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EPIDEMIOLOGY, DIAGNOSIS AND CONTROL OF POULTRY PARASITES

Food and Agriculture Organization of the United Nations

EPIDEMIOLOGY, DIAGNOSIS AND CONTROL OF POULTRY PARASITES

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PREFACE

Poultry products are one of the most important protein sources for man throughout the world and the poultry industry, particularly the commercial production systems have experienced a continuing growth during the last 20-30 years. The traditional extensive rural scavenging systems have not, however seen the same growth and are faced with serious management, nutritional and disease constraints. These include a number of parasites which are widely distributed in developing countries and contributing significantly to the low productivity of backyard flocks.

The handbook provide an overview of the parasites of major pathogenic and economic importance and presents procedures and techniques for their diagnosis, epidemiological studies, surveys 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 a wide distribution of the handbook will facilitate the standardization and improvement of diagnostic capabilities as well as stimulate the collection and use of epidemiological data, the foundation for effective disease control programmes.

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1 POULTRY AND PARASITES

1.1 POULTRY PRODUCTION IN CONTEXT

Poultry are kept in backyards or commercial production systems in most areas of the world. Compared to a number of other livestock species, fewer social and religious taboos are related to the production, marketing and consumption of poultry products. For these reasons poultry products have become one of the most important protein sources for man throughout the world.

The total number of poultry in the world has been estimated by the Food and Agriculture Organization of the United Nations (FAO, 1997) to be 14.718 million, with 1.125 million distributed throughout the African continent, 1.520 million in South America, 6.752 million in Asia, 93 million in Oceania, 3.384 million in North America and 1.844 million in Europe (Figure 1.1). The most commonly kept poultry are chickens (*Gallus* spp.), ducks (*Carina* spp.), geese (*Anser* spp.) and turkeys (*Meleagris* spp.). Among these, domestic chickens (*Gallus domesticus*) are the most important. This has been clearly demonstrated by numbers and the fact that during the last three decades egg production has doubled and poultry meat production has tripled, whereas the production of duck, goose and turkey meat has only recently started to expand. This expansion in poultry production is in part due to easy industrialisation, e.g. short turnover, low establishment costs and efficient disease prophylaxis, compared with production of other livestock. On a world basis, production of poultry meat has in the last 10 years increased from 20% to 30%. In Africa, poultry meat is estimated to represent almost 25% of all meat, in some areas it even accounts for 100% of the animal protein available. In Asia and Europe poultry meat accounts for more than 20% of all meat produced, whereas in North and Central America more than 40% of all meat produced is poultry meat.

Important factors in the continuing growth of the poultry industry in many countries include: the ease and efficiency of poultry to convert vegetable protein into animal protein, the attractiveness and acceptability of poultry meat, its competitive cost and the relative ease with which new technologies, such as health care systems, can be passed on to other countries and farmers.

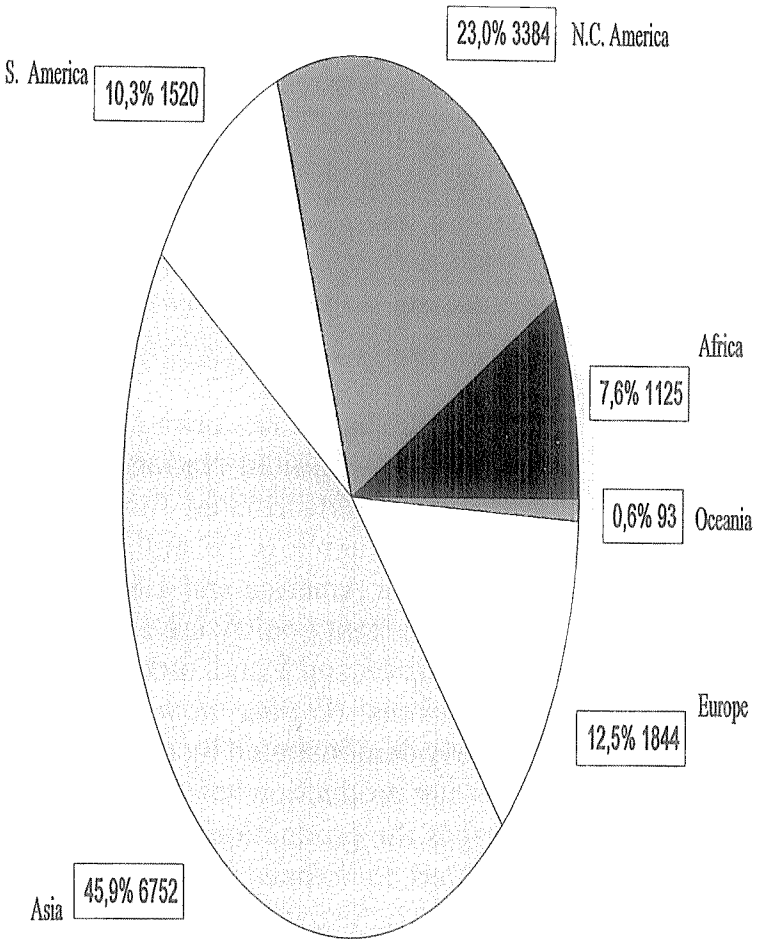

Figure 1.1 The world poultry population was estimated to be 14718 million in 1997(FAO, 1998). On all continents the most commonly kept poultry is the domestic chicken (*Gallus domesticus*).

1.2 POULTRY PRODUCTION SYSTEMS

Basically there are two production systems. The modern commercialized intensive system and the traditional extensive or rural scavenging system with numerous examples of modified systems in-between the two. Improved traditional systems are often called semi-scavenging production systems. Approximately 80% of the chickens in developing countries are kept in traditional, rural scavenging systems. These are characterized by few inputs in terms of housing, feeding and disease control. The output in terms of number of eggs is low, e.g., 40 - 60 eggs per hen/year and the weight gain for broilers is low with excess time (more than 6 months) required to produce a ready to slaughter broiler. Intensive commercial systems are costly, labour intensive and sophisticated in terms of housing, feeding and disease prophylaxis: Nevertheless, the output is high with regard to number of eggs per hen and weight gain for broilers, e.g., 280 - 320 eggs per hen/year and only 35 - 40 days needed to get broilers to their slaughter weight.

In many tropical areas traditional poultry production is often described as a low input/low output system, where poultry flocks of 10-20 animals are left scavenging around the house during daytime. Here they obtain what feed they can get from the environment such as insects and seeds. In addition they may be given leftovers from the kitchen and other types of offal.

In contrast to the traditional poultry production, modern poultry industry is concentrated on few big farms with flock sizes in the range of 5000-250000 (or more) animals. Total confinement, improvement of cleaning and disinfection procedures, production according to the "all in - all out" principle, and furthermore the prophylactic use of vaccines and drugs has reduced the significance of diseases in modern industrial poultry production.

1.3 CONSTRAINTS TO THE POULTRY PRODUCTION

The enormous expansion in the commercial poultry production sector has been possible through improved management in terms of management procedures such as total separation between different age groups, introduction of the "all in - all out" system, efficient housing systems,

routine vaccination programmes, proper feeding and avoidance of predators. The mortality in commercial systems is in the range of 10% or less per year. However, development of resistant infectious agents (e.g., *E.coli*, *Salmonella*, *Eimeria* spp. etc.) is introducing new problems to the commercial sector. Especially the aspect of zoonotic diseases such as *Salmonella* and *Campylobacter* is of great concern. The widespread use of drugs have led to resistance towards a range of antibacterial and anthelmintic drugs. Furthermore, concern about residues in poultry products is growing among consumers.

The low productivity in traditional systems is mainly due to high mortality, which is caused by mismanagement, diseases, lack of nutritional feeding and predators. In traditional systems the mortality has been estimated to be in the range of 80 - 90% within the first year after hatching! Specially chicks under 3 months of age have high mortality rates. This means that populations can hardly survive.

1.4 DISEASES

Diagnosis, treatment and/or prevention of diseases are of crucial importance, and in commercial production systems chickens are therefore routinely vaccinated against all major diseases such as Newcastle Disease, Infectious Bursal Disease (Gumboro), Mareks Disease, Infectious bronchitis (IB), Avian Influenza and others, depending on the specific disease situation in each country.

Table 1.1 Some commonly encountered diseases in poultry, excluding parasitic infections.

Viral infections	Bacterial infections	Others
Infectious Bronchitis	Avian Salmonellosis	Fungal infections
Newcastle Disease	Other Salmonella infections	Poisons and toxins
Infectious Laryngotracheitis	Avian Pasteurellosis	Nutritional deficiencies
Avian Encephalomyelitis	Avian Tuberculosis	Congenital disorders
Avian Influenza	Infectious Coryza	
Avian Adenovirus Infections	Avian Mycoplasmosis	
Leucosis/Sarcoma group	Erysipelas	
Mareks Disease	*Staphylococcos aureus*	
Infectious Bursal Disease	*Streptococcos* spp.	
Avian Pox	Chlamydiosis	
Chicken Anaemia Agent	*Escherichia coli*	
Duck Virus Hepatitis	*Campylobacter* spp.	

In the traditional system disease prophylaxis is rare. Among diseases in rural chickens, viral infections such as Newcastle Disease have attracted most attention, but resent studies have shown that a wide range of viral, bacterial and parasitic diseases are present in these systems. It appears that the prevalence of viral diseases such as Gumboro, Mareks Disease, Infectious bronchitis, avian influenza and chicken anaemia agent are higher than earlier presumed and have attracted far too little attention. Also bacterial agents such as *Pasteurella multocida* (Fowl cholera), *Salmonella* spp., *E.coli*, *Haemophilus paragallinarum* (Coryza), *Mycoplasma* spp., and others are very important (Table 1.1). In addition parasitic infections are frequent in traditional systems with prevalences close to 100 %. Knowledge related to parasitic infections is scarce and research in this area is almost non existing. This manual, therefore, will focus on the life cycle, epidemiology, diagnosis and control of parasitic infections.

1.5 PARASITIC DISEASES IN POULTRY

The prevalence of most parasitic diseases in poultry seems to have been reduced significantly in commercial indoor poultry production systems due to improved housing, hygiene and management. However, parasitic diseases continue to be of great importance in deep-litter and free-range commercial systems. In traditional systems throughout the world a number of parasites are widely distributed and contribute significantly to the low productivity. The most commonly mentioned parasites are *Eimeria* spp., *Ascaridia galli* and *Heterakis gallinarum* which is mainly due to the many studies carried out on these parasites.

1.5.1 The prevalence of parasitic diseases in various poultry production systems

Although it is known that parasites constitute a health problem in poultry, there are only a few reports on the prevalence and significance of endo-, ecto- and haemo-parasites in the different poultry production systems. From searches performed on databases such as CAB ABSTRACTS, AGRIS, AGRICOLA, MEDLINE AND DIALOG a number of references have been selected and compiled in Table 1.2.

Table 1.2 The prevalence (in %) of various parasitic diseases in Africa, Asia, the Americas and Europe reported in selected references.

Parasite	Africa	Asia	Americas	Europe
Oxyspirura mansoni	-	52.6	reported	-
Syngamus trachea	23.1	20	reported	reported
Gongylonema ingluvicola	68	2.3	1	-
Tetrameres spp.	66.7	2.3	10	-
Dispharynx nasuta	25	18	reported	-
Acuaria hamulosa	46.7	4	1	-

Parasite	Africa	Asia	Americas	Europe
Amidostomum anseris	-	reported	reported	-
Capillaria spp.	34.3	13.5	60	56.3
Ascaridia galli	66.7	60.5	90	63.8
Ascaridia columbae	23.2	-	reported	reported
Heterakis spp.	90.7	89	90	72.5
Allodapa suctoria	68.5	81.8	reported	-
Strongyloides avium	3.9	0.6	reported	reported
Acanthocephalus spp.	24	-	-	-
Metroliastes lucida	1	-	60	-
Raillietina spp.	81.4	84.2	69.2	3.3
Davainea proglottina	5.7	8	10	reported
Choanotaenia infundibulum	16.2	7	reported	3.3
Hymenolepis spp.	57.7	6.5	5	-
Amoebotaenia cuneata	39	12.7	reported	-
Cotugnia digonopora	47.8	20.8	-	-
Echinostoma spp.	-	reported	-	-
Prosthogonimus spp.	3.6	1.1	-	-
Brachylaemus commutatus	3.4	-	-	-
Notocotylus	-	6.6	-	-
Eimeria spp.	72	70	reported	reported
Histomonas meleagridis	-	1.1	-	reported
Plasmodium spp.	37.2	reported	-	-
Leucocytozoon spp.	97	1.1	-	-
Trichomonas spp.	73	-	-	-
Aegyptinella spp.	41.9	5.5	-	-
Haemoproteus spp.	-	4.5	-	-

Parasite	Africa	Asia	Americas	Europe
Cryptosporidium spp.	100	-	-	-
Sarcocystis spp.	6.6	-	-	-
Toxoplasma gondii	-	33.3	-	-
Argas spp.	41	1.1	-	-
Dermanyssus gallinae	39.2	reported	-	67
Cnemidocoptes mutans	12	-	-	reported
Mallophaga (lice)	100	74.9	1	reported
Siphonaptera (fleas)	50.8	reported	-	reported

In Africa and Asia most of the studies have been concerned with extensive backyard systems, whereas the studies from the Americas and Europe were carried out mainly on commercial systems. This might be the reason for the low number of parasites reported from Europe and the Americas compared to Africa and Asia. Furthermore, it should be noted that this table only represents the work that **has** been done. The missing figures might actually not reflect the true situation, but only indicate that studies have not been carried out in order to detect possible parasitic infections in poultry.

2 PARASITE GROUPINGS

In this manual it has been found convenient to divide the parasites into three broad groups with subgroups. These groups are **Endoparasites** (Table 2.1), **Ectoparasites** (Table 2.2) and **Haemoparasites** (Table 2.3). Furthermore, the three most important commercial bird species have been pictured in association with their most common parasites (Figure 2.1 to 2.3)
The list is not complete as it only includes those species which have a pathogenic and an economic importance. For a complete list please be referred to E.J.L. Soulsby (1982): Helminths, arthropods and protozoa of domesticated animals or other specialised books as mentioned under reference books (→ 9).

Table 2.1 Endoparasites of importance in poultry production.

Parasite	Hosts	Predilection site
Nematodes		
Oxyspirura mansoni	chickens, turkeys, guineafowls, peafowls	eye, lacrimal duct
Syngamus trachea	pheasants, chickens, turkeys, geese, guineafowls, quails, peafowls	trachea, lungs
Gongylonema ingluvicola	chickens, turkeys, partridges, pheasants, quails	oesophagus, crop
Tetrameres spp.	chickens, turkeys, ducks, grouse, pigeons, quails, guineafowls, geese,	proventriculus
Dispharynx nasuta	chickens, turkeys, grouse, guineafowls, partridges, pheasants, pigeons, quails	oesophagus, proventriculus
Acuaria hamulosa	chickens, turkeys, grouse, guineafowls, pheasants, quails	gizzard
Amidostomum anseris	ducks, geese, pigeons	gizzard

Nematodes		
Capillaria spp.	chickens, turkeys, geese, grouse, quails, guineafowls, partridges, pheasants, pigeons	entire intestinal tract
Ascaridia galli	chickens, turkeys, doves, ducks, geese	small intestine, occasionally oviduct
Ascaridia dissimilis	turkeys	small intestine
Heterakis spp.	chickens, turkeys, ducks, geese, grouse, guineafowls, partridges, pheasants, quails	caeca
Allodapa suctoria	chickens, turkeys, doves, ducks, grouse, guineafowls, partridges, pheasants, quails	caeca
Cestodes		
Raillietina spp.	chickens, turkeys, guineafowls, pigeons	small intestine
Davainea proglottina	fowls, pigeons	small intestine
Choanotaenia infundibulum	fowls, turkeys	small intestine
Hymenolepis spp.	fowls, ducks, geese	small intestine
Trematodes:		
Echinostoma revolutum	ducks, geese	rectum, caeca
Prosthogonimus spp.	fowls, ducks, geese	bursa Fabricius, oviduct, cloaca, rectum
Protozoa:		
Eimeria spp.	chickens, turkeys, ducks	small intestine
Histomonas meleagridis	turkeys, chickens,	caeca, liver

Table 2.2 Ectoparasites of importance in poultry production.

Parasite	Hosts	Predilection site
The fowl tick: *Argas persicus*	chickens, turkeys, pigeons, ducks, geese	skin
Mites: *Dermanyssus gallinae, Ornithonyssus sylviarum, O.bursa*	chickens, turkeys, ducks, wild birds	skin
Mite: *Cnemidocoptes mutans*	chickens, turkeys	under the skin on legs, occasionally on comb and wattles
Flea: *Echidnophaga gallinacea*	chickens and other birds	head

Table 2.3 Haemoparasites of importance in the poultry production.

Parasite	Hosts	Predilection site
Leucocytozoon spp.	chickens, ducks, geese, turkeys	leucocytes, erythrocytes
Plasmodium spp.	chickens, turkeys	erythrocytes
Haemoproteus spp.	ducks, geese, chickens	erytrocytes
Aegyptinella spp.	chickens, turkeys, ducks, geese	erythrocytes

