate and develop to infectivity during the winter (i.e. below 10-15°C), but the two latter may accumulate and then develop *en bloc* when favourable conditions turn up in the early summer. Under these circumstances it may be beneficial to move the pigs to a safe pasture just before the temperature increases in the spring, and then eventually use the contaminated areas for crops.

7.2.3 Mixed or alternate grazing

As pigs have only few helminths in common with ruminants, grazing

management may include mixed grazing (pigs grazing together with ruminants) or alternate grazing (pigs alternating with ruminants on the same pasture). It may be necessary to provide the sows with nose-rings to avoid rooting and other serious damage to the vegetation (see below). As seen in Tables 2.1 and 2.2, a few helminths may infect both pigs and ruminants, and therefore local identification of the helminth species present are recommended before starting this regimen. Some of the species (e.g. the flukes *Fasciola hepatica* and *Schistosoma japonicum*) may be controlled simply by avoiding contact with freshwater, but *Trichostrongylus axei* and *Dicrocoelium dendriticum* may still be present. Furthermore, when ruminants are using a pig contaminated area (outdoor, indoor), attention should be paid to the risk of liver and lung lesions caused by migrating *Ascaris suum*.

7.2.4 Hygiene of pens

When pigs are kept in concrete pens (outdoors, indoors), the dung should be removed daily to reduce the large majority of nematode eggs before they become infective. Furthermore, the floor should be kept as dry as possible, as free-living stages of all helminths (even *Ascaris suum*) require nearly 100% relative humidity to develop. The draining capacity, and thus the dry microclimate at floor level, may be the main reason why slatted floors in some intensive systems seem to be rather effective in reducing helminth transmission indoors.

It is often recommended to wash concrete floors (e.g. using high-pressure devices or steam cleaning), but this recommendation is questionable, as water may improve the general conditions for egg/larval development and survival (it is impossible to remove *all* eggs) and furthermore may help spreading the infective stages from developmental foci (sheltered crevices etc.) to all over the pens.

7.2.5 Dose and move

Before animals are moved to safe areas (outdoor, indoor), they may be dosed with an anthelmintic to remove any worms present, in order to keep the environment free of contamination for as long as possible (dose and move). This principle has been shown to be rather effective, although it unfortunately also increases the risk of development of anthelmintic resistance (see Section 7.6).

7.2.6 Routine deworming

Routine deworming programmes often appeal to farmers for reasons of convenience, and as a result worm treatments generally is the only control measure carried out. However, the effect of each treatment will be rather transitory if the pigs are re-infected continuously, while the effect is considerably prolonged if the transmission rate is low. Each treatment with a drug will increase the selection pressure for development of anthelmintic resistance, and therefore helminth control programmes should reduce the number of treatments to a minimum and rather increase other control measures. Nevertheless, some kinds of routine anthelmintic treatment are relevant in the control of nematodes in most managements systems.

Several programmes for deworming of pigs have been worked out, and most are adjusted to the age or the reproduction cycle. The standard procedure is treatment of sows shortly before farrowing, before a move to clean farrowing units. The objective is to eliminate the worms from the sows in order to prevent contamination of the environment of the newborn piglets. In dirty herds this treatment may be supplemented by an additional treatment of the sows at breeding (and treatment of boars and gilts). To reduce the infection rates of the offspring, it is often recommended to treat piglets at weaning and growing pigs once or twice during the fattening period, although it should be realized that in some intensive systems piglets and growing pigs do not need any routine drug treatment at all.

The choice of drug should partly depend on the worm species present. Some drugs have a broader spectrum of activity than others, some nematodes may be controlled only by certain drugs; some drugs are more expensive than others etc. (see Section 7.5). Furthermore, it is important to alternate between drugs with different modes of action in order to reduce the risk of developing anthelmintic resistance (see Section 7.6), and to avoid drugs against which resistance has already developed.

7.2.7 Nose-ringing of sows

In some geographical areas it is common to fix an iron ring in the snouts of sows kept on pasture, to reduce their rooting activity. As this is an effective way to keep the grass cover on the pasture, it may also reduce the uptake of soil-transmitted nematodes.

7.2.8 Adequate nutritional level

The overall effect of helminth infections may be reduced by ensuring an adequate level of nutrition (especially proteins), although this should be no substitute for a sound parasite control programme.

7.2.9 Genetic resistance

Very little is known about genetic resistance to helminth infections in pigs, although a clear difference in infection levels between two breeds has been described. Especially in sheep, genetic differences in susceptibility have been documented within and between breeds, and it is likely that such differences may exist in pigs as well.