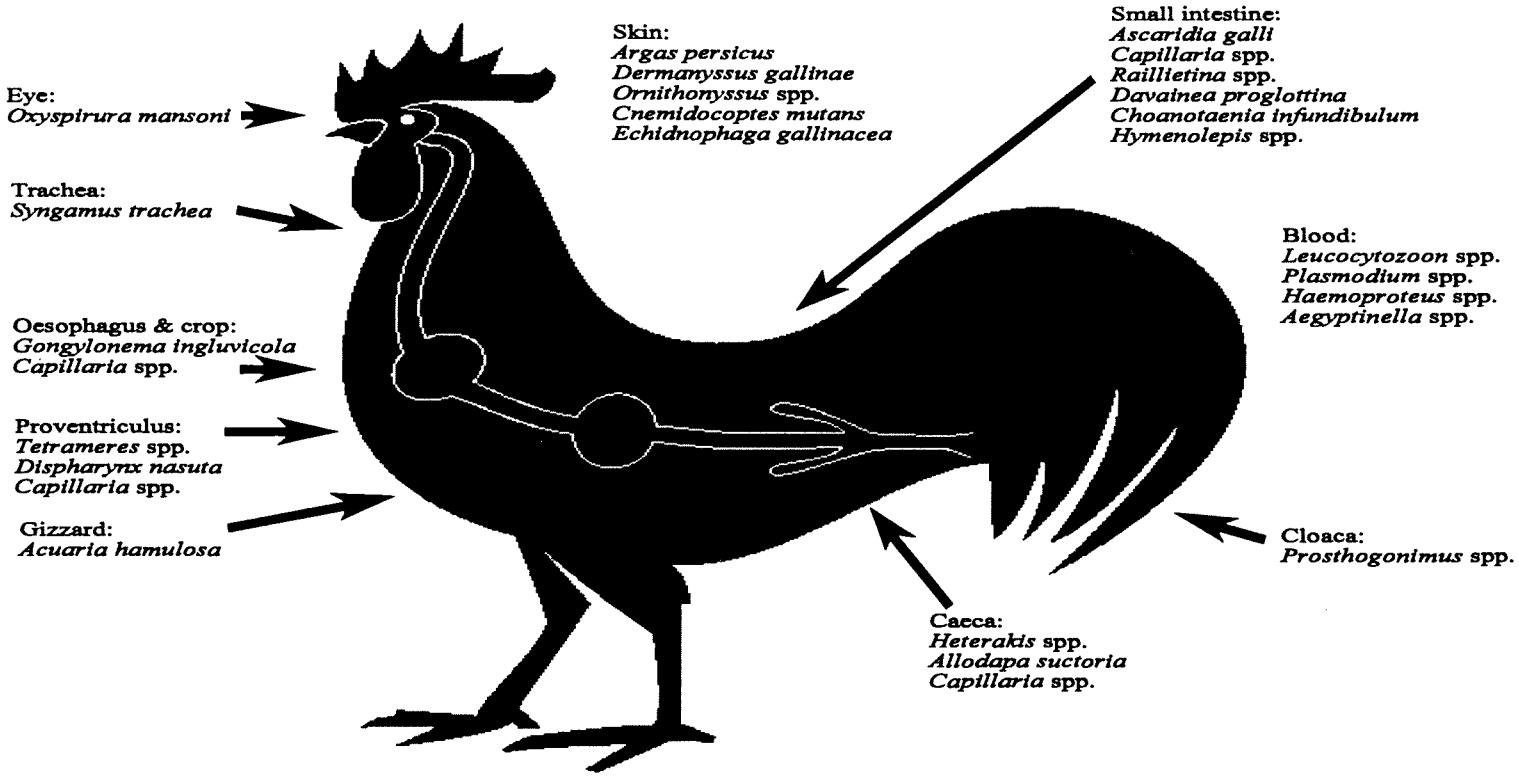

Figure 2.1 Common parasites in chickens

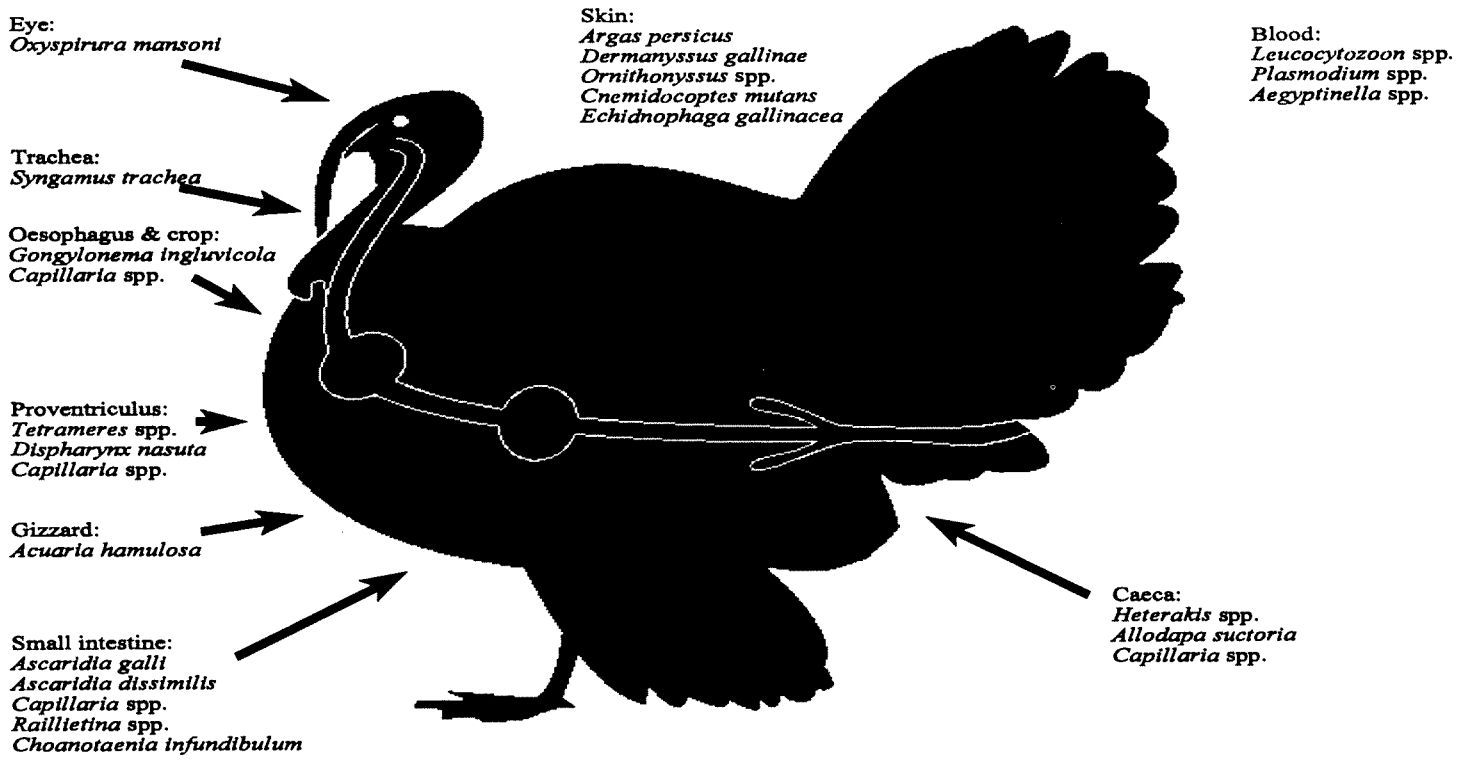

Figure 2.2 Common parasites in turkeys

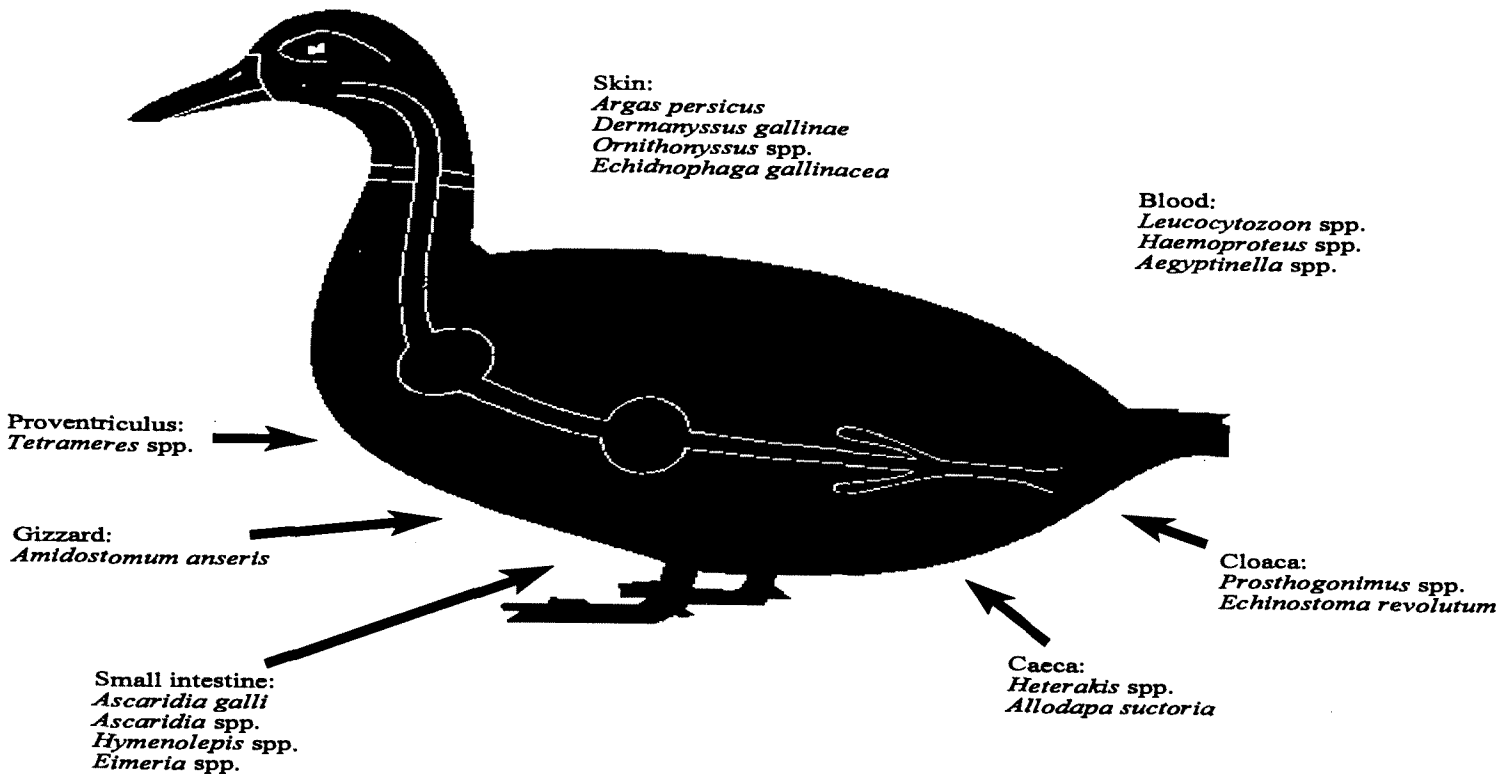

Figure 2.3 Common parasites in ducks

3 LIFE CYCLE AND EPIDEMIOLOGY OF POULTRY PARASITES

This chapter will in short describe the primary hosts, the predilection site of the parasites, morphology, the life cycle, epidemiology, clinical signs and the pathogenicity of the parasites of economic importance in poultry production. An accurate identification of the parasites is essential in order to design effective prophylactic measurements or treatment. Identification at species level may give directions to control measures aiming at eliminating the intermediate hosts and thus break the life cycle. Endoparasites with a direct life cycle may require treatments with anthelmintics or efficient hygienic measurements (as seen in some commercial indoor systems).

3.1 NEMATODES

Nematodes are the most common and most important helminth species in poultry. More than 50 species have been described in poultry. Of these the majority causes pathological damage to the host.
Nematodes belong to the phylum Nemathelminthes, class Nematoda. The nematodes of poultry are parasitic, unsegmented worms. The shape is usually cylindrical and elongated, but the cuticle may have circular annulations, be smooth, have longitudinal striations or ornamentations in the form of cuticular plagues or spines. All worms have an alimentary tract. The sexes are separate. The life cycle may be direct or indirect including an intermediate host.

3.2 NEMATODES OF THE DIGESTIVE TRACT

3.2.1 *Gongylonema ingluvicola* Ransom, 1904

Hosts: *G. ingluvicola* has been reported in chickens, turkeys, partridges, pheasants and quails in North America, Europe, Africa, Asia and Australia.

Predilection site: The adult nematodes are embedded in the epithelium of the crop, oesophagus and sometimes the proventriculus.

Morphology: The female worm is 32 - 55 mm long and the males measure 17 - 20 mm. The anterior end of the body has a varying number of characteristic round or oval thickenings called cuticular plagues on the cuticle (Figure 3.1). The eggs measure approximately 35 x 58 μm.

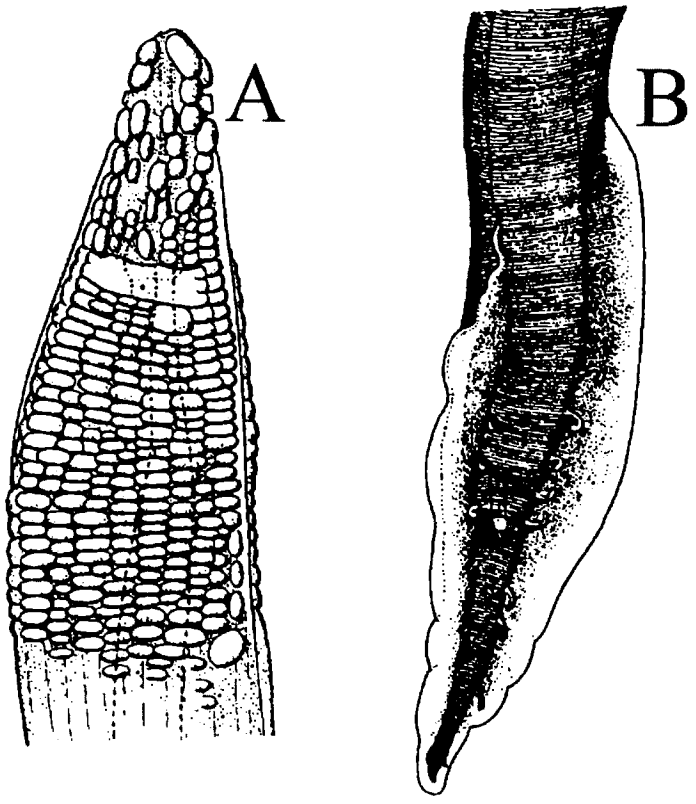

Figure 3.1 A: Head of *Gongylonema ingluvicola* B: Tail of male *G. ingluvicola* (Redrawn after Cram 1927).

Life cycle and epidemiology: The life cycle is indirect, including beetles of the species *Copris minutus* or cockroaches (*Blatella germanica*) as intermediate hosts. After eggs have been passed in the faeces they are occasionally eaten by beetles or cockroaches. After 30 days of development in the intermediate host the larvae become infective. Fowl become infected by eating the intermediate host containing the infective L_3 stages.

Clinical signs and pathogenicity: The adult parasites are moderately pathogenic, depending on the number of worms embedded in the epithelium. There may be an inflammation and hypertrophy with cornification of the epithelium in chronic infections.

3.2.2 *Tetrameres* spp.

T. americana Cram, 1927 and *T. fissispina* (Diesing, 1861).

Hosts: *T. americana* is a common parasite in chickens, turkeys, ducks, geese, grouse, pigeons and quails in North America and Africa. *T. fissispina* is a common parasite in chickens, turkeys, ducks, guineafowls, geese and pigeons worldwide.

Predilection site: The adult worm is found in the proventriculus where the worms are embedded deep in the glands. The females are easily seen from the serosal surface as dark red spots.

Morphology: There is a distinct sexual difference. The males are 5 - 5.5 mm long and 116 - 133 μm wide. The female is spherical and measure 3.5 - 4.5 mm in length by 3 mm in width. Four longitudinal furrows are present on the surface (Figure 3.2). The eggs measure 42 - 50 x 24 μm.

Life cycle and epidemiology: The eggs are passed with the faeces and hatch when swallowed by intermediate hosts such as grasshoppers (*Melanoplus femurrubrum* or *M. differentialis*) or cockroaches (*Blatella germanica*). Infection of the final host occurs when the intermediate host is eaten. Soon after ingestion the males and females migrate to the proventriculus where they embed themselves in the glands. After copulation the males leave the glands and die.

Clinical signs and pathogenicity: Infected fowls lose weight and become anaemic. In chickens with heavy infections (more than 10 females embedded in the glands) the proventriculus becomes thickened and edematous, and the lumen may be partially obstructed.

Other species: *T. crami* in wild and domestic ducks, *T. pattersoni* in quails.

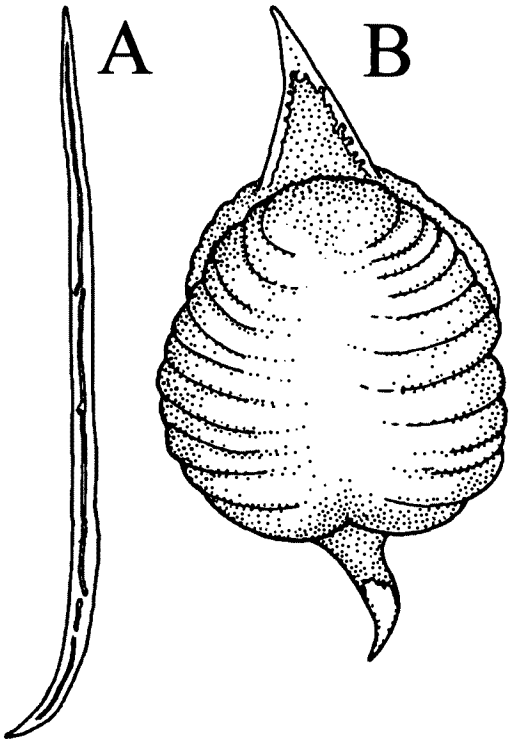

Figure 3.2 *Tetrameres americana* A: Male
B: Female (Redrawn after Cram 1927).

3.2.3 *Dispharynx nasuta* (Rudolfi, 1819)

Syn. *D. spiralis*, *Acuaria spiralis*

Hosts: Occurs in chickens, turkeys, grouse, guineafowls, partridges, pheasants, pigeons and quails in North and South America, Africa and Asia.

Predilection site: *D. nasuta* is located in the proventriculus and oesophagus, rarely in the small intestine.

Morphology: The males are 7 - 8.3 mm long and the females are 9 -10.2 mm. They have four characteristic cuticular cordons which recurve but do not anastomose or fuse and have a wavy pattern to the anterior end. The left male spicule is long and slender, 0.4 - 0.52 mm, and the right spicule is 0.15 - 0.2 mm (Figure 3.3). The vulva is found towards the posterior end. Eggs measure 33 - 40 x 18 -25 μm and are embryonated when passed.

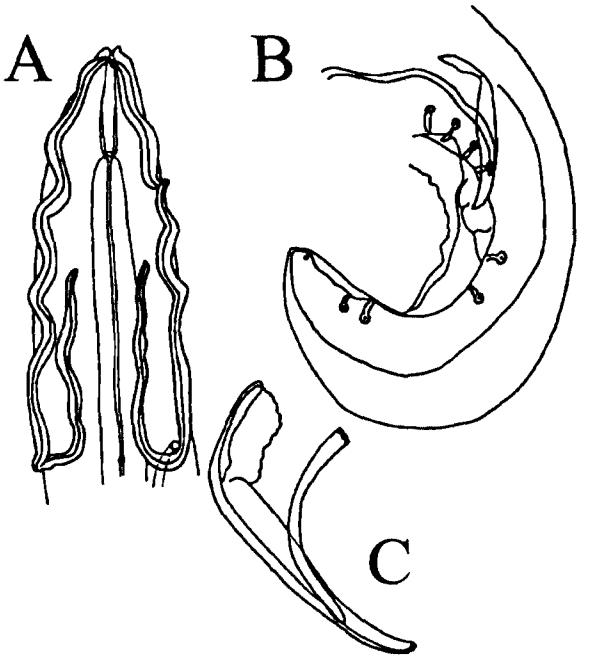

Figure 3.3 *Dispharynx nasuta*. A: Anterior end of male B: Male posterior end C: Male spicules (Redrawn after Gupta 1960).

Life cycle and epidemiology: The life cycle is indirect with pillbugs (*Armadillidium vulgare*), sowbugs (*Porcellio scaber*) or other isopods as intermediate hosts. When the embryonated eggs are laid and ingested by an intermediate host, the larvae develop in the body cavity of the isopod. When eaten by the final host the adult worms develop in the proventriculus.

Clinical signs and pathogenicity: Depending on the severity of the infection the birds become emaciated, weak and anaemic. In light infections inflammation and hypertrophy of the mucosa are seen. In heavy infections the adult worms penetrate the mucosa creating deep ulcers, inflammation and hypertrophy of the mucosal layer. Furthermore, destruction of the glands is seen.

3.2.4 *Acuaria hamulosa* (Diesing, 1851)

Syn. *Cheilospirura hamulosa*

Hosts: A. *hamulosa* occurs in chickens and turkeys in North and South America, Europe, Africa and Asia.

Predilection site: The adult worms are embedded in nodules or abscesses under the keratinised layer of the gizzard.

Morphology: The males measure 10 - 14 mm and the females 16 - 29 mm. They have four long cuticular cordons giving the worms a characteristic appearance (Figure 3.4). The cordons are irregular and wavy, extending approximately 2/3 down the body. The males have a long and slender spicule on the left side measuring 1.63 - 1.8 mm and on the right side only 0.23 - 0.25 mm long.

Life cycle and epidemiology: The life cycle is indirect. Grasshoppers of the species *Melanoplus*, *Oxya nitidula* or *Spathosternum prasiniferum*, various beetles, sandhoppers and weevils may act as intermediate hosts. The eggs are excreted with the faeces and develops into an infective stage (larva) after 3 weeks in the intermediate host. The final host is infected when ingesting the intermediate host. The prepatent period is three weeks.

Clinical signs and pathogenicity: Heavy infections cause droopiness, weakness, anaemia and emaciation. Rupture of the gizzard may occur. The worms are found lying in soft, yellow-red nodules. The keratinised layer may be destroyed or even necrotic in severe infections.

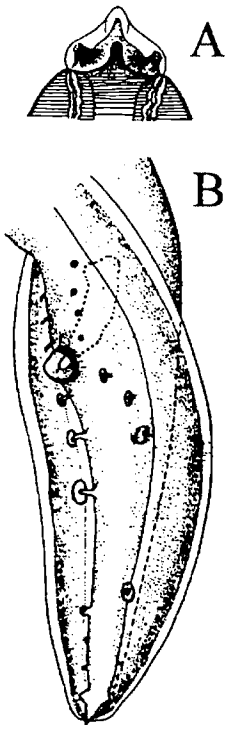

Figure 3.4 *Acuaria hamulosa* A: Head
B: Tail (Cram 1927).

3.2.5 *Amidostomum anseris* (Zeder, 1800)

Syn. *A. nodulosum*

Hosts: *A. anseris* is a cosmopolitan parasite in domestic and wild geese, ducks and pigeons.

Predilection site: The worm is found under the keratinised layer of the gizzard, in the proventriculus or in the oesophagus.

Morphology: The adult worm is slender and red in colour (Figure 3.5). The male is 10 - 17 mm long and 250 - 350 μm wide. The spicules are equal in length, 0.2 - 0.3 mm, both branching at the end. The female is 12 - 24 mm long, 200 - 400 μm wide with the thickest point around the vulva. The eggs contain an embryonated larvae when laid and measure approximately 100 x 50 μm.

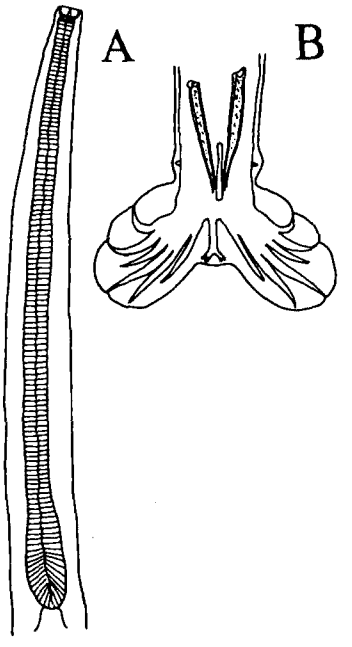

Figure 3.5 *Amidostomum anseris*
A: Head B: Male tail (Redrawn after Boulenger 1926 and Railliet 1909).

Life cycle and epidemiology: The life cycle is direct with a prepatent time of 14 to 25 days. After being deposited in the environment with the faeces, the larvae develop into the infective 3rd stage larvae in two to three days. The development may happen inside the egg or outside. Susceptible animals become infected by ingesting or drinking contaminated food or water.

Clinical signs and pathogenicity: Clinical signs include loss of appetite, emaciation, weakness and anaemia. The birds may develop diarrhoea and die if stressed. The worms are very pathogenic to young animals, while older animals become carriers. The adult parasite cause severe inflammation, haemorrhages and necrosis. Extreme blood losses may occur in heavily infected birds.

3.2.6 *Capillaria* spp.

Six species are commonly found in poultry: *C. annulata* (Molin, 1958). *C. contorta* (Creplin, 1839). *C. caudinflata* (Molin, 1858). *C. bursata* Freitas and Almeida, 1934 *C. obsignata* (Madsen, 1945), Syn. *C. columbae* and *C. anatis* (Schrank, 1790), Syn. *A. brevicollis*, *C. retusa*, *C. collaris*, *C. anseris*, & *C. mergi*.

Hosts: All six species have been reported to occur in domesticated and wild birds. Furthermore, all species are cosmopolitan in their distribution.

Predilection site: The *Capillaria* species are located throughout the intestinal tract. *C. annulata* and *C. contorta* are found in the crop and in the oesophagus. *C. caudinflata*, *C. bursata* and *C. obsignata* parasitizes the small intestine, whereas *C. anatis* occurs in the caeca.

Morphology: The worms of this genus are small and hairlike and difficult to detect in the intestinal content. The *C. annulata* males are 15 - 25 mm long and the females are 37 - 80 mm long. The characteristic eggs have bipolar plugs and measure 60 x 25 μm. *C. contorta* males are equal in size to the males of *C. annulata*, but the females are shorter only measuring 27 - 38 mm. The eggs of *C. contorta* are app. 60 x 25 μm. *C. caudinflata*, *C. bursata*, *C. obsignata* and *C. anatis* are all smaller only measuring 6 - 35 mm. The eggs measure 45 x 25 μm. For details concerning differentiation between the six species see Figure 3.6.

Life cycle and epidemiology: The life cycles of the *Capillaria* species may be direct or indirect. The eggs are deposited with the faeces unembryonated and develop into the first larval stage in 9 to 14 days. For *C. obsignata*, *C. anatis* and *C. contorta* the life cycle is direct, which means that the eggs are infective to susceptible hosts as embryonated L_1. After ingestion the eggs hatch at their predilection site and develop into adult worms without migration in the host. Eggs of the species *C. caudinflata*, *C. bursata* and *C. annulata* are swallowed by earthworms and develop into infective stages in 14 - 21 days. Birds are infected when ingesting the earthworms. The prepatent time for *Capillaria* spp. is approximately 3 weeks.

Clinical signs and pathogenicity: Infections with *Capillaria* spp. can be highly pathogenic for birds kept in deep-litter systems or in free-range

systems where big numbers of infective eggs may build up in the litter or in the soil. Light infections with *C. contorta* and *C. annulata* produce inflammation and thickening of the crop and oesophagus. Heavy infections produce marked thickening of the oesophagus and crop wall with catarrhal and croupous inflammation.
When infections occur in the small intestine or in the caeca (*C. caudinflata*, *C. bursata*, *C. obsignata* or *C. anatis*) the animals become emaciated, weak and anaemic. Bloody diarrhoea with haemorrhagic enteritis is seen in heavy infections. *C. obsignata* infections are very pathogenic in pigeons and may cause high mortality rates.

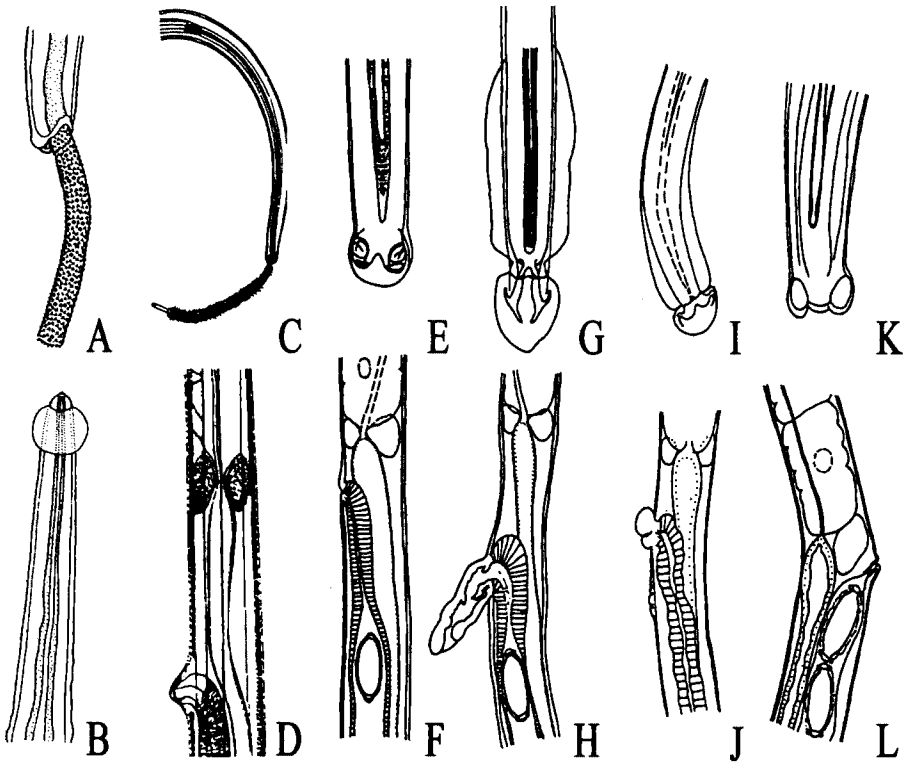

Figure 3.6 *Capillaria* spp. A, C, E, G, I & K: Male bursa. B: Female head. D, F, H, J & L: Female vulva. A & B: *C. annulata*, C & D: *C. contorta*, E & F: *C. obsignata*, G & H: *C. caudinflata*, I & J: *C. bursata*, K & L: *C. anatis* (Redrawn after Ciurea, Travassos 1915 and Wakelin 1965).

3.2.7 *Ascaridia galli* (Schrank, 1788)

Syn. *A. lineata*, *A. perspicillum*.

Hosts: *A. galli* is found in chickens, turkeys, geese, guinea fowl and a number of wild birds, the principal host presumably being the chicken. The first description of *A. galli* was made in Germany. Later, it was reported in chickens in Brazil, India, Zanzibar, The Philippines, Belgian Congo (Democratic Republic of Congo), China, Canada and the U.K. *A. galli* has subsequently been described in material collected from a number of countries in temperate, subtropical and tropical climates and is said to be a worldwide infection in poultry.

Predilection site: *A. galli* is a nematode occurring in the small intestine. In heavy infections, *A. galli* may cause partial or total obstruction of the duodenum or the jejunum. Adult worms may migrate through the lumina of the large intestine and cloaca and end up in the oviduct, where they can be incorporated into the hen's egg.

Morphology: The adult worms are semitransparent, the length of the female ranging from 72 - 116 mm and the male from 51 - 76 mm, and are therefore the biggest nematode in poultry (Figure 3.7). The oral opening has three prominent lips. The male with preanal sucker and two equal spicules of 1 - 2.4 mm long. The female open in the middle of the body. *A. galli* eggs are oval, with smooth shells and measure 73-92 by 45-57 μm. *H. gallinarum* eggs are similar in shape and appearance, but can be distinguished from *A. galli* eggs by their slightly smaller and parallel sides.

Life cycle and epidemiology: The life cycle of *A. galli* is direct, involving two principal populations; the sexually mature parasite in the gastrointestinal tract and the infective stage (L_3) in the form of an embryonated resistant egg in the environment (Figure 3.8). The eggs are passed with the faeces of the host and develop in the open, reaching the infective stage (L_3) in 10 to 20 days or longer depending on temperature and relative humidity, e.g. the minimum time required to reach the infective stage is five days at 32-34°C when the eggs are incubated in water. At temperatures between -12°C to -8°C, the eggs may die after 22 hours, however, the eggs can

survive a winter with moderate frost. Temperatures above 43 °C are lethal for eggs at all stages. In deep litter systems the eggs can probably remain infective for years depending on the temperature, humidity, pH and ammonium concentration. Occasionally earthworms can ingest *A. galli* eggs and transmit these to chickens, but this is not the principal route of transmission.

The life cycle is completed when the infective eggs are ingested by new hosts through contaminated water or feed. The eggs containing the L_3-larvae are mechanically transported to the duodenum. The larvae are protected by the three layers covering the eggs until they reach the duodenum or jejunum, where they hatch within 24 hours. During hatching the coiled larvae emerge from the anterior end of the egg through an opening in the shell moving out into the lumen of the intestine. The larvae then enter the histotropic phase where they embed themselves into the mucosal layer of the intestine. The histotropic phase has a duration of 3 to 54 days before the final maturation in the lumen. The histotropic phase is a normal part of the life cycle, where the duration of the histotropic phase depends on the number of ingested infective eggs. The more eggs the longer the histotropic phase. After the histotropic phase the worms settle down in the lumen of the duodenum. The prepatent period varies from 5-8 weeks.

Few epidemiological studies have been carried out to investigate the infection and transmission of *A. galli*. It is generally accepted that the establishment of worms in the intestine is influenced by many factors such as the age of the chicken, the size of the infective dose, the age of the infective eggs, the sex of the chickens, and the diet of the host.

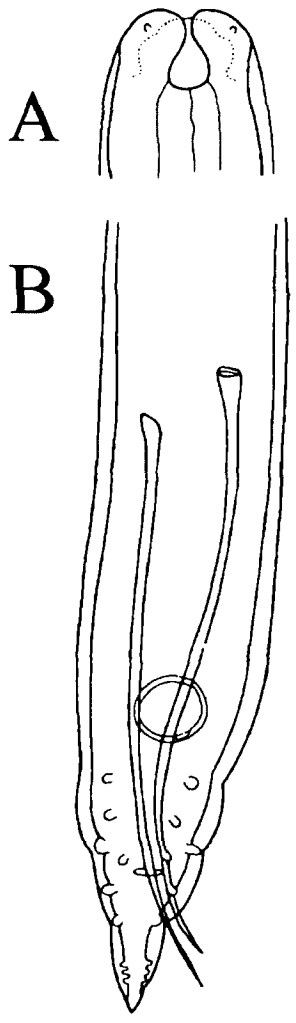

Figure 3.7 *Ascaridia galli* A: Anterior end B: Posterior end of male.

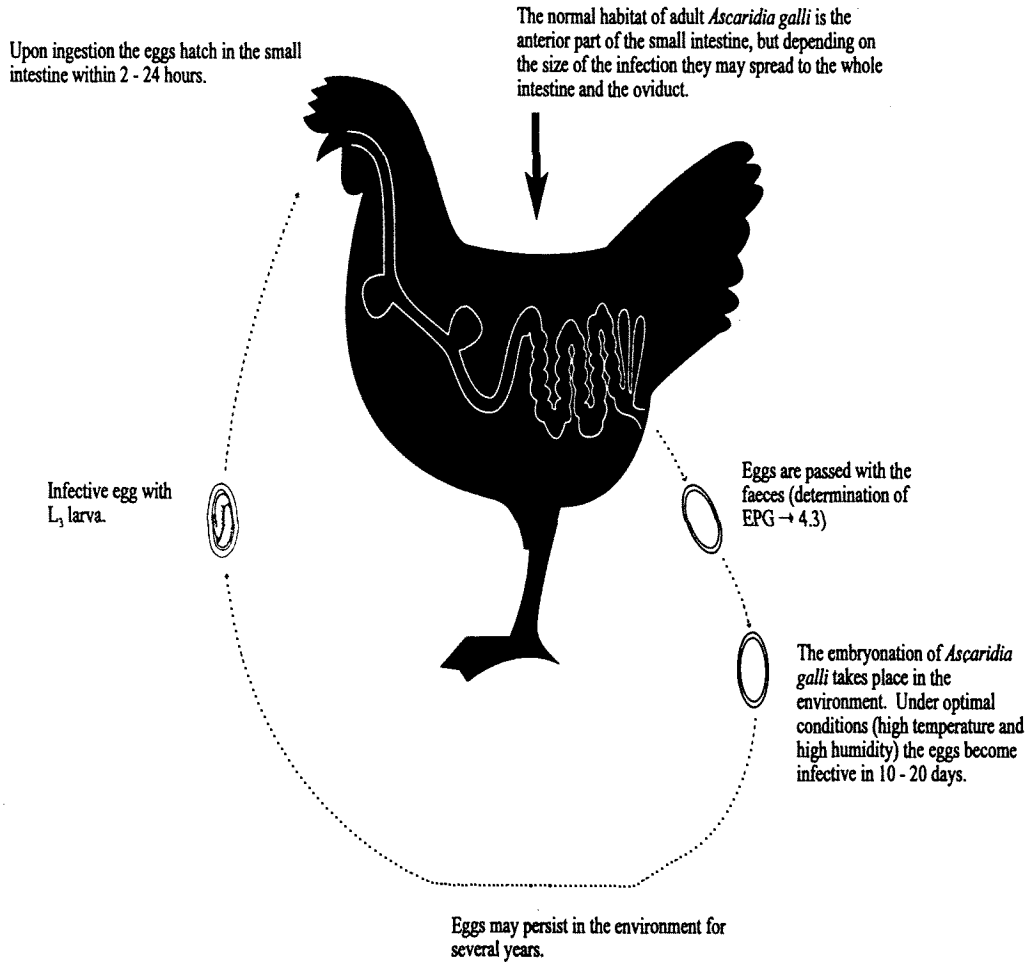

Figure 3.8 The life-cycle of *Ascaridia galli*. The life-cycle is direct. Occasionally earthworms can act as transport hosts. The prepatent time varies from 40 - 60 days (Permin & Ikjær 1998).

Clinical signs and pathogenicity: Infections with *A. galli* result in weight loss in the chicken, which correlates with an increasing worm burden. Clinical signs are more pronounced in chickens up to three months of age, after which the worm burden normally decreases. Generally, the clinical signs include loss of appetite, drooping wings, ruffled feathers, loss of weight, decreased egg production, anaemia, diarrhoea and mortality.

Enteritis or haemorrhagic enteritis may be seen when large numbers of young parasites penetrate the duodenal or jejunal mucosa. The duration of the histotropic phase is dose-dependent and may last up to 54 days after single dose infections. The embedded larvae cause haemorrhage and extensive destruction of the glandular epithelium. Furthermore, a proliferation of mucous-secretory cells may result in adhesion of the mucosal villi. Damage to the epithelia may not only be caused by the larvae, but also by the adult worms in the form of pressure atrophy of the villi with occasional necrosis of the mucosal layer. In chronic infections a loss of muscle tonus may be seen, and the intestinal wall may assume a flabby appearance when viewed *in situ*. During the histotropic phase, there is loss of blood, reduced blood sugar and the ureters frequently become distended with urates.

3.2.8 *Heterakis* spp.:

Three species are believed to be of importance in poultry. These are: *H. gallinarum* (Schrank, 1788), (Syn. *H. papillosa*, *H. vesicularis*, *H. gallinae*). *H. isolonche* von Linstow, 1906, (Syn. *H. bonasae*) and *H. dispar* (Schrank, 1870).

Hosts: *H. gallinarum* has been reported in chickens, turkeys, ducks, geese, grouse, guineafowl, partridges, pheasants and quails from most parts of the world. *H. isolonche* occurs globally in ducks, grouse, pheasants, quails and chickens. *H. dispar* affects chickens, geese and ducks in various parts of the world.

Predilection site: All three species are found in the lumen of the caeca. Larvae of *H. isolonche* live in the mucosa of the caeca before they, as adults, live in the lumen of the caeca.

Morphology: The three species are similar in appearance, *H. dispar,* though slightly larger than *H. gallinarum* and *H. isolonche*. The male *H. gallinarum* is 7 - 13 mm long and the female is 10 - 15 mm. Differentiation between the three species is based on the shape of the oesophagus and the length and shape of the spicules (Figure 3.9). The eggs measure 65 - 80 x 35 - 46 μm, and they have a thick, smooth shell and are difficult to differentiate from *A. galli* eggs.

Life cycle and epidemiology: The life cycle is direct. Earthworms and houseflies can act as mechanical transport hosts. The non-embryonated eggs pass out with the faeces and develop into infective eggs in approximately 2 weeks, depending on temperature and humidity. When infective eggs are ingested by susceptible hosts, the eggs hatch in the small intestine. Within 24 hours the larvae have reached the caeca through the lumen of the intestine where they develop into adult worms. *H. isolonche* larvae may have a tissue phase before becoming adult worms. The prepatent time is 24 - 30 days.

Clinical signs and pathogenicity: The caeca shows marked inflammation and thickening of the mucosa with petechial haemorrhages. Except for *H. isolonche* infections, clinical signs may not be seen. Infections with *H. isolonche* may produce nodular thyplitis, diarrhoea, emaciation and death. The most important role of *H. gallinarum* is its capability of transferring the protozoon *Histomonas meleagridis* to fowls. *H. meleagridis* organisms may remain viable in the eggs of *H. gallinarum* for years (→ 3.20).

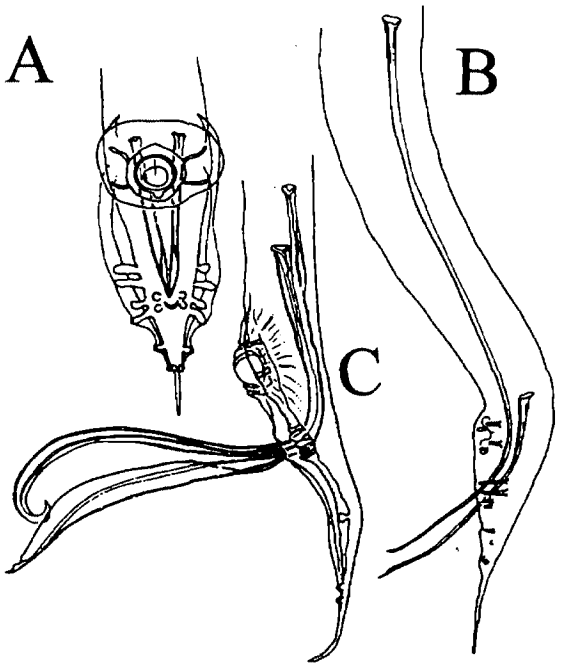

Figure 3.9 Male tails of A: *Heterakis dispar* (Redrawn after Madsen 1945) B: *H. gallinarum* (Redrawn after Lane 1914) and C: *H. isolonche* (Redrawn after Cram 1927).

3.2.9 *Allodapa suctoria* (Molin, 1860)

Syn. *Subulura brumpti*, *S. suctoria*

Hosts: *A. suctoria* is very common in chickens, turkeys, guineafowls, ducks, pheasants, grouse and quails in North and South America, Africa and Asia.

Predilection site: The adult worms occur in the lumen of the caeca.

Morphology: The males are 7 - 10 mm long and the females measure 9 - 18 mm. The eggs are spherical and thin-shelled, 52-64 x 41-49 μm. The adult worms are quite similar in shape and size to *Heterakis* spp. and can be differentiated by microscopical examination of the oesophagus and the spicules (Figure 3.10).

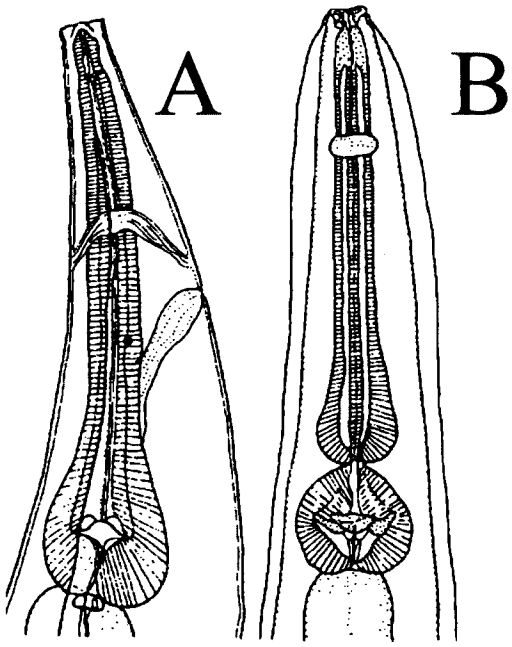

Figure 3.10 A: Anterior end of *Heterakis gallinarum*
B: Anterior end of *Allodapa suctoria* (Redrawn after Skrjabin and Shikhobalova, 1915).

Life cycle and epidemiology: The life cycle is indirect with cockroaches and beetles as intermediate hosts. After the eggs have passed with the faeces they develop in the intermediate hosts finally encapsulating in the intestinal wall after 7-8 days. After another 7 days in the intermediate host the

wall after 7-8 days. After another 7 days in the intermediate host the infective L_3 larvae have developed. The final host becomes infected when ingesting the infected beetles or cockroaches. The larvae migrate to the caeca and develop into adults in 6 weeks.

Clinical signs and pathogenicity: Clinical signs are rarely seen, but the worm is important as a differential diagnosis to *Heterakis* spp.

3.3 NEMATODES IN OTHER ORGANS AND TISSUES

3.3.1 *Oxyspirura mansoni* (Cobbold, 1879)

Syn. *O. parvorum*

Hosts: Infections occur in chickens, turkeys, guineafowl and peafowl in tropical and subtropical areas.

Predilection site: The parasite is located under the nictitating membrane, in the naso-lacrimal ducts or conjunctival sacs.

Morphology: The female worm is 12 - 19 mm long and the male is 10 - 16 mm. The worm is slender and the cuticle is smooth. The pharynx has the shape of an hourglass. The male tail is curved ventrally and has no alae. The two spicules are uneven in size; the left is slender, 3 - 3.5 mm long and the right is stout and 0.2 - 0.22 mm long. The vulva is to the posterior end of the female worm (Figure 3.11).

Life cycle and epidemiology: The life cycle is indirect. After the eggs have passed through the lacrimal duct, swallowed and passed out with the faeces, the intermediate stages develop in cockroaches (*Pycnoscelus surinamensis*). After ingestion of the intermediate host, the larvae migrate via the oesophagus, pharynx and lacrimal duct to the eye.

Clinical signs and pathogenicity: The eyes become irritated and the birds start to scratch them. After a while the affected birds develop ophthalmitis with inflamed and watery eyes.

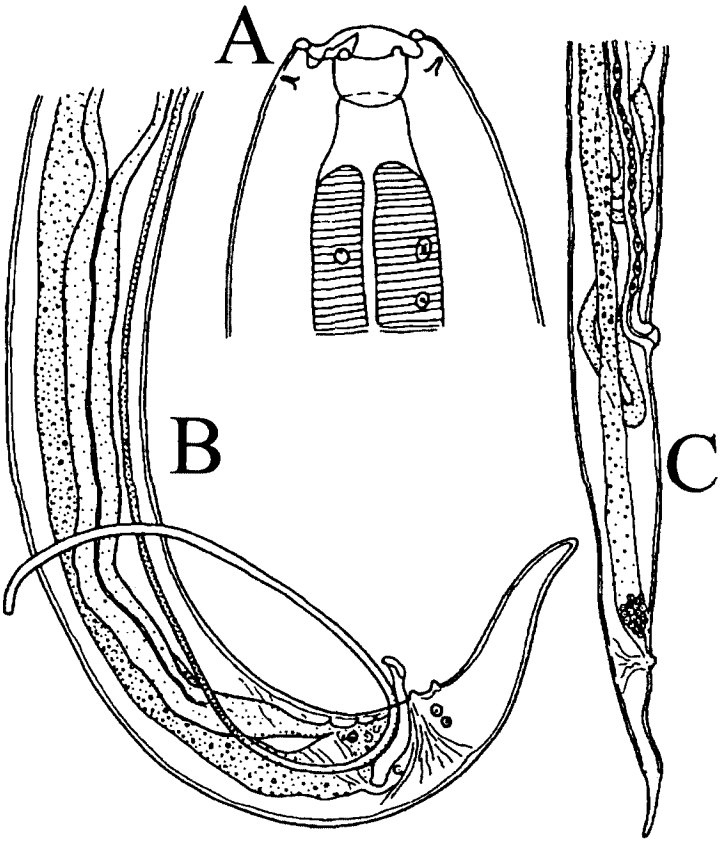

Figure 3.11 *Oxyspirura mansoni* A: Head B: Male tail and C: Female tail (Redrawn after Ransom 1904).

3.3.2 *Syngamus trachea* (Montagu, 1811)

Syn. *S. parvis*, *S. gracilis*. Also commonly called the Gapeworm

Hosts: *S. trachea* is found in chickens, turkeys, pheasants, guineafowls, geese and various wild birds throughout the world.

Predilection site: The adult worms are found in the trachea or in the lungs.

Morphology: The worms are red in colour and the two sexes are found in permanent copulation (Figure 3.12). The female is bigger than the male, measuring 5 - 20 mm, the male 2 - 6 mm. *S. trachea* has a wide mouth opening, without leaf-crowns. The buccal capsule is cup-shaped with six to ten teeth at the base. The males have two spicules which measure 53 - 82 μm (Figure 3.13). The eggs have a thick operculum in both poles and measure 70 - 100 x 43 - 46 μm.

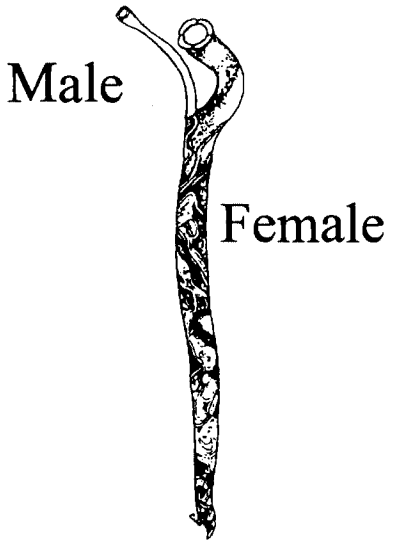

Figure 3.12 *Syngamus trachea:* Male and female in permanent copulation (Wehr 1937).

Life cycle and epidemiology: Infection happens when infective eggs or larvae are ingested. The life cycle may be direct or indirect as the larvae may be swallowed by earthworms, snails, flies or other arthropods. When these "intermediate" hosts are swallowed by poultry the infection is passed on. The larvae migrate through the intestinal wall and are carried by the blood to the lungs. Here they develop into the adult stage. The prepatent period is three weeks. Eggs are coughed up and swallowed and passed with the faeces. Depending on the temperature and humidity, the eggs become infective in 2 to 7 days.
Infections with *S. trachea* mainly affect young birds except for turkeys which are affected at any age. Pheasants and other reared game birds are highly susceptible.

Clinical signs and pathogenicity: The characteristic signs of *S. trachea* infections are: dyspnea and asphyxia, occurring when mucus accumulates in the trachea (gaping). Death follows when the mucus blocks the trachea. Emaciation, anaemia and weakness are also seen as clinical signs. At the *post mortem* examination the carcass is emaciated and anaemic and the adult worm is seen macroscopically when opening the trachea.

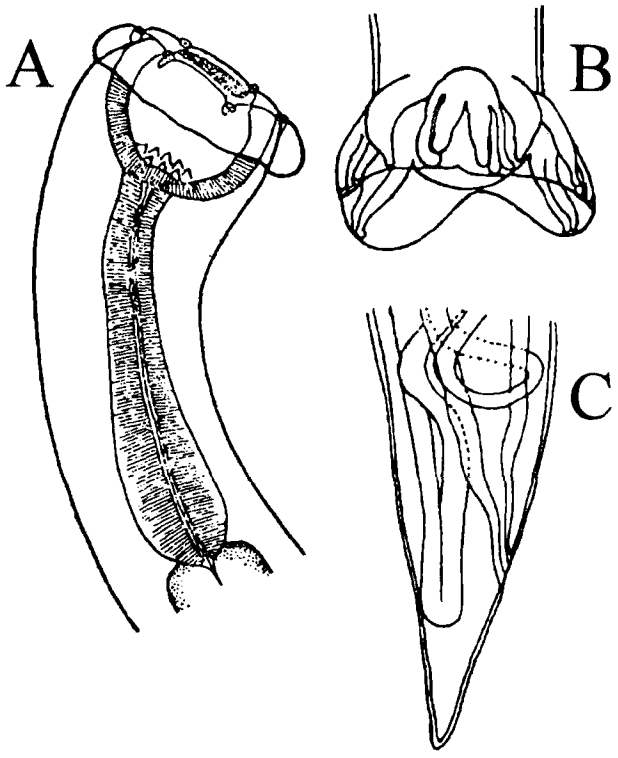

Figure 3.13 *Syngamus trachea.* A: Anterior end of male B: Male bursa C: Female posterior end (Ryzhikov 1949).

3.4 CESTODES

Poultry reared under free range conditions are likely to be infected with cestodes (tapeworms). All tapeworms of poultry have indirect life cycles with intermediate hosts such as earthworms, beetles, flies, ants or grasshoppers. The intermediate hosts are essential to perpetuate the life cycle and infections are therefore rare in indoor systems.

More than 1400 tapeworm species have been described in domesticated poultry and wild birds. The pathogenicity of the majority of these tapeworms is unknown. A great number are harmless or have a mild pathogenicity. Few species cause severe reactions in the host.

Tapeworms belong to the phylum Platyhelminthes, class Cestoda. The tapeworms of poultry are all endoparasitic, hermaphroditic worms with a flat, long segmented body without an alimentary tract or body cavity. Poultry tapeworms may reach a length of 30 - 50 cm. They have a scolex (the head) followed by a neck. The rest of the body is called the strobila consisting of a number of proglottids (segments) developing from the neck. Each segment contains a set of reproductive organs. The number of segments differs between species. The segments furthest away from the neck mature and are detached from the body. These gravid segments contain numerous eggs which are released to the environment with the faeces.

3.4.1 *Raillietina* spp.:

Three species of this genus are important parasites of poultry: *Raillietina echinobothrida (*Megnin, 1881). *Raillietina tetragona* (Molin, 1858) and *Skrjabinia cesticillus* (Molin, 1858).

Hosts: Infections with *R. echinobothrida* are found in chickens and turkeys, *R. tetragona* occurs mainly in chickens, guineafowls and pigeons, whereas *S. cesticillus* is common in domestic chickens. All three species are cosmopolitan in their distribution.