7.2.10 The 'gilt-only' system for control of *Stephanurus dentatus*

The kidney worm *Stephanurus dentatus* has a very long prepatent

period (6 to 19 months), and therefore the mature worms are generally found in brood sows older than 2 years. The 'gilt-only' system simply means that all breeding animals are consistently slaughtered after having produced their first litter, and this management practice has proven effective in the eradication of kidney worms from a pig population.

7.2.11 Control of *Trichinella spiralis*

Pigs become infected with *Trichinella* by eating infected meat (wild or domestic animals). Therefore, prevention of infection will depend on the prevention of cannibalism, by avoiding any animal tissue being fed to pigs without adequate cooking (boiling for 30 minutes), and control of rodents.

7.3 CONTROL OF TREMATODES

Most digeneans which infect pigs include freshwater snails in their life cycles (*Fasciola hepatica*, *Fasciolopsis buski*, *Schistosoma* spp., *Opisthorchis noverca*, see Tables 2.1 and 2.2, Chapter 2), and these infections may be controlled simply by avoiding contact between pigs and freshwater reservoirs (even small ponds and temporary pools), or by preventing pigs from eating raw fish (*Opisthorchis*).

The only trematode which is not controlled in this way, is *Dicrocoelium dendriticum*, as this fluke is transmitted terrestrially via slugs and ants. However, *Dicrocoelium* may be controlled by keeping the pigs in concrete yards or indoors.

7.4 CONTROL OF CESTODES

All cestodes infecting pigs have carnivorous mammalians as final

hosts, and the metacestodes in the internal organs of pigs are difficult to eliminate with drugs. Therefore, treatment for cestodes in pigs is not included in any control programme.

All control strategies rely on the prevention of contact between pigs and infected faecal matter. In the case of the zoonotic *Taenia solium,* community sanitation is the ultimate control measure, preferably supplemented by drug treatment of infected human. Similarly, *T. hydatigena* in pigs may be controlled by preventing contact between pigs and faeces of dogs (or other canids), although this parasite seldom becomes a problem calling for special control activities. Finally, *Echinococcus* spp. will never be regarded as merely a pig infection, as it is clearly a community health problem, involving especially dogs (and wild canids) as the main target for control efforts. A well-coordinated eradication programme includes information of the public in order to obtain routine drug treatment of all dogs and to prevent re-infection of the dogs (and wild canids) by inactivating all animal tissues/organs (thorough boiling or freezing) when slaughtering domestic animals, including pigs. Furthermore, eradication programmes must include control of wild canids if these contribute significantly to the completion of the life cycle of the parasite in the area.

7.5 ANTHELMINTICS

7.5.1 Definition

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

Currently a series of safe anthelmintics are 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, including dormant larvae.

Due to their cost, their tendency to delay or interfere with natural host immunity mechanisms, and not least the rapidly increasing prevalence of anthelmintic resistance, 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 an inevitable and often the only available method of controlling helminth parasites. But, they should not be used indiscriminately.

7.5.2 Characteristics of an ideal drug

An ideal drug should have a broad spectrum activity against adult and larval helminth parasites. A number of factors influence the efficacy of an anthelmintic drug. The individual pigs often harbour several different helminth species, which do not have the same sensitivity to a given anthelmintic. In addition, there is usually a difference in sensitivity between adult and larval stages, with immature stages and especially dormant larvae being less sensitive than the adult parasites. Furthermore, recent observations indicate that the concentration of a drug *in situ* may depend profoundly on the composition of the diet and the feeding regimen, with restricted feeding increasing the concentration, and thus the efficacy, of some orally administered drugs in the gastro-intestinal tract.

The ideal drug should also be metabolized rapidly in order to avoid metabolic residues in pigs slaughtered for human consumption, and thus to reduce the slaughter withdrawal period. Furthermore, the long-lasting presence of subtherapeutic concentrations of a drug may constitute a severe risk factor for the development of anthelmintic resistance.

A good drug has low toxicity to the host, and the ratio of the therapeutic dose to the maximum tolerated dose of pigs should be as large as possible.

There should be no unpleasant side-effects to the pigs, the operator or to the environment. Some drugs may cause inappetence or pain at the injection site.

The selected drugs should be competitively priced and ready to use correctly in an easy way. Furthermore, they should be stable and not loose activity on exposure to normal ranges of temperature, light and humidity.