Predilection site: The worms are located in the small intestine where the scolex is embedded into the mucosa.

Morphology: *R. echinobothrida* and *R. tetragona* may reach a length of 10 - 25 cm, while *S. cesticillus* measures 9 - 13 cm (Figure 3.14 & 3.15). The size of the eggs of all three species is identical, 74 x 93 μm, but the number of eggs in each gravid segment varies. The highest number of egg capsules is found in the gravid proglottid of *R. tetragona*. The morphology of the gravid segments of *R. echinobothrida* and *R. tetragona* differ from *S. cesticillus* in that the segments of the first two is replaced by many fibrous-walled egg capsules each containing several eggs while in *S. cesticillus* there are many thin-walled egg capsules each with a single egg.

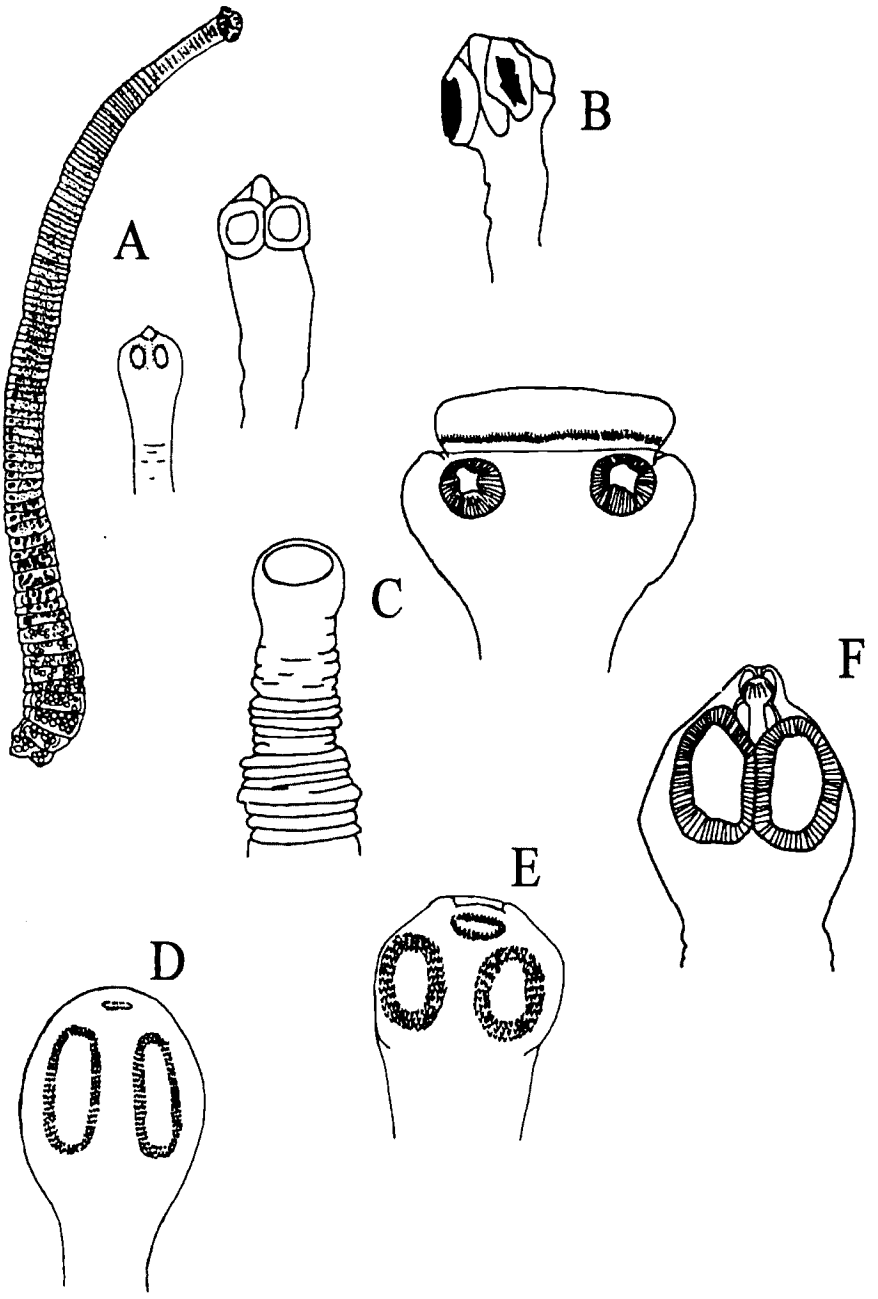

Figure 3.14 Tapeworms of poultry - Scolex A: *Hymenolepis cantaniana* B: *Hymenolepis carioca* C: *Skrajabinia cesticillus* D: *Raillietina tetragona* E: *Raillietina echino-bothrida* F: *Choanotaenia infundibulum* (Modified after Ackert, Monning, Neveu-Lemaire, Ransom & Wehr).

Life cycle and epidemiology: The gravid proglottids are passed with the faeces and eggs may survive for a considerable time (years?). Intermediate hosts such as ants (*Pheidole* and *Tetramorium*), beetles (*Calathus*, *Amara*) and others become infected by ingesting individual eggs. The embryo (larva) hatches from the egg in the intestine of the intermediate host. The larva changes into a cysticercoid and remains in the body cavity of the intermediate host until eaten by the final host. Activated by the bile in the final host, the cysticercoid attaches to the mucosa in the small intestine. Development of proglottids starts immediately. The prepatent period varies between 2 to 3 weeks.

Clinical signs and pathogenicity: Chronic infections are characterized by reduced growth, emaciation and weakness. Of the three species *R. echinobothrida* is the most pathogenic. Nodules and hyperplastic enteritis may develop at the site of attachment. This phenomenon is named "Nodular tapeworm disease" and may occur in heavy infections. Infections with *R. tetragona* are less pathogenic but can cause reduced weight gain.

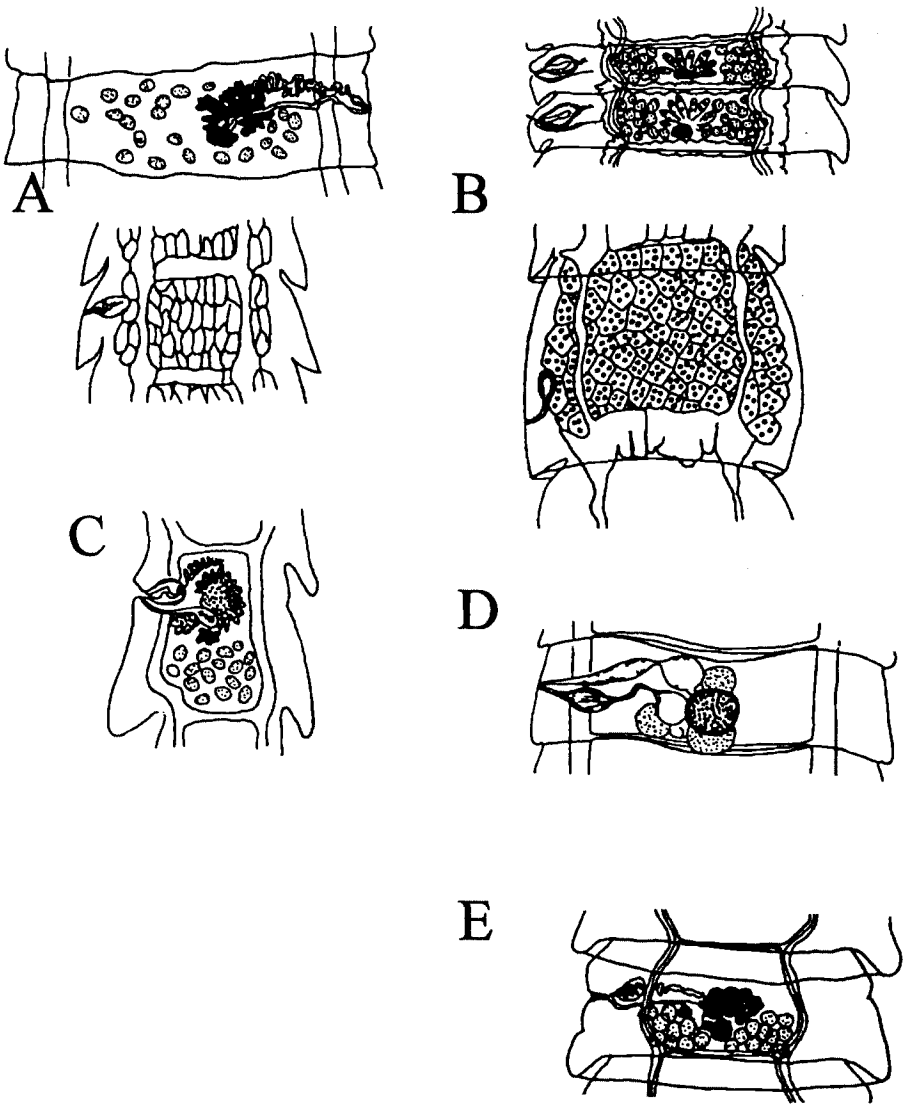

Figure 3.15 Tapeworms of poultry - Segments. A: *Raillietina tetragona* B: *Raillietina echinobothrida* C: *Choanotaenia infundibulum* D: *Hymenolepis carioca* E: *Skrajabinia cesticillus* (Modified after Fuhrmann, Lang, Monnig, Neveu-Lemaire, Ransom, Sawada).

3.4.2 *Davainea proglottina* (Davaine, 1860)

Hosts: *D. proglottina* infections are found in fowls, pigeons and other birds in most parts of the world.

Predilection site: The worms are buried in the mucosa of the duodenum.

Morphology: The adult tapeworms are small, 0.5 - 3 mm, with 4 to 9 proglottids (Figure 3.16). The eggs measure 28 - 40 μm.

Life cycle and epidemiology: The gravid proglottids are passed out with the faeces. The eggs hatch after being swallowed by various species of gastropod molluscs such as *Limax*, *Cepaea*, *Agriolimax* and *Arion*. Cysticercoids develop after 3 weeks and develop into adult tapeworms in 2 weeks upon ingestion by the final hosts.

Clinical signs and pathogenicity: *D. proglottina* is, despite of the small size, one of the more pathogenic species, especially in young birds and particularly if it occurs in large numbers. Clinical signs include dull plumage, slow movements, reduced weight gain, emaciation, dyspnea (difficulties in breathing), leg paralysis and death. Microscopically thickened mucosal membrane with haemorrhages, fetid mucus and necrosis are seen.

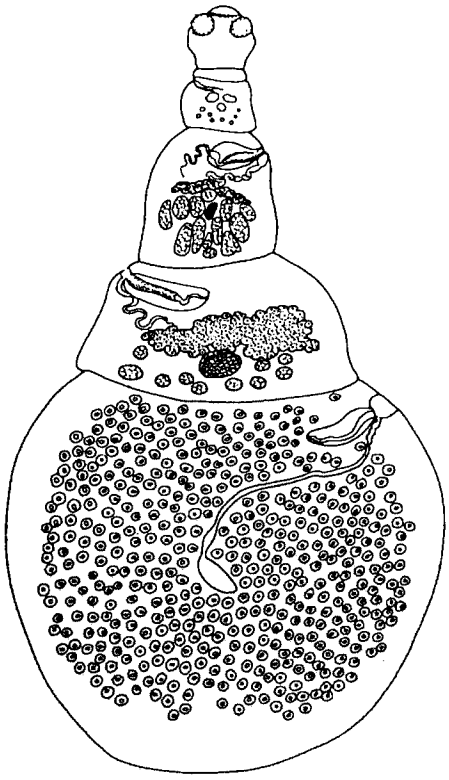

Figure 3.16 Adult *Davainea proglottina* (Jones & Bray, 1994).

3.4.3 *Choanotaenia infundibulum* (Bloch, 1779)

Hosts: Infections occur in fowls and turkeys in most parts of the world.

Predilection site: The worms are attached to the mucosa in the upper half of the small intestine.

Morphology: The mature worms reach a length of up to 23 cm and may be 1.5 - 3 mm wide. The segments are clearly wider at the posterior end of the parasite (Figure 3.14 & 3.15). The eggs have a distinctly long filament and measure 47 x 54 μm.

Life cycle and epidemiology: After the eggs have been deposited with the faeces, they hatch in the gut of the intermediate hosts following ingestion. The intermediate hosts are among others beetles of the genera *Tribolium*, *Geotrupes*, *Aphodius* or *Calathus* and the house fly, *Musca domestica*. After development in the intermediate host the cysticercoids are infective for the final host. After ingestion of an intermediate host gravid segments are released with the faeces of the host within 2 weeks.

Clinical signs and pathogenicity: The adult tapeworms are moderately pathogenic causing weight loss.

3.4.4 *Hymenolepis* spp.:

Three species have a pathogenic and economic importance. These are *H. carioca* (de Magalhaes, 1898). *H. cantaniana* (Polonio, 1860) and *Drepanidotaenia lanceolata* (Bloch, 1782).

Hosts: Infections with *H. carioca* and *H. cantaniana* are seen in fowl in most parts of the world. *D. lanceolata* occurs in ducks and geese and has a cosmopolitan distribution.

Predilection site: The worms are located in the small intestine.

Morphology: *H. carioca* is a slender threadlike tapeworm which can reach

a length of 8 cm. *H. cantaniana* is smaller and may reach a length of 2 cm. The adult worms of *D. lanceolata* may become 13 cm long and 18 mm wide, with segments wider than long (Figure 3.14 & 3.15).

Life cycle and epidemiology: The life cycle of the hymenolepids resembles other cestodes. Beetles (Scarabeidae) are the intermediate hosts of *H. carioca* and *H. cantaniana*, whereas water crustaceans are intermediate hosts of *D. lanceolata*. The prepatent time is 3 - 4 weeks. Several thousand adult worms may be found in the intestine.

Clinical signs and pathogenicity: Clinical signs with catarrhal enteritis, diarrhoea and death may be seen in heavy infections with thousands of adult worms in the intestine. Specially *D. lanceolata* causes severe reactions in ducks and geese.

3.5 TREMATODES

Trematodes or flukes are dorsoventrally flatened, unsegmented and leaflike parasites. They belong to the phylum Platyhelminthes, class Trematoda with two subclasses: Aspidogastrea and Digenea. All poultry trematodes belong to the subclass Digenea.
A mouth and an intestinal tract are present, an anus is rarely found. The reproductive system is hermaphroditic. All trematodes parasitizing poultry have an indirect life cycle and are thus called Digenea in contrast to the Monogenea, which do not require an intermediate host. Molluscans are intermediate hosts for all digenea. Some species require a second intermediate host or even a third. More than 500 species are known from birds, but only few are known to be pathogenic.
Digenean life cycles vary in complexity and can involve up to four hosts, but two or three is more common. After the hatching of the egg in water (usually) or in the gut of the host after ingestion of the egg (more rare) the miracidium is released and penetrates the tissues of a mollusc and develops into a mother sporocyst. Germinal cells in the mother sporocyst give rise to daughter sporocysts or rediae. Germinal cells in the daughter sporocysts or rediae develops into cercariae. The cercariae leave the snail, may encyst in the open or after penetrating another host, or may not encyst at all. Each cercaria gives rise to one metacercaria which in turn gives rise to one adult after it enters the gut or other appropriate site in the final host.

3.5.1 *Echinostoma revolutum* Frölich, 1802

Hosts: The principal hosts are ducks and geese, the parasite may occur in other aquatic birds, pigeons, fowl and humans. The parasite is seen worldwide.

Predilection site: The adult worms are located in the rectum and caeca.

Morphology: *E. revolutum* is 10 - 22 mm long and up to 2.25 mm wide. Echinostomes have a head-collar armed with spines, which is the major recognition feature for the family (Figure 3.17). The eggs measure 90 - 126 by 59 - 71 μm.

Life cycle and epidemiology: For *E. revolutum* the eggs pass with the faeces and mature in 3 weeks if the conditions are favourable, i.e., high humidity and high temperatures. The miracidium penetrates a snail, the intermediate host (*Lymnaea* spp., *Stagnicola palustris*, *Helisoma trivolvis*, *Physa* spp. or *Planorbis tenuis*). But also *Bulinus, Biomphalaria, Succinea, Pseudosuccinea* and *Corbiculina* may act as intermediate hosts. In the snail cercariae develop in 2 - 3 weeks and these may either encyst or escape and enter into another snail. The birds become infected when ingesting infected snails. The prepatent period is 15 - 19 days.

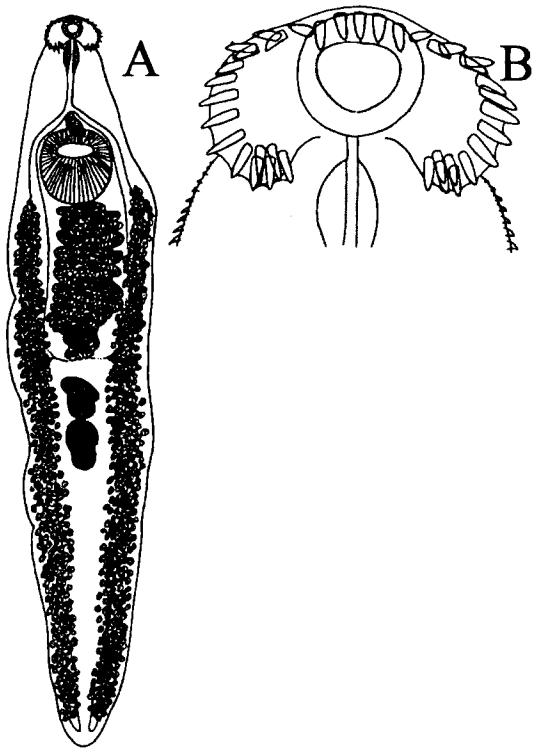

Figure 3.17 *Echinostoma revolutum* A: Adult worm B: Head of adult (Redrawn after Macy 1934).

Clinical signs and pathogenicity: Heavy infections with *E. revolutum* may cause emaciation and catarrhal enteritis. Death may occur in young animals.

3.5.2 *Prosthogonimus* spp.:

Three species are of interest. These are *P. pellucidus* (von Linstow, 1873), syn. *P. intercalandus* (Szidat, 1921), *P. macrorchis* Macy, 1934 and *P. ovatus* (Rudolphi, 1803).

Hosts: *P. pellucidus* occurs worldwide in fowls and ducks. *P. macrorchis* has been reported from poultry and ducks in North America, whereas *P. ovatus* is seen in fowl and geese in Africa, Europe, Asia as well as in North and South America.

Predilection site: The adult worms may be located in the bursa of Fabricius, the oviduct and in the cloaca/rectum.

Morphology: The adult worms measure 8 - 9 by 4 - 5 mm being broad in the posterior end. *P. ovatus* is slightly smaller, measuring 3 - 6 by 1 - 2 mm. The eggs of *P. pellucidus* measure 26 - 32 x 10 - 15 μm and the eggs of *P. ovatus* measure 22 - 24 x 13 μm.

Life cycle and epidemiology: The eggs are excreted with the faeces and hatch in the free. The miracidium enters the snail and becomes a mother sporocyst whish produces daughter sporocysts. The sporocysts then produce cercariae without forming rediae. The cercaria are then excreted from the snail and enters dragonfly larvae. In the dragonfly, the cercaria encysts, thus becoming a metacercaria. The final hosts become infected when eating the larval or adult stage of dragonflies.

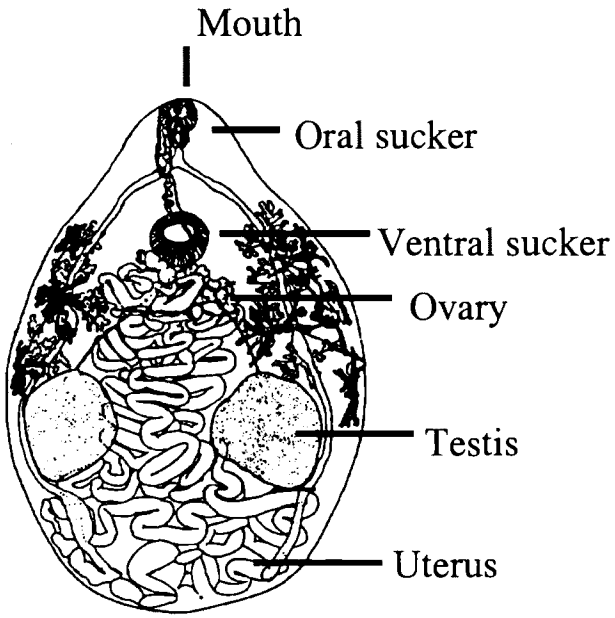

Figure 3.18 *Prosthogonimus macrorchis* (Redrawn after Macy 1934).

Clinical signs and pathogenicity: *Prosthogonimus* infections are considered the most pathogenic trematode infection of fowls and ducks. Clinical signs are most often seen in birds. Birds infected with *Prosthogonimus* spp. have a tendency to sit on the nest. Furthermore, there may be a milky discharge from the cloaca, and they may lay eggs with soft shells or without any shell. In chronic cases peritonitis may develop.

3.6 ENDOPARASITIC PROTOZOAN INFECTIONS

Protozoa are common in poultry and may produce moderate to severe clinical symptoms. Phylum *Sarcomastigophora* and phylum *Apicomplexa* contain most of the protozoa important for poultry (see also → 3.8). The *Sarcomastigophora* include the genera *Histomonas*, *Trypanosoma* and *Entamoeba* among others.

Coccidiosis is probably the most widespread and important parasitic disease in commercial as well as backyard poultry operations and as such responsible for major economic losses in the poultry industry. Coccidiosis in poultry is caused by protozoans from the following three genera: *Eimeria*, *Tyzzeria* and *Wenyonella*. All three belong to the phylum *Apicomplexa*. A typical life cycle of the genera *Eimeria* is shown in Figure 3.19. The haemoparasites also belong to this phylum (→ 3.8).

The life cycle of coccidia is direct and very short compared to helminths, and often completed in less than 7 days (Figure 3.19). The oocysts are expelled with the faeces and sporulate in 1 - 2 days. When the sporulated oocysts are ingested by a bird, sporozoites are released. These enter the intestinal epithelial cells and become 1st generation schizonts. Merozoites are produced by the schizonts in the epithelial cells and break out of the host cell. The merozoites may develop into 2nd generation schizonts, which again produce 2nd generation merozoites or they may develop into micro- and macrogametes. The macrogametes are fertilized by the microgametes and become a zygote, which turns into young oocysts. These break out of the host cell and are passed out with the faeces.