7.5.3 Dosing methods

Oral dosing is by far the most common and easiest way of administration of anthelmintics to pigs, because pigs, in contrast to e.g. ruminants on pastures, depend on daily feeding in troughs. Thus, many drugs are simply admixed to fodder, while oral dosing of individual animals, as commonly done in ruminants, is not necessary. This implies that pigs often are group-treated which unfortunately sometimes results in a subtherapeutic dose in individual animals, when the drug is only administered once. When drugs are administered over several days, this risk is less. Furthermore, the efficiency of a drug (especially Class I and III drugs, see below) may be considerably increased by low dosing for several days.

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

No anthelmintics are until now available in a formulation for external application ("pour on" preparations) to pigs.

7.5.4 Anthelmintic classes

On the basis of their mode of action, anthelmintic drugs can be subdivided into 5 classes.

Class I anthelmintics: Benzimidazoles and pro-benzimidazoles. These drugs exert their action on the intracellular polymerization of the tubulin molecules to microtubules. As the cellular functions are disrupted, the worms die. Examples of Class I compounds are thiabendazole, fenbendazole, parbendazole, flubendazole, febantel, and thiophanate.

Class II anthelmintics: Imidazothiazoles and tetrahydropyrimidines. These drugs act on the acetylcholine receptor in the neuromuscular system of the worms, causing a persistent depolarization of muscle cells and a spastic paralysis of the worms, which are then removed by gut motility. Examples of Class II drugs are levamisole, pyrantel, and morantel.

Class III anthelmintics: Avermectins and milbemycins. The compounds act on the nervous system of the worms, causing flaccid paralysis and removal by gut motility. Class III consists of the avermectins (ivermectin, doramectin) and the milbemycins (moxidectin) that also have some effect against ectoparasites, e.g. mange mites.

Class IV anthelmintics: Salicylanilids and substituted nitrophenols. These drugs are typically used against bloodsucking parasites and are not important in pigs.

Class V anthelmintics: Acetylcholine esterase antagonists. The Class V drugs are organophosphorous compounds, which are only used to a limited extent. Examples are dichlorvos and neguvon.

Piperazines have previously been classified as Class III anthelmintics. These drugs act on the GABA receptors, causing flaccid paralysis of the worms. However, recent knowledge indicates that their mode of action is different from that of avermectins and milbymicins, and cross resistance has not been documented.

7.5.5 Which drug to use ?

It is important first to identify the nature of the parasitic problem in order to select the appropriate drug. As an example, control of *Strongyloides ransomi*, which is transmitted to piglets via colostrum, may be exerted by administration of avermectins to the sows, and this may also be the drug of choice if both helminths and mange (*Sarcoptes* spp.) should be controlled simultaneously. However, as it will be emphasized below it is important to change regularly between drugs of different anthelmintic classes, in order to delay development of anthelmintic resistance, and if anthelmintic resistance against a given drug has already developed, *all* drugs belonging to this anthelmintic class should be completely avoided.

7.6 ANTHELMINTIC RESISTANCE

7.6.1 Definition and underlying mechanism

Anthelmintic resistance is defined as a significant increase in the ability of individuals within a strain of parasites to tolerate doses of a compound which would prove lethal to the majority of individuals in a normal population of the same species.

Anthelmintic resistance constitutes a widespread and rapidly increasing problem in helminth control programmes. The mechanism behind anthelmintic resistance is simple selection. No drugs are able to remove 100 % of the parasites exposed to the drug, and a few worm individuals (the least susceptible) will survive, while the large majority (the most susceptible) will be eliminated. When selection continues repeatedly, the resistance genes will accumulate in the worm population and the drug will loose its effect. It has been shown that the general fitness of resistant worm populations is high. Therefore, once field isolates have developed a solid anthelmintic

resistance, the likelihood of reversion to susceptibility is low, and worm populations remain resistant for many years, even without further selection.

Until recently, anthelmintic resistance was primarily confined to trichostrongyle/strongyle nematodes of grazing small ruminants and horses. In these host species, resistance to one, two or more classes of anthelmintics (see Section 7.5) is now so widespread that several farmers are left without any means of drug control of helminths of grazing animals. The situation regarding helminths in pigs does not seem to be quite so serious yet, but recent European reports indicate that anthelmintic resistance may be rather common among *Oesophagostomum* spp. in housed pigs.

7.6.2 Detection of anthelmintic resistance

Many cases of anthelmintic resistance, including resistant *Oesophagostomum* strains in pigs, have been diagnosed after specific investigations rather than after experiencing a breakdown of control at farm level. This is probably attributable to the subclinical course of most helminth infections.

The *Faecal Egg Count Reduction Test* (FECRT) is the most important test to be used under field conditions, as it is applicable for all types of anthelmintics and all species of helminths in which eggs are shed in faeces. FECRT is simple to carry out:

* Collect faecal samples from 20-30 identified animals which have not been treated for at least 2-3 months (if anthelmintic resistance is suspected for *Ascaris* or *Trichuris* or other helminths with long prepatent periods, the pigs should have been left untreated for considerably longer).

* In the laboratory, the faecal samples are subjected to a McMaster egg count procedure (Chapter 3.4).

* Distribute the animals by faecal egg counts, if practicable, into 2 groups of at least 10 animals each.

* The animals of one group should be carefully weighed and dosed according to weight with the drug under suspicion (the pigs of the other group are left untreated).

* 10-14 days post treatment, new faecal samples are collected from the same individuals. Care should be taken to reduce the risk of false positive egg counts (see Section 3.7.1) by housing the pigs in clean pens.

* The faecal samples are subjected to a McMaster egg count procedure, and larval cultures are set up in order to differentiate between species which have eggs of the strongylid type (Chapter 3.5).

* Calculate the post-treatment arithmetric mean for egg counts of the treated ($\bar{x}t$) and the control group ($\bar{x}c$) and calculate the 95% confidence interval. The *Faecal Egg Count Reduction* (FECR) is 100 (1 - $\bar{x}t/\bar{x}c$).

* An anthelmintic is regarded as efficient if FECR>95%. Resistance is present if the FECR<95% *and* the 95% confidence level is less than 90%. If only one of the two criteria is met, resistance is suspected.

* Larval cultures will reveal which species are present after treatment, and possibly indicate which species that might be resistant.

If more than one drug (class of anthelmintic) is suspected to have reduced efficiency, additional treatment group(s) must be included in the trial. It is necessary to determine to which drug (class of anthelmintics) the parasites are susceptible and immediately change to an efficient drug. To ultimatively confirm the presence of anthelmintic resistance, groups of pigs may be experimentally

infected with the isolate and subsequently subjected to treatment, slaughter and worm counts. Two standard experimental designs called *Controlled slaughter assay* and *Critical slaughter assay* exist, but both are expensive and time-consuming. Additionally, a number of *in vitro* procedures have been elaborated to detect anthelmintic resistance by incubating isolated trichostrongyle/strongyle eggs or larvae in serial concentrations of drugs and thereafter measure the hatching, motility or survival of the parasites (*Egg hatch test, Larval development assay,* etc.), or by using advanced biochemical techniques (e.g. *Tubulin binding assay*). These techniques require much experience and special laboratory equipment. None of these have gained widespread application in the field.

A description of the *in vivo* methods for detection of anthelmintic resistance is found in the recommendations from the World Association for the Advancement of Veterinary Parasitology (Coles et al. 1992, *Veterinary Parasitology* 4, 35-44).

7.6.3 Risk factors for development of anthelmintic resistance

Theoretically, a series of risk factors for development of anthelmintic resistance has been recognized, and many of them have proven to be important in practice. The most essential risk factors are listed below.

Frequency of anthelmintic treatment. A number of surveys on anthelmintic resistance unanimously conclude that the more frequently parasitized animals are treated with anthelmintics, the higher the risk for development of anthelmintic resistance. If the intervals between treatments approach the prepatent period, development of resistance may be rapid, as only individuals surviving consecutive treatments will mate and produce more resistant offspring. This is probably the main reason why the problem is so widespread in especially horses, sheep, and goats, as these animal species are often treated 5-12 times a year. In comparison, pigs are normally treated 2-4 times a year, or less.

Use of the same class of drugs for extended periods. As pointed out in Section 7.5, there are many drugs and trademarks, but actually only five classes of anthelmintics exist. As anthelmintics within the same class have an identical mode of action, anthelmintic resistance developed against one drug means that the resistant worm population is also more or less resistant to the other drugs of the class (*side resistance*). Therefore, treatments with drugs of the same class for extended periods of time expose the worm population to a consistent high selection pressure. This will evidently result in a more rapid accumulation of resistant genes than if there had been a systematic alternation between drugs with different modes of action.

Time of treatment. If only repressive treatments are used, i.e. the infected pigs are treated while they remain in a heavily contaminated environment (e.g. permanent pasture), resistant worms surviving the treatment will produce resistant progeny, but due to the high number of external infective stages, the resistant genes will quickly be 'diluted' with sensitive genes. However, most integrated control programmes (e.g. strategic treatment at turnout and the dose and move system) include treatment(s) before the animals are moved to a clean pasture/pen, and consequently only survivors of the treatment will contribute to the following generations, and the frequency of resistant genes will increase more rapidly.