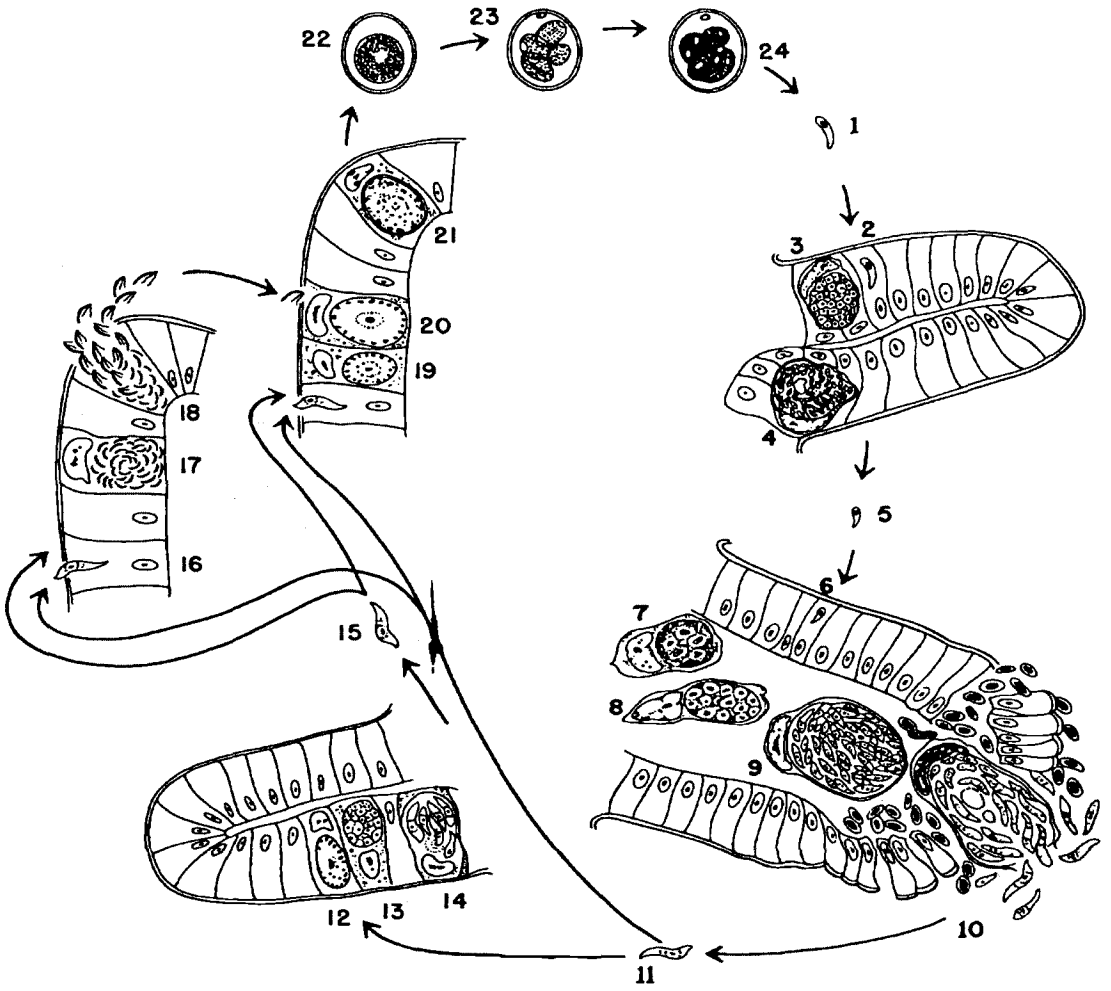

Figure 3.19 Life cycle of *Eimeria tenella*. A sporozoite (1) enters an intestinal epithelial cell (2), rounds up, grows and becomes a 1st generation schizont (3). This produces a large number of 1st generation merozoites (4), which break out of the host cell (5), enter new intestinal epithelial cells (6), round up, grow and become 2nd generation schizonts (7, 8). These produce a large number of 2nd generation merozoites (9, 10), which break out of the host cell (11). Some enter new host intestinal epithelial cells and round up to become 3rd generation schizonts (12, 13), which produce 3rd generation merozoites (14). The 3rd generation merozoites (15) and the great majority of 2nd generation merozoites (11) enter new host intestinal epithelial cells. Some become microgametocytes (16, 17), which produce a large number of microgametes (18). Others turn into macrogametes (19, 20). The macrogametes are fertilized by the microgametes and become zygotes (21). The zygotes mature and become young oocysts, these break out of the host cell and pass out with the faeces (22). The oocysts begin to sporulate (23, 24). When the sporulated oocyst is ingested by a chicken, the sporozoites are released (1) (Levine 1973).

3.6.1 Coccidiosis in chickens

Hosts: Nine species of *Eimeria* have been described in chickens, but only some of these cause severe clinical disease (Table 3.1). *E. tenella*, *E. brunetti* and *E. necatrix* are the most pathogenic ones. Other species such as *E. maxima* and *E. acervulina* are less pathogenic. The species belonging to the genera *Eimeria* are usually host specific, which means that different poultry species do not infect each other.

Morphology: When the oocysts are expelled in the faeces they are spheric in shape and not embryonated. They measure 16 x 42 μm. During sporulation 4 sporocysts are formed each containing two sporozoites.

Life cycle and epidemiology: The life cycle is directly involving a sexual phase and a non sexual phase (Figure 3.19). The life cycle is completed in 5 - 7 days, which ensures a rapid spread of infections in flocks.

Clinical signs and pathogenicity: Clinical signs are highly variable in flocks and range from decreased growth to a high percentage of visibly sick animals with diarrhoea and high mortality. Infections can be seen in all age groups. Usually there is decreased feed and water consumption. Weight loss and decreased egg production are observed. Survivors of severe infections recover in 10 - 14 days, but may require even more time to recover to normal production. The degree of immunity acquired prior to the development of clinical disease may influence the severity and development of a flock infection.

Table 3.1 *Eimeria* spp. species of importance infecting chickens.

Species	Predilection site	Macroscopic lesions	Severity
E. brunetti	posterior part of small intestine	coagulation necrosis, mucoid and bloody enteritis	very pathogenic
E. necatrix	small intestine	ballooning, white spots , petechial haemorrhages, mucoid blood filled exudate	very pathogenic
E. tenella	caeca	haemorrhages into lumen, thickening whitish mucosa, cores of clotted blood.	very pathogenic
E. acer-vulina	posterior part of small intestine	light infec.: whitish round lesions heavy infec.: plagues coalescing, thickened intestinal wall	pathogenic
E. maxima	small intestine	thickened intestinal wall, mucoid exudate, petechial haemorrhages	pathogenic

3.6.2 Coccidiosis in turkeys

Hosts: Coccidiosis is common in turkeys, but infections are not so pathogenic as compared to chickens. Several species infect turkeys, but the four species mentioned in table 3.2 are important. Turkeys of all ages are susceptible to primary infections, but birds older than 6 - 8 weeks are more resistant.

Morphology: When the oocysts are excreted in the faeces they are ovoid to ellipsoid in shape and not embryonated. They measure from 16 x 19 μm to

21 x 26 μm.

Life cycle and epidemiology: The life cycle is direct and short.

Clinical signs and pathogenicity: Typical signs of infection are watery or mucoid diarrhoea, depression, ruffled feathers and anorexia.

Table 3.2 *Eimeria* spp. species of importance infecting turkeys.

Species	Predilection site	Macroscopic lesions	Severity
E. adenoides	caeca, lower intestine	caecal cores consisting of caseous material, whitish appearance, petechial haemorrhages, swollen and edematous wall, mucus secretion.	very pathogenic
E. melea-grimitis	caeca	cream-coloured caseous caecal cores, thick mucosa, petechial haemorrhages.	slightly pathogenic
E. gallopar-vonis	posterior small intestine	inflammation, edematous intestine, soft white caseous necrotic material in lumen.	pathogenic
E. dispersa	mid part of small intestine	dilation of intestine with secretion of cream-coloured mucoid material, edema, congestion of capillaries, necrosis of villi.	slightly pathogenic

3.6.3 Coccidiosis in ducks

Hosts: More than 13 species of coccidia have been reported in wild and domestic ducks, but the descriptions are insufficient to use in diagnosis.

Morphology: Thin walled oocysts measuring 10 - 12.3 x 9 - 10.8 μm. Eight sporozoites are formed in the oocyst.

Life cycle and epidemiology: The life cycle is direct.

Clinical signs and pathogenicity: Clinical signs of infections with *Tyzzeria perniciosa* include anorexia, weight loss, weakness, distress, high morbidity and high mortality.

Table 3.3 *Eimeria* spp. species of importance infecting ducks.

Species	Predilection site	Macroscopic lesions	Severity
Tyzzeria perniciosa	anterior part of small intestine	haemorrhages into lumen, bloody or caseous exudate, cores of clotted blood.	pathogenic

3.6.4 *Histomonas meleagridis* (Smith, 1895)

Syn. Blackhead, Infectious enterohepatitis.

Hosts: Mainly turkeys, but also chickens and guineafowls can be infected. The infection is cosmopolitan.

Predilection site: The infection is located in the caeca and the liver.

Morphology: *H. meleagridis* is spherical or pleomorphic in shape, 3 - 16 μm in diameter (Figure 3.20).

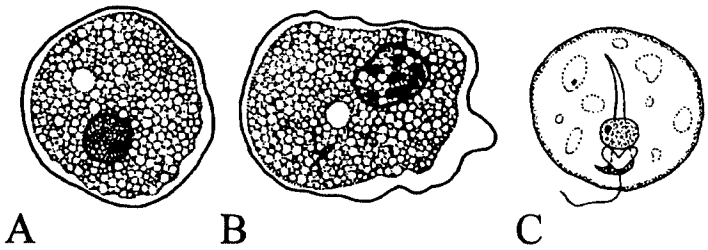

Figure 3.20 A, B & C: Different forms of the protozoa *Histomonas meleagridis.*

Life cycle and epidemiology: The life cycle of *H. meleagridis* may be direct but the organism is often transmitted to susceptible animals through eggs of *Heterakis gallinarum* or through earthworms.

Clinical signs and pathogenicity: Clinical signs are mainly seen in poults (young turkeys), whereas chickens are less affected. Early signs are weakness, anorexia, listlessness, closed eyes, dropped and ruffled feathers, emaciation. Occasionally the head may be cyanotic. Sulphur-yellow faeces is often seen in turkeys, whereas chickens have a bloody caecal discharge. The caeca are enlarged and thickened. The mucosa may be inflamed with necrotic areas. Serous and haemorrhagic exudate arising from the mucosa fills the lumen of the caeca. The exudate becomes caseous or cheesy. The liver is enlarged with dark brown necrotic areas. The necrotic areas are depressed compared to the surface and have a diameter of 0.5 - 2 cm.

3.7 ECTOPARASITES

The ectoparasites, Arthropods, are divided into two main classes: *The Arachnida* (arachnids) including the order Acari (ticks and mites) and *The Insecta* including the orders Phthiraptera (lice), Hemiptera (bugs), Siphonaptera (fleas) and Diptera (flies and mosquitoes). The latter order is characterized by having a body divided into head, thorax and abdomen, one pair of antennae attached to the head, three pairs of legs attached to the thorax and air tubes for breathing. Adults may have wings. The Arachnida are characterized by having fused body divisions, no antennae, three pairs of legs as larvae and four pairs of legs as adults.
Ectoparasites are very common in free-range systems, whereas they are usually controlled (although not eradicated) in commercial systems. The ectoparasites may constitute a clinical problem in themselves, but may also transmit a number of infectious diseases to poultry, such as *Pasteurella multocida*, *Aegyptinella* spp., *Borrelia anserina*, *Plasmodium* spp., *Leucocytozoon* spp., Newcastle Disease, Fowl pox or they act as transport/intermediate hosts of a range of helminth infections such as *Heterakis gallinarum*, *Choanotaenia infundibulum*, *Hymenolepis* spp. etc.. Only the most clinically and pathologically important species will be described in this section.

3.7.1 *Argas persicus* (Oken, 1818)

Syn. The fowl tick

Hosts: *A. persicus* infects chickens, turkeys, pigeons, ducks, geese and many wild birds in tropical and sub-tropical countries. Other species e.g., *A. walkerae*, *A. reflexus hermanni* are seen in West Africa.

Predilection site: On the skin, but most of the time the ticks hide in cracks or under tree bark, away from the host.

Morphology: The fowl tick belong to the soft-bodied ticks, the family *Argasidae*. In contrast to the hard ticks, the fowl tick has no dorsal shield (scutum), and except for the larvae stage they feed intermittently at all stages. The ticks have three stages: the larvae, the nymph and the adult. The adult mature female measures about 10 x 6 mm when they are engorged.

The unfed ticks are easy to recognize by the flat ovoid shape and the brown/reddish colour. There is very little difference between the female and male. The differentiation between the sexes can only be made on comparison of the genital pore. The opening is placed in the anterior end on the ventral surface and is larger in the female (Figure 3.21).

Life cycle and epidemiology: The eggs are laid in cracks in houses or under the bark of trees. The larvae hatch within 3 weeks and attach to a host under the wings. They engorge in about 5 days. The larvae have 3 pairs of long legs and circular bodies. They become spherical after engorgement. After dropping from the host they moult into the nymphal stage in about 7 days. There are two nymphal stages. The first stage feeds on the host for about 10 - 15 minutes and hides for 5 - 8 days before moulting into the second stage. After another 5- 15 days they feed on a host before moulting into an adult in about one week. The adults feed once a month and the females lay eggs after each meal. One batch consists of 20 - 100 eggs. The nymphs and adults are nocturnal in their behaviour and may survive without a blood meal for more than 5 years in cracks or other suitable places.

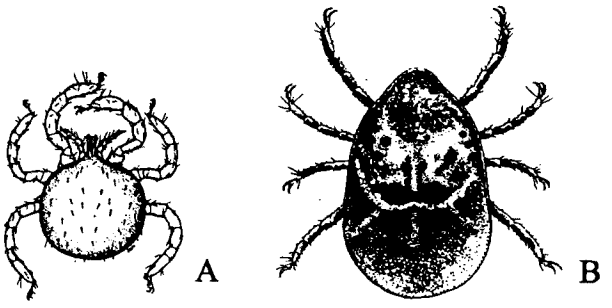

Figure 3.21 *Argas persicus* A: Larva, B: Adult. Not drawn to scale (Redrawn after Soulsby 1982)

Clinical signs and pathogenicity: Infections with fowl ticks may cause ruffled feathers, poor appetite (anorexia), diarrhoea, emaciation and lowered production. Heavy infections with *Argas* spp. can cause loss of blood leading to anaemia and eventually death. Furthermore, ticks are known to transmit haemoparasites and bacterial and viral diseases such as *Leucocytozoon* spp., *Aegyptinella* spp., *Pasteurella multocida*, *Avian encephalomyelitis* and possibly other diseases.

3.7.2 Skin mites:

Dermanyssus gallinae (DeGeer, 1778) Syn. The red mite, *Ornithonyssus sylviarum* (Canestrini & Fanzago, 1877) Syn. The northern fowl mite and *Ornithonyssus bursa* (Berlese, 1888) Syn. The tropical fowl mite.

Hosts: Chickens, turkeys, ducks and other domestic and wild birds are infected with mites throughout the world. *D. gallinae* is cosmopolitan in distribution, whereas *O. sylviarum* is found in temperate areas and *O. bursa* is found in sub-tropical and tropical areas.

Predilection site: *D. gallinae* is only found on the host during night time, due to a nocturnal behaviour, and may be found anywhere on the skin. During daytime the mites hide in cracks or other suitable places. *O. sylviarum* and *O. bursa* can be found on the skin throughout the day.

Morphology: The engorged adult female mites measure up to 1 mm in length. The colour varies from gray to deep red, depending on when the mites have been feeding blood. The mites can be distinguished on the shape of the dorsal plate (Figure 3.22).

Life cycle and epidemiology: The adult females lay eggs soon after a blood meal. *D. gallinae* lay their eggs off the host in cracks and crevices, whereas *O. sylviarum* and *O. bursa* lay their eggs on the host or in the surroundings. Six-legged larvae hatch in 48 - 72 hours from the eggs and moult into first stage nymphs. After 24 - 72 hours the first stage nymph moult into the second stage nymph. This nymph then moult into the adult stage within 24-48 hours. The entire life cycle can be completed in less than 7 days.
Chicken mites can live up to 8 months without a blood meal, making it difficult to control infections.

Clinical signs and pathogenicity: Infected birds may have a change of behaviour due to the itching effect of the mites. Loss of weight, reduction in egg production, anaemia and death are clinical signs frequently seen.
Furthermore, these mites can transmit a number of diseases such as *Pasteurella* spp., fowl pox, Newcastle disease and possibly *Chlamydia*.

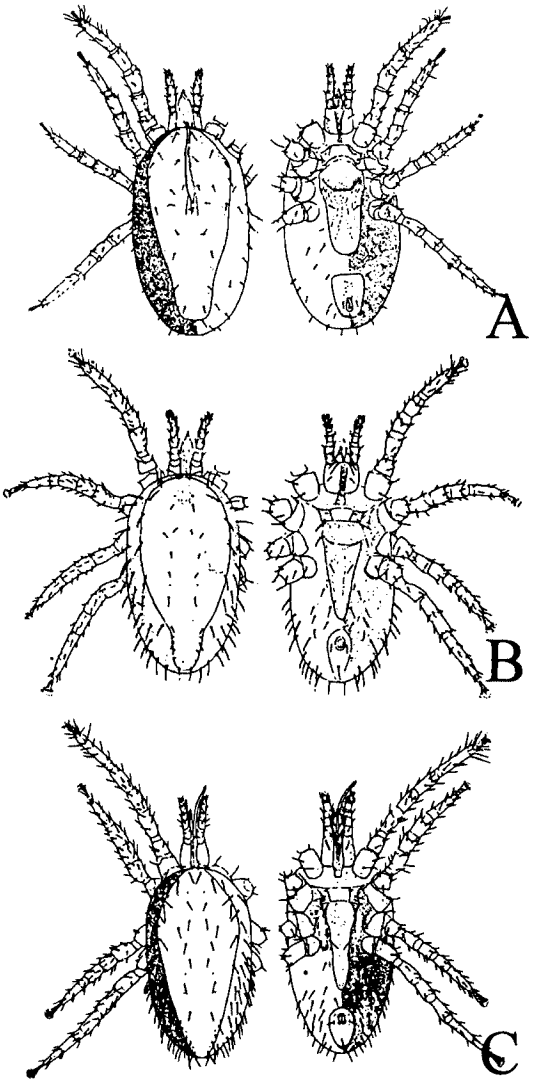

Figure 3.22 A: *Dermanyssus gallinae* B: *Ornithonyssus sylviarum* C: *Ornithonyssus bursa* (Soulsby 1982)

3.7.3 *Cnemidocoptes mutans* (Robin, 1860)

Syn. Scaly leg, burrowing mite.

Hosts: Chickens and turkeys in most parts of the world may harbour this parasite.

Predilection site: The parasites are found under the scales of the legs, thus the name Scaly leg, but can occasionally be seen on the comb, wattles and neck. The birds get infected from the ground and the infection spreads from the toes upwards.

Morphology: Adult mites are almost spherical in shape (Figure 3.23) with short legs. The adult females measure 0.5 mm.

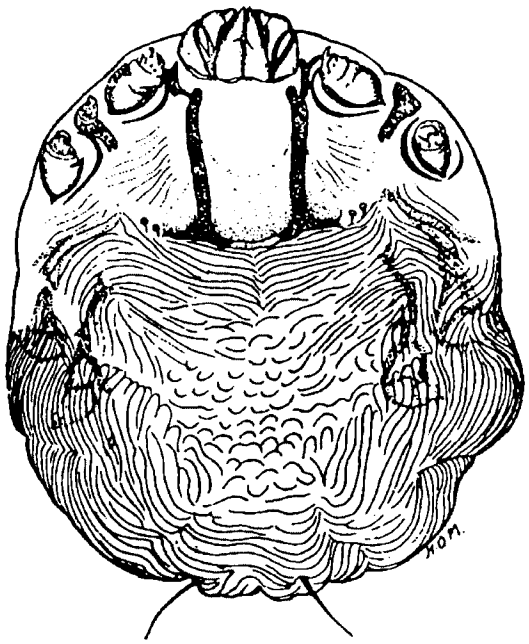

Figure 3.23 Dorsal view of *Cnemidocoptes mutans* (Scaly leg mite) adult female (Soulsby 1982)

Life cycle and epidemiology: The mites pass through their entire life cycle on the host. Chickens get infected through contact with infected soil or animals.

Clinical signs and pathogenicity: The parasites burrow the skin underneath the scales of the legs, causing inflammation with exudate and subsequently keratinization of the legs (i.e., hypertrophy of stratum corneum causing dermatitis hypertrophicans). In chronic cases lameness and malformation of the feet are seen.

3.7.4 *Echidnophaga gallinacea* Westwood, 1875
Syn. Sticktight flea

Hosts: The sticktight flea is common on chickens and other birds in tropical and subtropical areas throughout the world.

Predilection site: The adult flea attaches to the skin of the head, often around the eyes in clusters of hundreds.

Morphology: The colour of the adult flea is brown to black measuring about 1 mm (Figure 3.24)

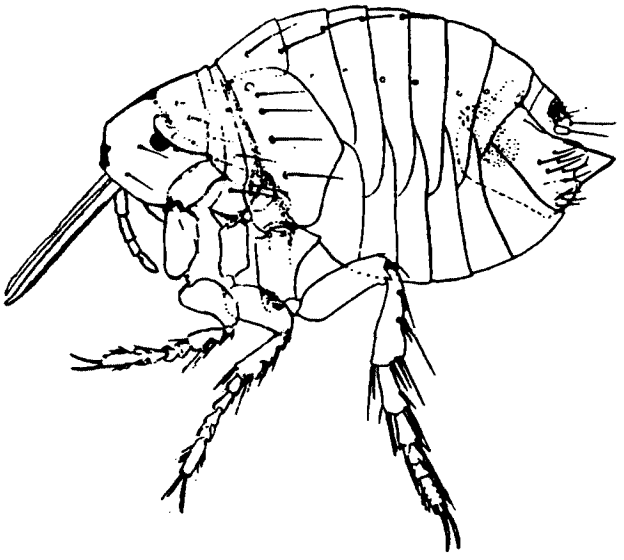

Figure 3.24 Adult *Echidnophaga gallinacea*
(Redrawn after Soulsby 1982)

Life cycle and epidemiology: The adult females eject several eggs per day either by force or passively. The eggs incubate in the surrounding litter and hatch within 1 - 2 weeks. The larvae feed on dry blood, faeces and other organic matter before they turn into a cocoon. The duration of the pupal stage varies from 1 week to several months. Emerging from the cocoon the fleas seek a host. After a blood meal they are ready to lay eggs. Immature fleas may survive for weeks or months without feed. Also the adults survive for weeks without feeding, but can survive for many months if a host is available.

Clinical signs and pathogenicity: Irritation, blood loss and anaemia may affect the birds severely and death may occur. The flea is not known to transmit infectious diseases.

3.7.5 Mosquitoes and flies

The order Diptera includes among others the families *Culicidae* (mosquitoes), *Simuliidae* (black flies), *Ceratopogenidae* (midges) and *Muscidae* (house flies and stable flies). The majority irritate or they suck blood from the hosts. All domesticated and wild birds are potential hosts. Except for the annoyance there is little clinical significance. The great importance lies in the role as an intermediate host or as a mechanical vector. The mosquitoes (*Aedes* spp., *Anopheles* spp. and *Culex* spp.) may act as intermediate hosts for *Plasmodium* spp. (→ 3.8.7) but can also mechanically transfer fowl pox virus. The Black flies are intermediate hosts of *Leucocytozoon* spp. (→3.8.2). *Leucocytozoon* spp. may also be transmitted by biting midges as well as fowl pox, avian infectious synovitis and *Haemoproteus* spp.. The Muscidae may transfer Newcastle Disease virus, *Heterakis gallinarum* (→ 3.2.8), *Pasteurella multocida* and *Mycobacterium avium* to non-infected birds, and may also act as intermediate hosts for tapeworms, i.e., *Choanotaenia infundibulum* (→ 3.4.3) or *Hymenolepis carioca* (→ 3.4.4).

3.8 BLOOD PARASITES (HAEMOPARASITES)

Poultry kept in free range systems or on pastures are exposed to a number of vectors/final hosts transmitting protozoan diseases. The haemoparasites or blood parasites are mainly found in poultry in tropical areas and the following genera *Plasmodium* spp., *Leucocytozoon* spp., *Haemoproteus* spp., *Aegyptinella* spp., *Eperytrozoon* spp., *Trypanosoma* spp. and microfilaries of nematodes belonging to the suborder *Filariata* have been recorded in poultry. Over 10 species of these are of pathogenic and economic importance.

3.8.1 *Leucocytozoon caulleryi* (Mathis and Leger, 1909)

Syn. *Leucocytozoon schueffneri.*

Hosts: Chickens are the only hosts of *L. caulleryi.* Infections are common in birds in Eastern and Southern Asia.

Morphology: The mature gametocytes (15.5 x 15 μm) are round or oval in shape and found in young and mature erythrocytes. The size of the host cell is approximately 20 μm in diameter. Full-grown gametocytes push the nucleus of infected host cells out (Figure 3.25).