Dose size. Until recently, a correctly administered drug had to eliminate only 80-90% of the worm population in order to be recognized as an efficient anthelmintic. Now there is a general agreement that all worms of an anthelmintic sensitive population should be eliminated by a correct treatment. This tightening of the criteria for an efficient drug is clearly based on the above-mentioned fact that those few worms which are able to survive a treatment constitute the basis for the development of anthelmintic resistance. Similarly, underdosing has been shown to be a potential risk factor. Common reasons for underdosing are that the farmer does not know the weight of his animals, that he uses an average dose for all animals in the flock (including the heaviest individuals), or that he uses mass treatment by mixing the drug into the fodder or drinking water, whereby some individuals may get too low doses.

Pharmacokinetic behaviour of the drug. After administration, anthelmintics show varying pharmacokinetic behaviour, i.e. when and for how long time the drug concentrations are above the therapeutic level, and for how long time a subtherapeutic, but still selective, concentration persists. Furthermore, drugs will not reach identical high concentrations everywhere in the body of the host, and hence also the anthelmintic resistance selection pressure will differ with parasite species.

Spread of resistant strains. The most important way of geographical spread of anthelmintic resistance is by transport of host animals harbouring resistant worm populations.

7.6.4 Prevention of anthelmintic resistance

There is an urgent need for development and adoption of strategies to prevent anthelmintic resistance from being developed in pig helminths, and to prevent the spread of already developed (but often undiscovered) anthelmintic resistance.

Even though anthelmintic resistance in pig helminths is apparently not a widespread problem at the present, it should be recognized that new anthelmintics with a novel mode of action may possibly not be expected on the market within the next decades. Hence, the source of anthelmintics in the near future is the already existing one, and it is very important to increase the life span of these anthelmintics by reducing the risk of development of resistance.

Knowledge of risk factors (see above) provides veterinary advisers with several practical recommendations which may delay the development of anthelmintic resistance in pigs.

Reduce the dosing frequency/include alternative methods of control. Anthelmintics should be used only when necessary, and should be based on parasitological data and information about management and hygiene. In the large majority of cases anthelmintic intervention may be justified, but it should be combined with improved grazing

management, pen hygiene etc. in order to reduce the number of treatments.

Use correct doses. When anthelmintic treatment is recommended, care should be taken that the animals receive at least the full recommended dose according to live weight. If the pigs are treated individually, and not all of them are weighed, then the pigs should be treated with a dose corresponding to the live weight of the heaviest animal. A special problem arises when the pigs are treated flockwise with a drug mixed up in the fodder or the drinking water. Here it may be suspected that some pigs will be underdosed if the drug is administered for only one day, while the risk of underdosing may be reduced by administering the drug over several days in succession. Many formulations of anthelmintics are easily adulterated. Furthermore, it is strongly recommended that only registered drugs from authorized sources should be purchased.

Rotate between anthelmintic classes. When anthelmintic treatment is suggested, present information recommends that the anthelmintics from different classes (different modes of action) should be used in a rotation scheme on a yearly basis. Such a programme could start with the use of an anthelmintic from class I the first year, then a compound from class II the following year, and thereafter a drug from class III the third year. In the 4th year a benzimidazole (class I) could be used again, etc. If resistance against one class has been recorded, all drugs belonging to this class should, of course, be abandoned from the rotation scheme.

Treat new animals effectively and establish quarantine. When new animals, e.g. breeding animals, are to be introduced to the herd, they should be kept separate from the rest of the herd for the first 3-7 days. It is wise to treat them a few days before arrival and a few days after arrival, when they are still in quarantine. They should be treated with anthelmintics, possibly with two or three drug classes, each at the recommended dose.

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Helminth parasites of swine are ubiquitous. Although no precise information is available on their economic impact for pig producers, there is little doubt that they are important, causing reduced feed conversion efficiency and slower weight gain. The difficulties in substantiating the losses are associated with the fact that the infections are chronic and less dramatic than other diseases of swine. Although great improvements have been made in reducing the prevalence of the most important swine helminths, they still occur with great frequency, particularly in backyard operations in developing countries, and it is very common to find pigs infested with more than one species. Some of these parasites are also a health hazard to humans and thus cause suffering and economic losses to pork producers from condemned meat or reluctance of consumers to eat pork meat. There is therefore a need for advice and guidance in the diagnosis and control of swine parasites. This handbook reviews the epidemiology of economically important helminth parasites of swine and presents procedures and techniques for their diagnosis, survey and control. The book is designed for routine use in all types of animal health institutions where diagnostic parasitology is performed, including universities, research institutes and field laboratories. It is hoped that it 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.

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