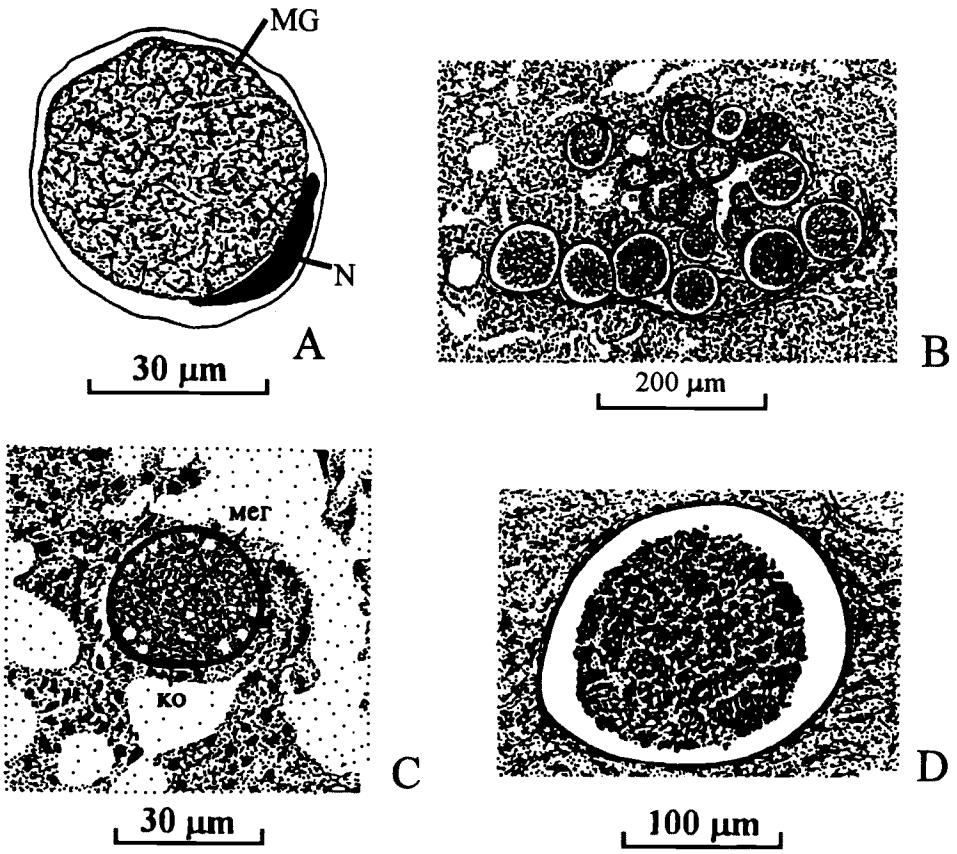

Figure 3.25 Exoerytrocytic stages of *Leucocytozoon caulleryi* in domestic chickens. A: First generation meront in cell from a spleen (N: nucleus of host cell, MG: macrogamont B: Megalomeronts in lung-tissue. C: Growing megalomeront in lung-tissue (MER: meront, KO: membrane). D: Mature megalomeront in heart-tissue (Redrawn after Valkiunas, 1997).

Life cycle and epidemiology: The vectors of *L. caulleryi* are biting midges, *Culicoides* spp.. After transmission to the birds, the sporozoites develop into schizonts. The schizogony takes place in the brain, liver, spleen, lungs and many other organs. Merozoites are then released and may enter a new cycle or may enter erythrocytes or erythroblasts to develop into gametocytes (Figure 3.26). The gametocytes are only found in erythrocytes. The parasite organism lies next to the host cell nucleus and has a round form (Figure 3.25). In the vector the zygote elongates into an ookinete , which passes through the intestinal wall to form oocysts under the basal lamina. In the oocysts sporozoites are formed and introduced into new hosts when bitten. Infections occur frequently when numbers of *Culicoides* spp. are abundant.

Clinical signs and pathogenicity: Clinical signs are seen in chickens 2 weeks after infection. Depending on the strain of *L. caulleryi* the mortality may reach 100 %. Affected chickens are listless, anaemic, the combs and wattles may be pale and the egg production may drop. Haemorrhages may be seen in the liver, lungs and kidneys. Extensive haemorrhages are seen in the kidneys when the merozoites are released. At post mortem examinations haemorrhages, splenomegaly and hepatomegaly are common findings.

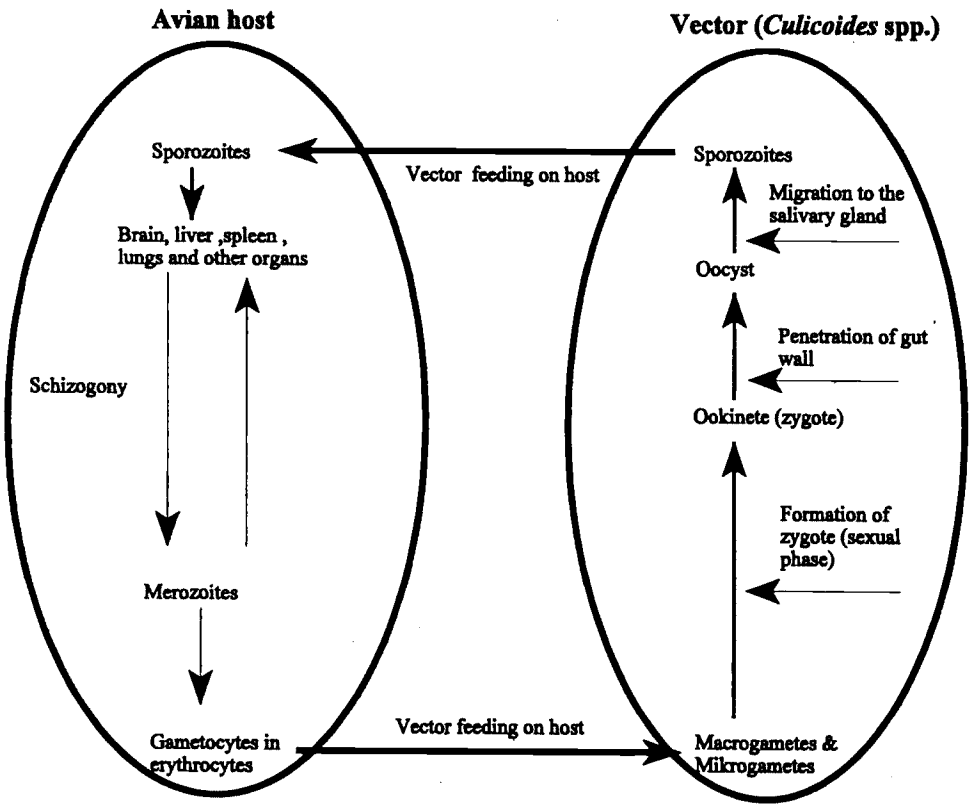

Figure 3.26 Life cycle of *Leucocytozoon caulleryi*

3.8.2 *Leucocytozoon macleani* Sambon, 1908

Syn. *Leucocytozoon sabrazesi*

Hosts: Domestic chickens and wild galliformes, especially pheasants in Southeast and South Asia.

Morphology: The merozoites enter erythroblasts and probably mononuclear leucocytes and form ovoid (10 x 15μm) and elongated gametocytes (24 x 4 μm). The host cells (67 x 6 μm) with elongated gametocytes become spindle-shaped with the nuclei appearing as thin bands beside the parasite.

Life cycle and epidemiology: The life cycle has only partly been studied. Most likely, it is similar to the life cycle of *L. simondi*. Vectors of *L. macleani* belong to the *Simuliidae*

Clinical signs and pathogenicity: Anaemia, pyrexia, diarrhoea, thick oral discharge and paralysis are often seen.

3.8.3 *Leucocytozoon simondi* (Mathis and Leger, 1910)

Syn. *L. anatis* and *L. anseris*

Hosts: Ducks, geese and wild anseriform birds in Europe, North America and Asia.

Morphology: The gametocytes measure 14 - 22 μm, are elongated when found in lymphocytes and monocytes and round when found in erythrocytes (Figure 3.26). The host cells become elongated, 45 - 55μm long, with a long, thin nucleus along one side. Round host cells are found with micro- and macro-gametocytes inside.

Life cycle and epidemiology: The birds become infected when bitten by blackflies (*Simulium* spp.). After entering the blood stream the sporozoites enters hepatocytes where the first generation of schizonts develop. Subsequent generations of schizonts may develop in the brain, heart, lung, liver, kidney, gizzard, intestine and lymphoid tissue forming megaloschizonts. The megaloschizonts (up to 500 μm) develop possibly in lymphoid or macrophagic cells. The megaloschizonts contain numerous cytomeres in which merozoites develop. At some stage the megaloschizonts rupture and the merozoites are released into the blood. This cycle may continue indefinitely or the merozoites may enter lymphocytes, monocytes and erythrocytes forming micro- and macro-gametocytes. In the vector the macro-gametocytes are fertilized by the micro-gametocytes forming oocysts. In the stomach of the blackflies the oocysts mature producing sporozoites.

The sporozoites break out of the oocysts and pass to the salivary glands. The prepatent period is approximately 2 weeks.

In young animals the morbidity may be high with a high mortality, whereas mortality in adults usually is low. Birds recovering from infections become life long carriers of the disease as for all species of *Haemosporidia.*

Clinical signs and pathogenicity: Infections with *L. simondi* are highly pathogenic for young ducks and geese. Per acute to acute infections appear in young animals. The animals are listless, anorectic, anaemic and have a laboured breathing. CNS symptoms may be seen before death. In adult birds the infections are usually chronic in character with similar symptoms as mentioned above. At *post mortem* examination splenomegaly and hepatomegaly is seen.

3.8.4 *Leucocytozoon smithi* (Leveran and Lucet, 1905)

Syn. *L. peaolopesi*

Hosts: Domestic and wild turkeys in North America. The parasite was introduced in Africa and Europe.

Morphology: The gametocytes (Figure 3.27), present only in leucocytes, are rounded before becoming elongated, 20 - 22 μm in length. After infection, the host cells become elongated, up to 50 μm in length.

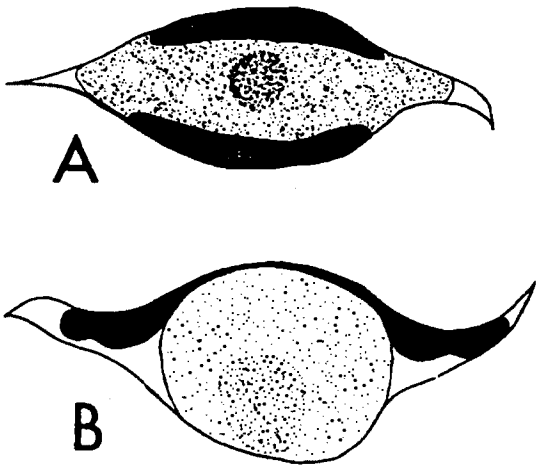

Figure 3.27 A. *Leucocytozoon smithi* macrogamete from turkey. B. *Leucocytozoon simondi* microgametocyte from duck (Levine 1973).

Life cycle and epidemiology: The life cycle is similar to other species of *Leucocytozoon* with a prepatent period of 9 days. The intracellular schizogony occurs in the liver. Megaloschizonts have so far not been recorded in *L. smithi*, but they are common in *L. simondi*.
The vectors of *L. smithi* are blackflies (*Simulium* spp.). Morbidity and mortality in birds is high. Birds recovering from infections may become life long carriers and can die if stressed.

Clinical signs and pathogenicity: Infections are more serious in poults than in adult turkeys. Affected birds are anaemic, anorectic and droopy with a tendency to sit. At later stages the affected birds may suffer from incoordination, fall over and die. In severe cases the duration of the disease is 2 - 3 days. At *post mortem* enlargement of spleen and liver is seen with inflammation in the duodenum.

3.8.5 Avian malaria

Plasmodium gallinaceum (Brumpt, 1945) and *Plasmodium juxtanucleare* (Versiani and Gomes, 1941)
Syn. *P.metatataticum* and *P.japonicum*, respectively.

Avian malaria is caused by protozoans belonging to the genus *Plasmodium*. About 38 species have been described. Generally avian malaria is not of major veterinary importance in commercial production systems, but may cause losses. However, among free ranging chickens high mortality rates can been seen due to *Plasmodium* spp. infections and more important an immuno suppression might take place.

Hosts: *P. gallinaceum* infections are seen in chickens in Asia and probably Africa, whereas *P. juxtanucleare* parasitizes chickens and turkeys in South America, Africa and Asia.

Morphology: Both gametocytes and schizonts of *P. gallinaceum* can be round, oval or irregular in shape. The nucleus of host cells is rarely expelled during infection, but may be displaced by the parasite. Each schizont produces from eight to thirty-six merozoites (Figure 3.28). The schizonts of *P. juxtanucleare* are round, ovoid or irregular and small compared to *P. gallinaceum*'s schizonts and usually in contact to the hosts cell nucleus (Figure 3.29). Two to seven merozoites are produced. In average there are 16 - 20 merozoites in erythrocytic schizonts of *P. gallinaceum* and 3 - 5 in

P. juxtanucleare. The gametocytes are usually round or ovoid, but may be irregular or slightly elongated. Often the host cell is distorted.

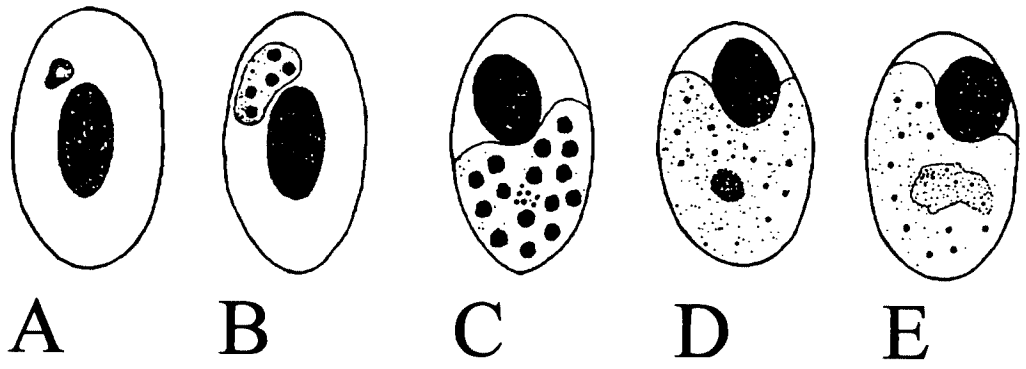

Figure 3.28 *Plasmodium gallinaceum* A: Young trophozoite (ring stage) B: Older trophozoite C: Mature trophozoite (segments) D: Macrogamete E: Microgametocyte (Redrawn after Levine 1973).

Life cycle and epidemiology: The vectors of avian malaria are mainly the mosquitoes *Mansonia* spp., *Aedes* spp., *Culex* spp. and *Armigeres* spp.. During blood sucking sporozoites are transferred from the mosquito salivary glands to the bloodstream of the host infecting macrophages and fibroblasts. In the liver cells merozoites are produced. After two generations, the merozoites are released into the bloodstream where they enter erythrocytes. The first stage of the merozoites in the erythrocytes, the trophozoite, appears as a circular form (except for *P. juxtanucleare*). Several divisions of the parasite nucleus lead to the formation of mature schizonts with a variable number of nuclei. The host cell ruptures and releases merozoites which infect new erythrocytes and can induce subsequent generations of schizonts in internal organs. After several asexual cycles, micro- and macro-gametocytes are formed. When ingested by mosquitoes the micro- and macro-gametocytes merge forming zygotes leading to oocysts and subsequently sporozoites in the salivary glands of the mosquitoes (Figure 3.30).
The gametocytes are located in erythrocytes. Exoerythrocytic stages are found in endothelial cells throughout the body.

Wild birds are relatively resistant to infections with avian malaria. Serious outbreaks may occur in domestic chickens when they are introduced into

glands to the bloodstream of the host infecting macrophages and fibroblasts. In the liver cells merozoites are produced. After two generations, the merozoites are released into the bloodstream where they enter erythrocytes. The first stage of the merozoites in the erythrocytes, the trophozoite, appears as a circular form (except for *P. juxtanucleare*). Several divisions of the parasite nucleus lead to the formation of mature schizonts with a variable number of nuclei. The host cell ruptures and releases merozoites which infect new erythrocytes and can induce subsequent generations of schizonts in internal organs. After several asexual cycles, micro- and macro-gametocytes are formed. When ingested by mosquitoes the micro- and macro-gametocytes merge forming zygotes leading to oocysts and subsequently sporozoites in the salivary glands of the mosquitoes (Figure 3.30).
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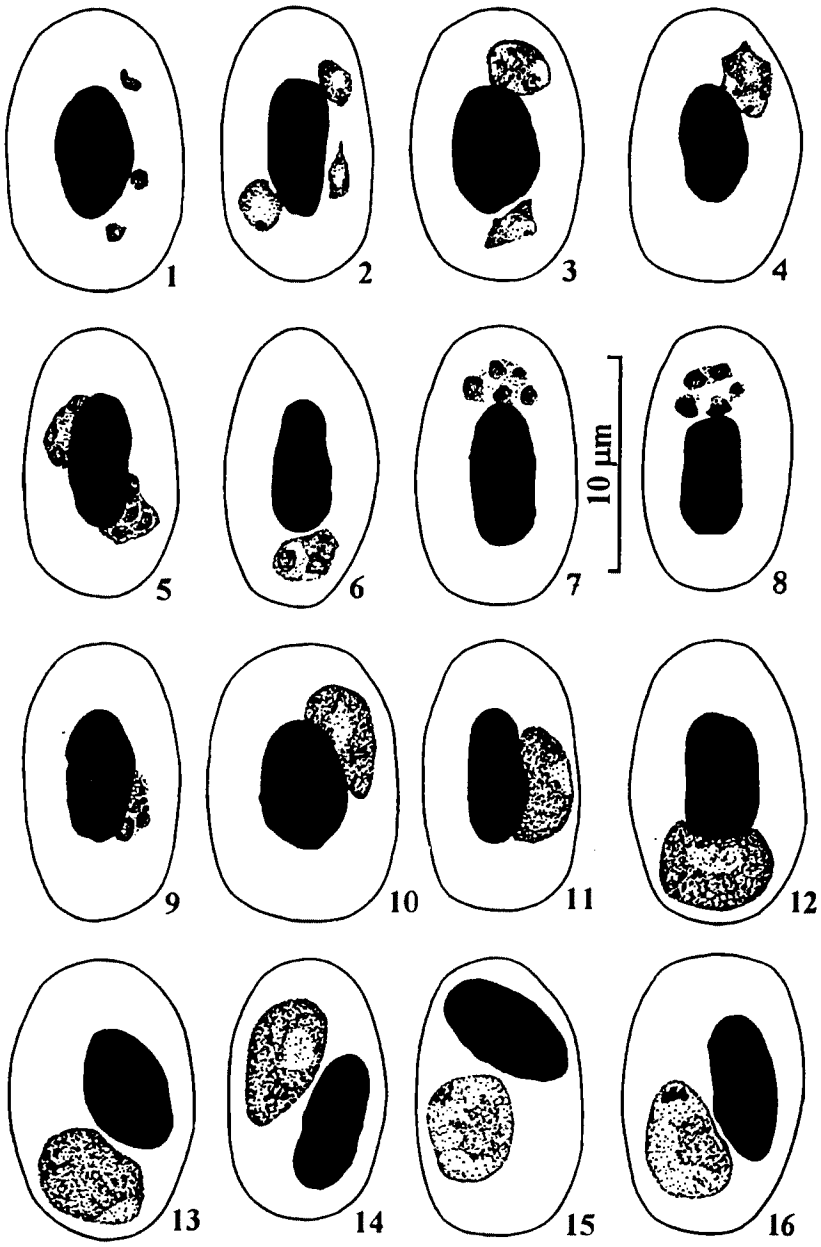

Figure 3.29 Different stages of *Plasmodium juxtanucleare* inerytrocytes from domestic chickens. 1-2: trophozoite. 3-9: merozoites. 10-14: macrogametocytes. 15-16: microgametocytes (Redrawn after Valkiunas, 1997).

Clinical signs and pathogenicity: Depending on the strain the clinical signs range from no apparent signs to severe anaemia and death. *P.gallinaceum* and *P. juxtanucleare* are most pathogenic and may cause high mortality, up to 90%. The presence of exoerythrocytic stages of *P. gallinaceum* may block capillaries in the brain producing CNS symptoms and sudden death.

3.8.6 *Aegyptinella* spp.:

A. pullorum (Carpano, 1929) and *A. mushkovskii* (Schurenkova, 1938)

Hosts: Chickens, turkeys, ducks, geese and other birds in Africa, Asia and Southern Europe.

Morphology: The initial bodies occur in the erythrocytes as trophozoites. They appear as small, 05 - 1.0 μm, round to oval bodies in the erythrocytes

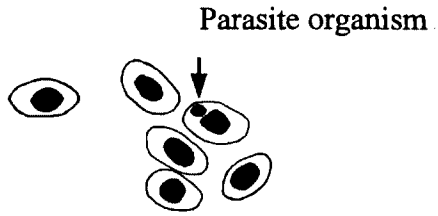

Figure 3.31 *Aegyptinella pullorum* in erythrocyte

Life cycle and epidemiology: *Aegyptinella* spp. belongs to the genus *Rickettsiales*. They are transmitted by the soft tick *Argas persicus* (→3.7.1). The life cycle is "simple", being a multiplication of the organism. The parasites are seen in erythrocytes as either initial bodies or as marginal bodies (Figure 3.31).
Newly introduced animals into endemic areas may die within a few days. Indigenous chickens are resistant to infections with *Aegyptinella* spp..

Clinical signs and pathogenicity: Affected birds have ruffled feathers, they become anorectic, droopy and may suffer from diarrhoea and hyperthermia. At *post mortem* examinations anaemia, jaundice, hepatomegaly, spleenomegaly and haemorrhages on the serosa may bee seen.

4 DIAGNOSTIC METHODS

To diagnose parasitic infections in poultry is a time consuming job, but it can be done with relatively simple tools. This chapter presents diagnostic techniques which can be performed by most laboratories for identification and quantification of parasitic infections through clinical and *post mortem* inspections, and the examination of faecal material and blood smears. The more sophisticated immunological and molecular biology techniques have not yet been developed for diagnosis of poultry parasites.

4.1 CLINICAL EXAMINATION OF CHICKENS

Clinical examinations of individual animals may be of limited value. But when investigating a disease problem in a poultry flock it is often beneficial to examine a few animals. Before the examination is commenced, information related to the flock and the anamnesis (disease history) should be recorded, e.g. the flock size, management practices, vaccination procedures, feeding practices, trading habits, other poultry in the flock, symptoms before death, clinical signs, number of animals dead, time of death etc.. Make notes on the information and examine live animals as follows:

Procedure

Flock:

☞Observe the flock: The general attitude of live birds and all abnormal conditions should be carefully noted.

☞Are there any signs of depression, anorexia, respiratory signs, blindness, incoordination, tremors, paralytic conditions, abnormal gait and leg weakness etc. in the flock.

☞It may be useful to observe the waste disposal area (e.g., have any dead animals been thrown there by the farmer?).

Individual animal:

☞Look for ectoparasites on the head, under the wings and thighs (lice and mites can be found on the bird, whereas other species leave the animals during daytime)

☞Examinations should be made for diarrhoea, respiratory discharges, conjunctival exudates, nasal discharges, feather and comb conditions, skin changes, body condition, dehydration, abscesses, signs of cannibalism, tumors etc..

☞If necessary, blood samples and blood smears are made, swabs are made, faecal samples are collected etc.

☞If *post mortems* are required a number of chickens should be sacrificed and examined according to the procedure described in section 4.6

4.2 Faecal examination

Identification of helminth eggs in faeces is an easy and cheap way to diagnose many helminth infections and to get an impression of the infection level at individual as well as population level. All helminths must find a way for their eggs to become available for a new host. In poultry, the eggs simply pass with the faeces. Newly deposited faeces may contain unembryonated eggs or eggs with well-developed embryos, e.g., *Tetrameres* spp., *Acuaria hamulosa*, *Allodapa suctoria* and *Gongylonema ingluvicola*. Hatched larvae are not seen in poultry faeces. Therefore, a complete examination of fresh poultry faeces does not include the Baermann technique for isolation of parasite larvae.

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

4.2.1 Collection of faecal samples

In order to maintain a standardized approach to counting and quantification of the parasite burden the examinations should always be carried out on fresh faeces.

Equipment

☞Cages for placement of individual animals

☞Plastic gloves (cheap plastic gloves are often preferable to the more expensive latex gloves) or plastic bags (big enough for the hand to fit)

☞Marking pen (waterproof)

☞Big 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 from identified birds to ensure that they are completely fresh. As samples cannot be taken directly from the cloaca, the bird is placed in a cage and the faeces is collected. This will also allow for registration of the age, sex and reproductive status of the bird and avoid repeated samples from the same individual.

☞Occasionally, if the helminth status of individual birds is not required, pooled samples can be taken by collecting a number of fresh droppings from the poultry house or pen.

☞The samples may be stored in the plastic bags or gloves by turning them inside out. Each sample should be unambiguously labelled with animal identification, date and location 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. Adult chickens produce about 5 - 8 g in one dropping.

☞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 keep them fresh. 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 a cooling box is not available, the samples may be placed in plastic containers with tight lids, and 3% formalin should be added 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 due to the dilution.

☞Immediately in the laboratory, the samples should 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, but a much shorter time is recommended, as even a few days storage may lead to larval development. Faecal samples should never be kept in a freezer as the eggs may be damaged.

4.2.2 Qualitative techniques for faecal examinations

A large number of different procedures are available for demonstrating helminth and coccidia eggs in poultry faeces. Three methods will be described below, all of them providing results that are only qualitative (or, at the most, semi-quantitative), 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, cestode 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 and cestode eggs will flotate in saturated NaCl,(salt) some nematode and cestode species have eggs which will flotate only in fluids with higher specific gravities, such as saturated $MgSO_4$ or saturated NaCl + glucose (sugar) (as used below). Among poultry helminths, little is known about this fact, and therefore it is recommended to standardize and continuously use only one of the flotation fluids with high specific gravity, e.g, the saturated salt and sugar solution (→ 8.1).

Trematode eggs are in general so heavy that the flotation principle does not work. Therefore, these eggs are concentrated by sedimentation techniques (→4.2.6), which, however, are not fully efficient and highly variable in their sensitivity.

4.2.3 Direct smear method

Identification of helminth eggs and coccidia oocysts is possible by using a direct smear method, where a thin smear of emulsified faeces is examined in a microscope.

**Direct microscopic examination of intestinal mucosa (→ 4.5) can only be used in animals which have been killed or found dead. It can be used to find the intracellular and extracellular stages of coccidia, other protozoa, small nematodes (e.g., *Capillaria* spp.), small trematodes, cestodes and cestode scolices.

Equipment

☞Slides

☞Cover slips

☞Microscope with 40 - 100 x magnification

☞**Scissors

Procedure

The *Direct smear* method is illustrated in Figure 4.1.

☞A small quantity of faeces is placed on a slide

☞A few drops of water are added and mixed with the faeces

☞A cover slip is placed on top

☞The slide is examined in a microscope using 40 - 100 x magnification (→ 4.2.11)

☞**If the mucosa is to be examined, a deep scraping is made of the suspected mucosa with one end of the slide. The material is spread in a very thin layer on a new slide and covered with a cover slip.

☞Examine the slide in a microscope at 40 - 100 x magnification.

Note: this method can be used for the detection of helminth eggs and coccidia oocysts when the concentration of eggs is high. Furthermore, it can be used to detect cestode and trematode eggs.

Note: this is not a quantitative method.

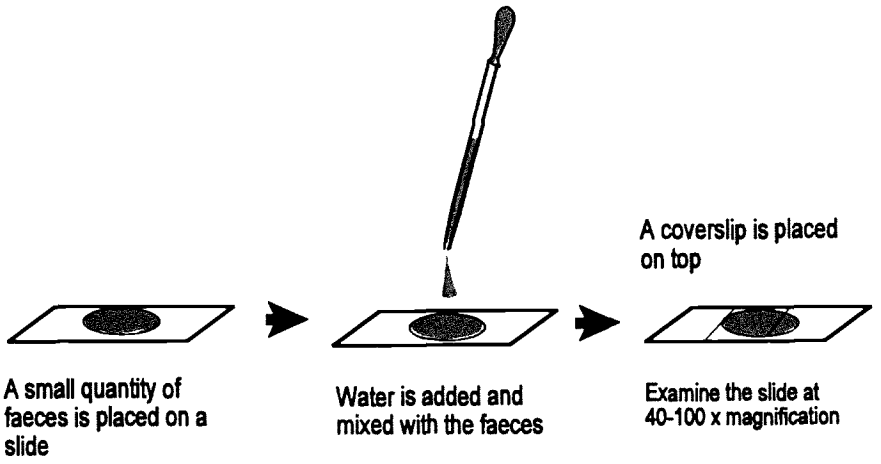

Figure 4.1 Direct smear method.

4.2.4 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 pre-calibrated teaspoon (3 g)

☞Flotation fluid: Saturated NaCl with 650 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

☞Cover slips and microscope slides

☞Microscope with 40-100 x magnification

Procedure

The *Test Tube Flotation* procedure is illustrated in Figure 4.2

☞Transfer approximately 3 g faeces (weigh out or measure with pre-calibrated 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 strained faecal suspension from plastic container 2 into a test tube, which is placed in a 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 cover slip on the top of the test tube.

☞Leave the test tube for about 20 minutes. The helminth eggs and the coccidia oocysts will flote and thus accumulate just beneath the cover slip.

☞Lift off the cover slip 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 cover slip must be done very carefully in order to retain as many eggs as possible. Place the cover slip on a microscope slide, and examine the sample at 40-100 x magnification in a microscope.

Note: this method is qualitative and not quantitative.

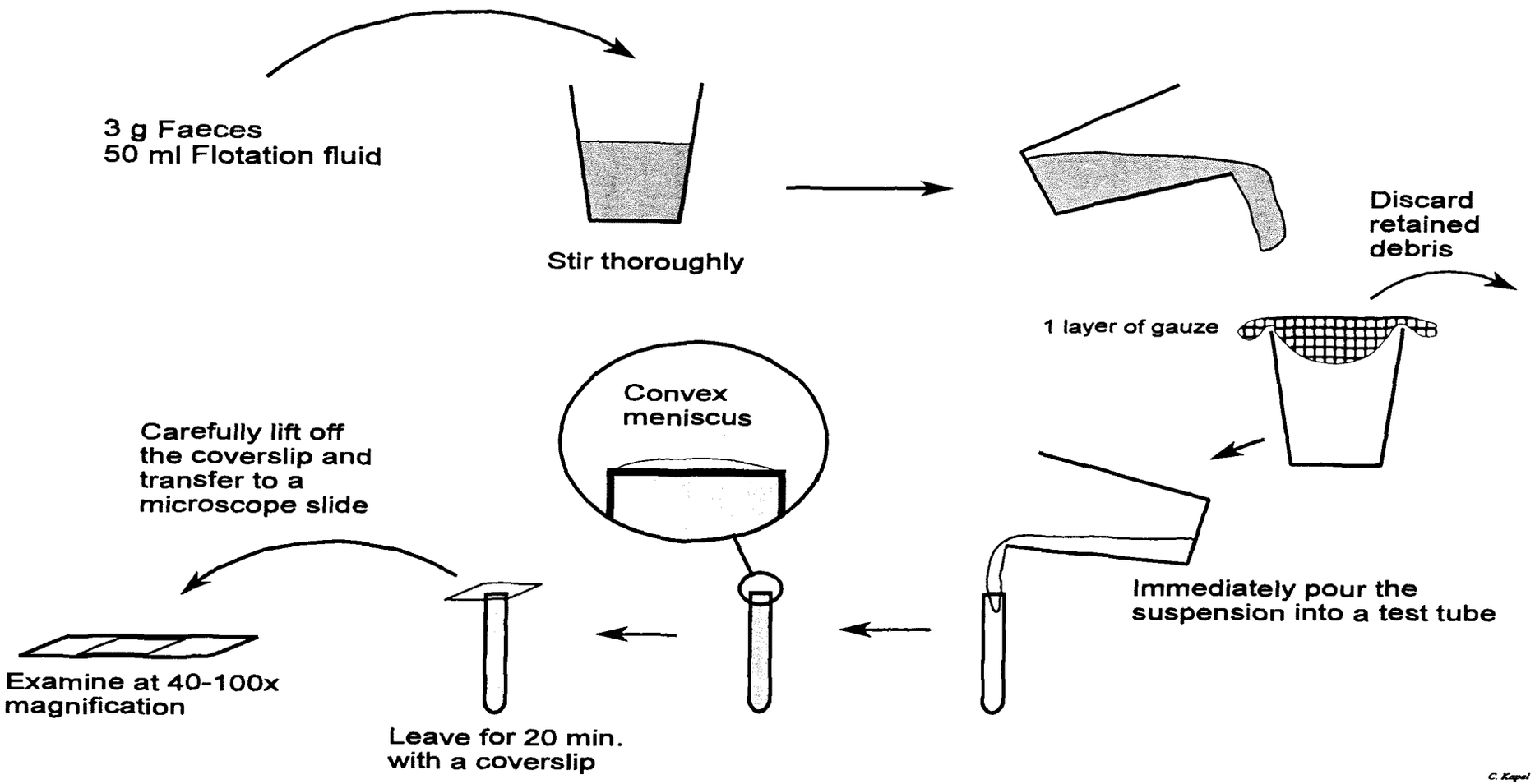

Figure 4.2 Test tube flotation

4.2.5 Simple flotation

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

Equipment

- ☞2 beakers or plastic containers (disposable or recyclable)
- ☞Weight or a pre-calibrated teaspoon (3 g)
- ☞Flotation fluid: Saturated NaCl with 650 g glucose per litre
- ☞Measuring cylinder
- ☞Stirring device (fork, tongue depressor)
- ☞Nylon tea strainer or a single layer of cotton gauze
- ☞Test tube (dry)
- ☞Cover slips and microscope slides
- ☞Microscope with 40-100 x magnification

Procedure

The *Simple Flotation* procedure is illustrated in Figure 4.3

- ☞Transfer approximately 3 g faeces (weigh out or measure with pre-calibrated 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 flote 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 in the drops of surface layer which adhere 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 cover slip on the microscope slide, and examine the sample at 40-100 x magnification in a microscope.

Note: this method is qualitative and not quantitative.

The test tube method and the simple flotation method are in principle equal, but one may discuss which of the methods is best. It is the opinion of the authors that the simple flotation method is superior to the test tube method due to the fact that the surface is bigger and that the distance the eggs have to travel to reach the surface is shorter.

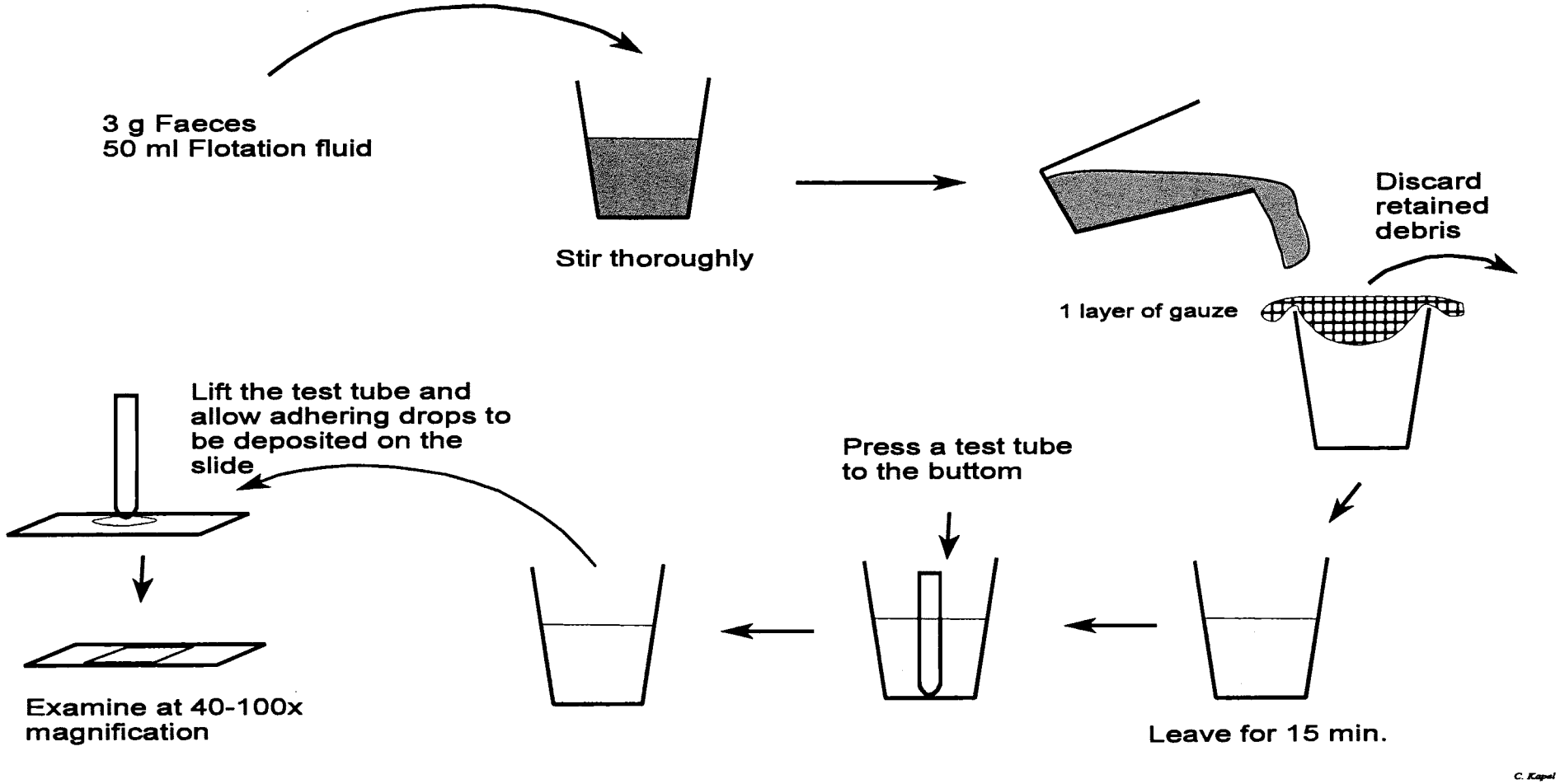

Figure 4.3 Simple flotation

4.2.6 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 poultry trematodes, this applies to *Echinostoma revolutum* and *Prosthogonimus* spp.

The technique described below is merely a combination of washing and sieving of faeces to remove the smallest and the largest faecal particles. This 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 recyclable)
- ☞Weight or a pre-calibrated 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 should be replaced by conic sedimentation beakers of glass, but they are rather expensive. The figures illustrate the technique using conical beakers.
- ☞Methylene Blue (1% solution) or Malachite Green (1% solution)
- ☞Cover slips and microscope slides
- ☞Microscope with 40-100 x magnification

Procedure

The *Sedimentation Procedure* is illustrated in Figure 4.4

☞Transfer approximately 3 g faeces (weigh out or measure with pre-calibrated 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 approximately 10 ml of the filtered suspension into a test tube 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.

☞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 cover slip on the microscope slide, and examine the sample at 40-100 x 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.

Note: the sedimentation technique is qualitative and not quantitative.

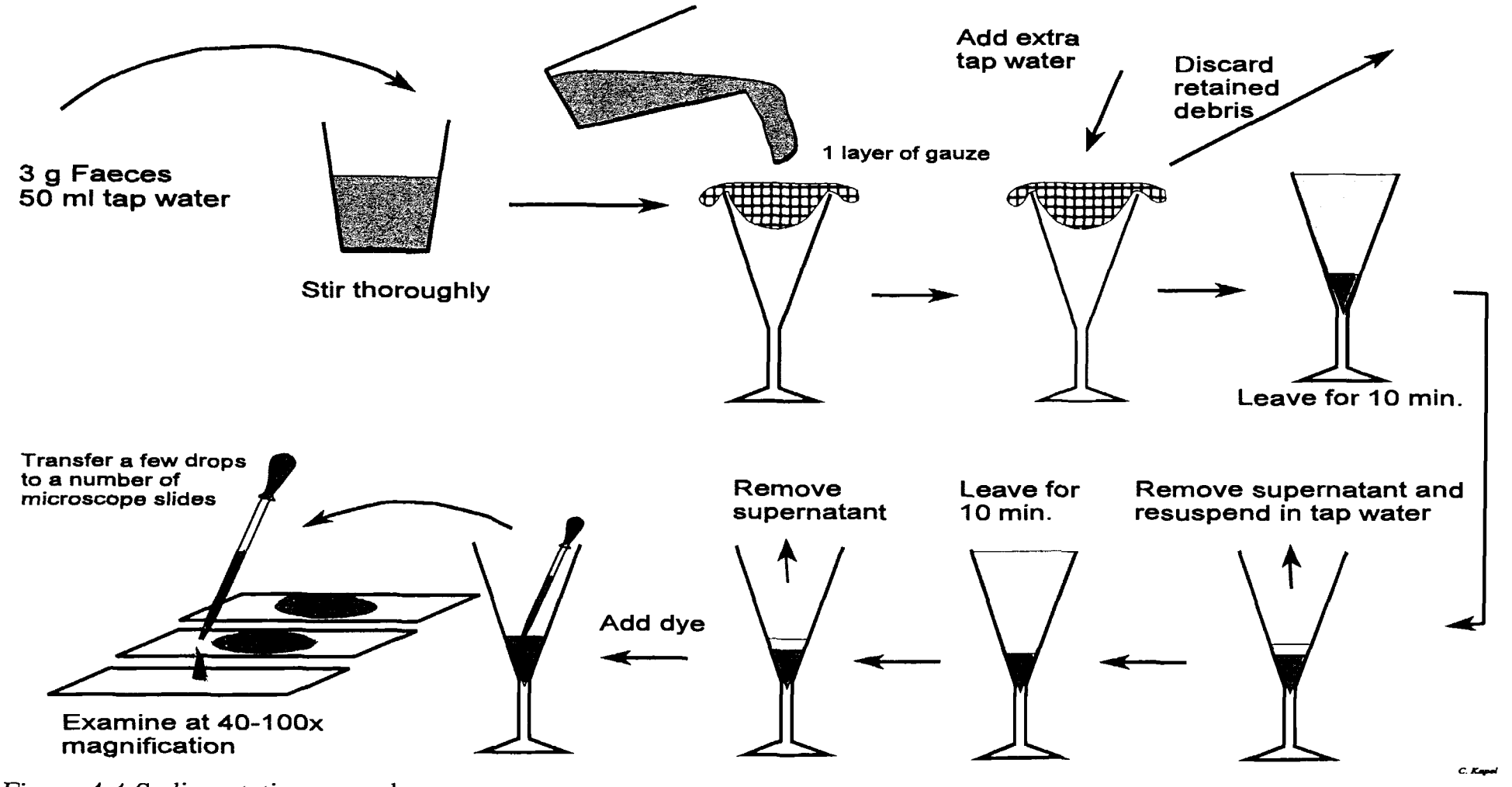

Figure 4.4 Sedimentation procedure

4.2.7 Quantitative techniques for faecal examinations

The qualitative flotation techniques, which are used for nematode eggs, cestode eggs and coccidia oocysts, have been elaborated to become quantitative, when the eggs are allowed to flote 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.

4.3.8 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)
- ☞Weight
- ☞Measuring cylinder
- ☞Flotation fluid: Saturated NaCl with 650 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 clearer. 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 inexpensive that they may then be discarded; or, alternatively, the scratched outer surface may be polished (e.g. by a watchmaker).

☞Filtering paper cut into approximately 1 cm wide strips

☞Microscope with 40 - 100 x magnification

Procedure

The *Simple McMaster Technique* is illustrated in Figure 4.5

☞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 flotation fluid by means of a measuring cylinder. If less than 4 g faeces is weighed, 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. Care should be taken to avoid air bubbles.

☞Leave the filled McMaster chamber to rest on the table for 3-5 minutes before counting to allow all eggs to flote.

☞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 4.2.10 *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.

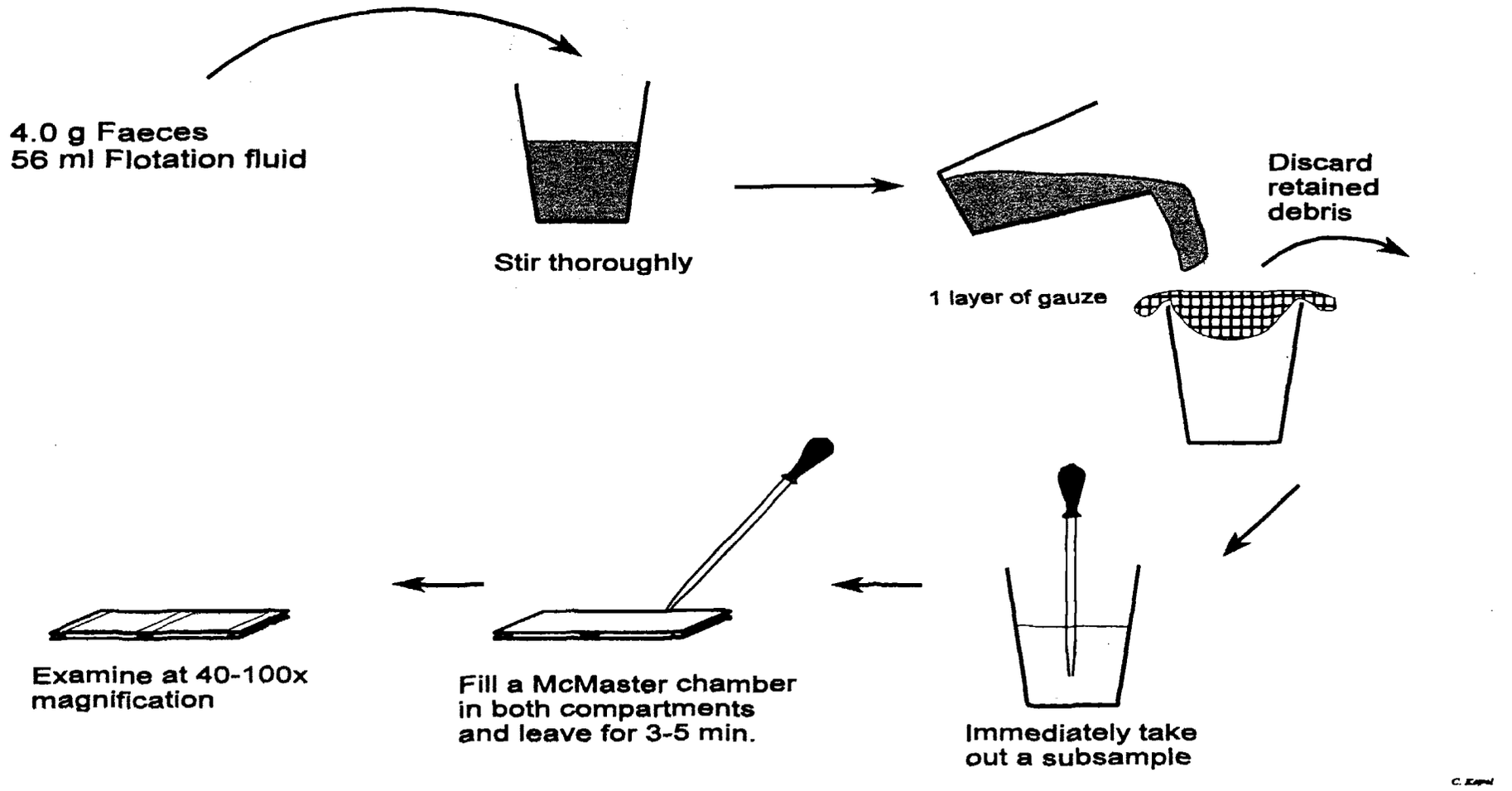

Figure 4.5 Simple McMaster technique

4.2.9 Concentration McMaster technique

This technique is somewhat more complicated than the *Simple McMaster Technique*, but the recovery of eggs is better and the sensitivity is higher (20 eggs per gram of faeces). Therefore, this technique is 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 650 g glucose per litre
- ☞Pasteur pipettes and rubber teats
- ☞McMaster counting chamber.
- ☞Filtering paper cut into approx. 1 cm wide strips
- ☞Microscope with 40-100 x magnification

Procedure

The *Concentration McMaster Technique* is illustrated in Figure 4.6

☞Weigh out 4.0 g of 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 correspond 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 hard clumps of faeces will be completely dissolved, but the step may not be necessary if the faeces are soft and rich in water. 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.

☞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 closing 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, thereafter 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 careful, by sucking up and down in 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 flote, 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 4.2.10 *Counting the McMaster chamber*).

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

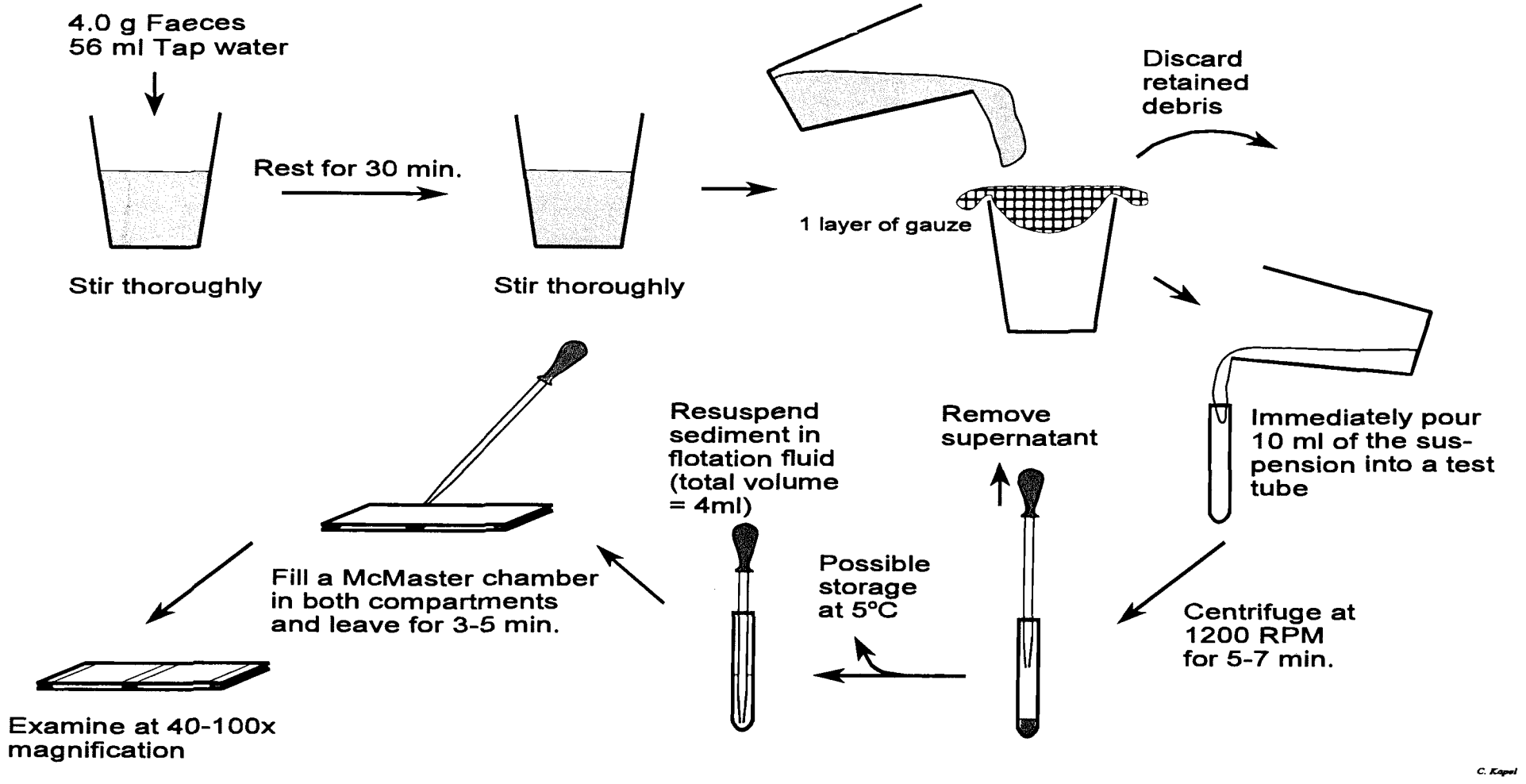

Figure 4.6 Concentration McMaster technique

4.2.10 Counting the McMaster chamber

Procedure

☞The filled McMaster chamber must rest on the table for at least 3-5 minutes to allow all eggs to flote, i.e. accumulate just below the upper glass of the chamber. It is important that the eggs have enough time to flote in order to avoid an underestimate in 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.

☞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 4 x 10 magnification, while all others are recommended to use a 10 x 10 magnification until they are completely familiar with all types of eggs. If coccidia oocysts are counted, a 10 x 10 magnification should always be used, as poultry 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, cestode egg or coccidia oocyst 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 1 x 1 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 represent 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 represent 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 the first side of the chamber, and 22 eggs are counted in the second side.
If the *Simple McMaster Technique* has been used, EPG = (18+22) x 50 = 2000.
If the *Concentration McMaster Technique* has been used, EPG = (18+22) x 20 = 800.

4.2.11 Identification of eggs

Helminth eggs found in poultry faeces may have a characteristic appearance, which permits an unambiguous identification.

Some of the most common eggs are shown in Figure 4.7. As coccidia are quite common in poultry some oocysts are also shown. Furthermore, some characteristics are briefly listed in Table 4.1

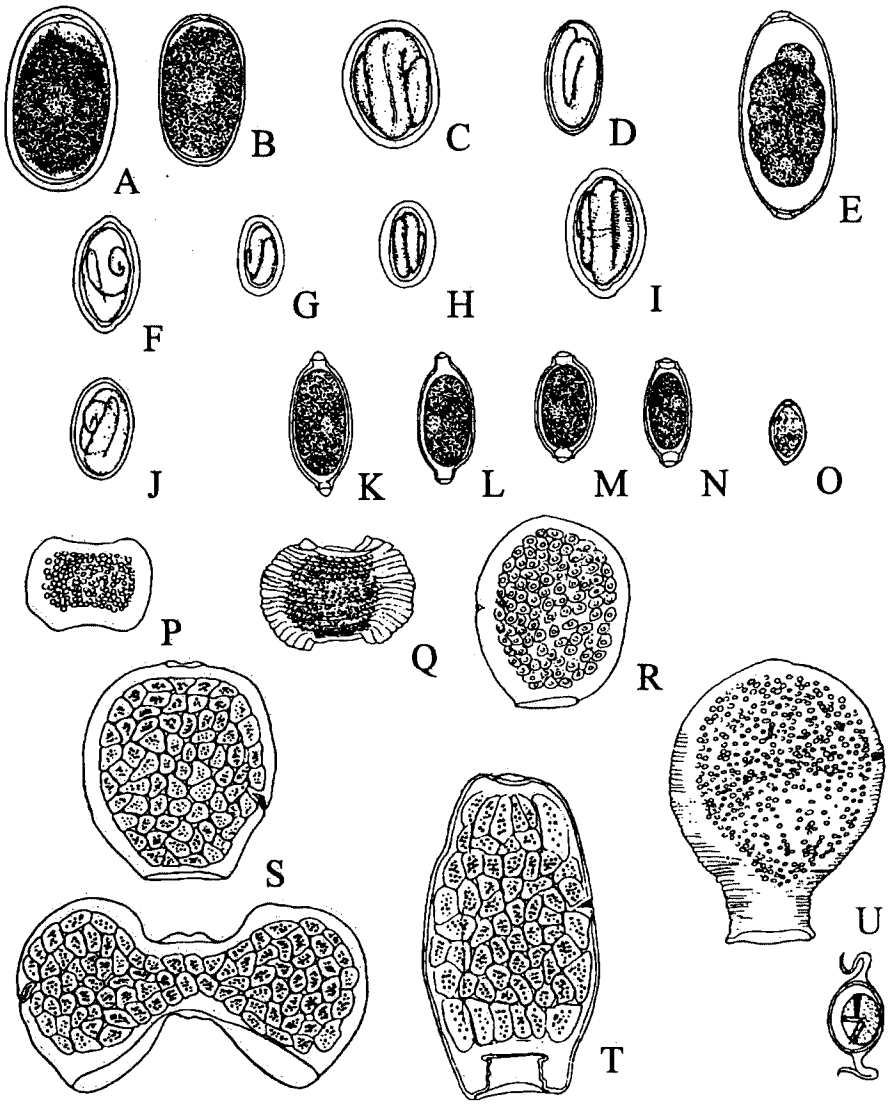

Figure 4.7 The most important helminth eggs and segments of cestodes. A: *Ascaridia galli* B: *Heterakis gallinarum* C: *Allodapa suctoria* D: *Strongyloides avium* E: *Syngamus trachea* F: *Tetrameres americana* G: *Acuaria* spp. H: *Acuaria hamulosa* I: *Gongylonema ingluvicola* J: *Oxyspirura mansoni* K: *Capillaria annulata* L: *Capillaria anatis* M: *Capillaria obsignata* N: *Capillaria contorta* O: *Prosthogonimus* spp. P - U: Segments of cestodes P: *Amoebotaenia cuneata* Q: *Hymenolepis carioca* R: *Raillietina cesticillus* S: *Raillietina echinobothrida* T: *Raillietina tetragona* U: Segment of *Choanotaenia infundibulum* and a single egg (Modified after Soulsby 1982).

Table 4.1 A list of characteristics of the most common helminth eggs and coccidia oocysts.

Parasite	max. size: width x length (µm)	contents of the fresh egg	morphological characteristics of eggs
EGGS			
Nematodes			
Oxyspirura mansoni	65 x 45	larvae	ovoid embry. egg
Syngamus trachea	90 x 49	larvae	ellipsoid, operculated
Gongylonema ingluvicola	58 x 35	larvae	
Dispharynx nasuta	40 x 25	larvae	ellipsoid
Tetrameres spp.	60 x 30	larvae	-
Acuaria hamulosa	40 x 27	larvae	thick shell
Amidostomum anseris	110 x 82	embryo	-
Capillaria spp.	70 x 30	not embryonated	operculated
Ascaridia spp.	94 x 55	not embryonated	ellipsoid, thick shell
Heterakis spp.	75 x 50	not embryonated	ellipsoid, thick shell
Allodapa suctoria	86 x 76	larvae	spherical
Cestodes			
C.infundibulum	-	seg. with eggs	elongate filaments
Davainea proglottina	28 x 40	seg. with eggs	spherical
Hymenolepis spp.	62 x 55	seg. with eggs	oval
Raillietina spp.	25 x 50	seg. with eggs	oval, spherical
Trematodes:			
Prosthogonimus spp.	27 x 18	not embryonated	operculated w/spine
Echinostoma revolutum	126 x 70	not embryonated	ellipsoid

Coccidia oocysts			
Eimeria acervulina	20 x 16	one cell	ovoid oocysts
E.brunetti	30 x 24	one cell	ovoid oocysts
E.maxima	42 x 30	one cell	subspherical oocysts
E.necatrix	22 x 18	one cell	ovoidal oocysts
E.tenella	26 x 23	one cell	broadly ovoid
E.adenoides	32 x 21	one cell	oocysts
E.meleagrimitis	27 x 22	one cell	ellipsoidal oocyst
E.galloparvonis	33 x 20	one cell	ovoid
E.dispersa	31 x 24	one cell	ellipsoidal
Tyzzeria perniciosa	12 x 11	one cell	broadly oval
			thin-walled oocysts

4.2.12 Interpretation of the faecal 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 was possible to predict the worm burdens from the egg counts, one would still have the problem of how to interpret the effect of the worm burden.
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), and the management. Therefore, only the false negative and positive results, and the relationship between egg counts and worm burdens, will be discussed below.

4.2.13 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 poultry has recently been moved from a clean to a contaminated area, it may harbour a heavy worm burden of young immature larvae 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. The prepatent period of *Eimeria* spp. is less than 1 week < *Capillaria* spp.: 2 - 3 weeks < *Heterakis* spp.: 3 - 4 weeks < *Ascaridia galli*: 4 - 8 weeks etc.

When the transmission rate is high, the poultry will respond immunologically to the parasites, and a depression in egg output may take place. This is particularly common when the helminth species are strongly immunogenic. *Ascaridia galli* is a good example. But also lightly immunogenic species, such as *Heterakis* 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, or when adult worms have a low fecundity, or when the test used is not sufficiently sensitive.

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 non-embryonated 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. Poultry are exceptionally good candidates for false positive samples, as they may scavenge around in faeces or contaminated soil.

4.2.14 The relationship between egg counts and worm burdens

Even though the EPG cannot be used to estimate the worm burden directly in either experimental infections or in natural infections, the described methods are adequate for monitoring helminth infections at individual as well as population level. The EPG may be influenced by the number of adult worms in the GI tract, worm age, host immunity, host age, host sex, stages of infection, fecundity of the worm, feed composition and consistency of the faeces and time of day of collecting the faeces. The faecal consistency may have a rather great influence, as dry, hard faeces with relatively small content of water will generally have higher EPG values than softer and more watery faeces due to a simple diluting effect.

As mentioned, poultry respond immunologically to helminth infections. Thus, *Ascaridia galli* females seem to have the highest egg output when only few pairs are present, while the individual fecundity decreases with increasing worm burdens. For less immunogenic species 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 lying becomes reduced or may even stop completely.

4.3 Diagnosis of haemoparasites

The diagnosis of *Leucocytozoon* spp., *Plasmodium* spp. and *Aegyptinella* spp. infections is based on direct microscopic identification of gametocytes or schizonts and initial bodies (*Aegyptinella* spp.) in erythrocytes or leucocytes in thin blood smears (Leucocytozoonosis → 3.8.1-5, Avian malaria → 3.8.6, Aegyptinellosis → 3.8.7).

4.3.1 Blood smears

Equipment for blood smears

☞Slides with or without frosted ends. Different slides are available on the market. Some are pre-cleaned and some have frosted ends. If they are not clean, it is advisable to clean them with 95 % alcohol and dry them with a piece of clean cloth. Smears with frosted ends are recommended if a large number of slides are to be made.

☞Giemsa stain

☞Staining jar

Procedure

The preparation of a blood smear is shown in Figure 4.8

☞Information on the animal and date of sampling should be written with a pencil in the frosted area.

☞While cleaning and writing the needed information, the slides should be held by their edges to avoid fingermarks.

☞A small drop of blood is placed on the end of one slide which is held horizontally (A).

☞The end of the other slide (B) is placed on top of the drop to allow the blood to spread. The angle between the horizontal slide and the other should be between 30° and 45°.

☞Slide B is then pushed quickly along slide A so the blood is pulled along. An even thin blood film should be the result. In the microscope the erythrocytes should be seen lying "shoulder-to-shoulder".

☞Slide A is then dried by placing it vertically in a box or by waving it in the air. It is very important that dust is avoided on the slide.

☞After drying the blood film is fixed by dipping it in absolute methyl alcohol.

☞Allow the alcohol to evaporate.

Procedure for staining

☞A 10% Giemsa stain is prepared according to the suppliers recommendations (→ 8.2)

☞For the best staining result, the slides are stained in a jar in which the slides can be entirely dipped for ½ hour.

☞The slides are removed from the straining jar and washed twice in buffered or ordinary tap water.

☞After washing the slides are dried in air.

☞When dry they are ready for examination in a microscope using oil and 400 x magnification.

☞If the staining has proved not to be good enough, the slides can be additionally stained.

Note: Poultry erythrocytes contain a nucleus and it may be difficult for the inexperienced researcher to produce thin blood smears. It is thus recommended to practice before making blood smears for examination.

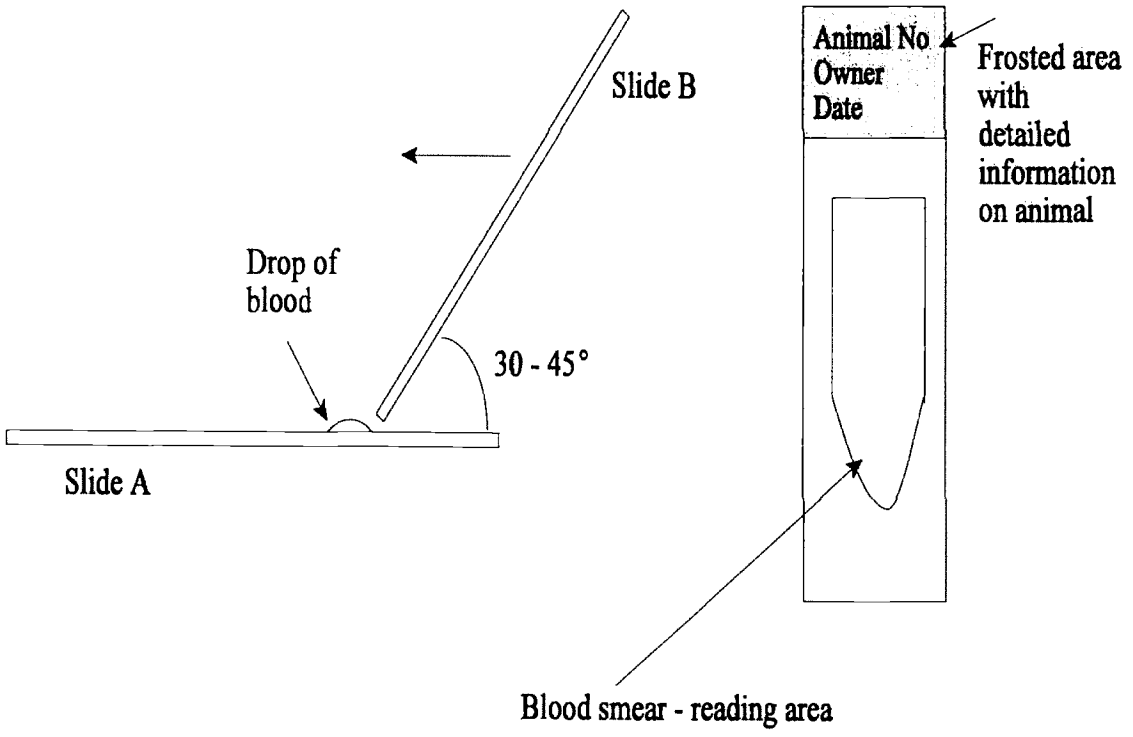

Figure 4.8 Procedure for preparation of a blood smear.

4.4 Diagnosis of ectoparasites

The ectoparasites are identified by distinguishing between body divisions, the head, the thorax and the legs (ticks → 3.7.1, mites → 3.7.2-3, fleas → 3.7.4).

4.4.1 Direct examination

Infected feathers from the vent or under the wings are placed in a screw-capped bottle. In the laboratory a few ectoparasites can be picked up with a needle and transferred to saline or Berlese's fluid (→ 8.2) and examined under a microscope. Berlese's fluid is convenient for mounting specimens for direct examination.

4.4.2 Skin scraping

Examination for the presence of mites can be done by scaping the skin and examine in a microscope.

Equipment

- ☞Scalpel blade
- ☞Slides
- ☞Cover slips
- ☞10% KOH (Potassium hydrate)

Procedure

- ☞Scrapings are taken from the edge of the affected area.
- ☞Feathers should be removed from the chosen area.

☞Before scraping liquid paraffin is used to moisten the skin and to ensure that the scraping adhere to the scalpel.

☞Scraping is done until a slight amount of blood oozes from the surface.

☞The material is transferred to a slide and some drops of 10% KOH is added and the specimen should be allowed to clear for 5 - 10 minutes.

☞A cover slip is placed on top, and the sample is ready for examination under low magnification.

4.5 Post-mortem examination of chickens

Equipment

☞Disinfectants

☞Scissors and knife

☞Pair of forceps

☞Tray measuring 40 x 30 cm

☞Containers with formalin (if tissue samples are needed for histological examination) or 70 % alcohol (if specimens of parasites are isolated for further identification)

☞Sterile cotton swabs for bacteriological examinations

☞Media for viral examination

☞Slides for impression smears

Post mortem procedure

1) Examination of the general body condition through palpation (symmetry, crepitation, ascites etc.). Evaluation of the general condition - is the weight normal, below or above normal.

2) Examine the plumage, skin, legs, feet etc. Indications of the effect of management and the environment where the poultry come from, e.g., inflammation of the feet: poor litter, wet bedding, chronic *Pasteurella multocida* infection of the head, fowl pox, ectoparasites etc..

3) Cloaca region to receive special attention (enteritis, salphingitis, uraemia etc.)

4) During 1 - 3 special attention is given to ectoparasites, specially around the head, on the body under the wings and thighs.

5) Mucosa of openings (conjunctiva, oral cavity and cloaca) examined (anaemia, inflammation, avitaminosis A).

6) The bird is placed on its right side with the head pointed towards the examiner.

7) Sinus infraorbitalis are opened and examined (Mycoplasma infections, Haemophilus paragallinarum, NDV, AI, ILT, IBV etc.).

8) The beak is opened and the blunt part of the scissors inserted into the oral cavity which is cut open. Continue down the neck to open the oesophagus and the crop. Oral cavity, pharynx, oesophagus and crop to be examined (inflammation, avitaminosis A, fowl pox, ILT etc.).

9) Larynx and trachea are subsequently opened and examined (NDV, ILT, *Syngamus trachea* etc.).

10) Inspect and incise thymus.

11) The bird is placed on its back with the legs towards the examiner.

12) Skin incised transversely behind processus xiphoideus and s/c incision towards both knees are done to enable the removal of skin over the pectoral muscles to be pulled in cranial direction.

13) Bursa praesternalis inspected (inflammations associated with immobile birds and mycoplasma and reovirus infections).

14) Pectoral muscles incised to inspect for tumours (MD, bleedings as seen in septicaemic carcasses, deep pectoral necrosis and IB variant manifestations.

15) Legs and hips are bent outwards until each head of femur is

dislocated (luxated) from its acetabulum.

16) Transverse incision behind processus xiphoideus to open into the abdominal cavity.

17) Incisions are made on both sides of the thorax up to the brachial region/shoulders (apertura thoracis cranialis).

18) Sternum with the pectoral muscles may now be rotated 180° upwards and cranially to expose the abdominal and thoracic cavities, but before this is done sternum is lifted slightly to see if there are indications of bacteriological/virological infections (swollen organs, bleedings, exudates etc.). In that case tissue samples are removed or swabs taken as sterile as possible.

19) All airsacs are inspected *in situ* (inflammation).

20) Other visible organs are subsequently inspected *in situ*.

21) The heart with the pericardial sac is removed and examined. The necessary incisions are made to examine all parts (form and colour evaluated).

22) The liver and the gall bladder are removed and examined.

23) The spleen is removed and examined (enlarged liver (with rounded edges) and spleen typical of septicaemia).

24) A transverse incision cranially to the proventriculus is made and the whole intestinal tract is removed in a caudal direction. To remove the whole intestinal tract a transverse incision is made 1-2 cm cranial to the cloaca. The oesophagus and the crop are removed by separation from the other tissue and pulled in a cranial direction.

25) The intestinal tract is examined from the serosal surface and opened in a caudal direction starting from the proventriculus. During this procedure pancreas is investigated. When examined for endoparasites the whole intestine is placed in a tray.

26) Examinations for parasites are carried out (proventriculus, ventriculus/gizzard (- remove the keratinised layer) and intestine (duodenum loop, mid intestine, rectum and cloaca). Look for nematodes, cestodes, trematodes and coccidia oocysts (→ 4.6).

27) The mucosa is examined (necrotic enteritis, salmonellosis (typhlitis), NDV, bleedings, ulcerations).

28) Incision through the cloaca in the midline in cranial direction to inspect the whole abdominal cavity including reproductive tract, testicles, kidneys and airsacs.

29) In female poultry (♀) the reproductive tract is cut free of its ligaments in a caudal direction and subsequently opened through infundibulum, magnum, isthmus, uterus and vagina which are inspected both from serosal and mucosal surface (productive inflammations often caused by *E.coli*).

30) In young poultry Bursa Fabricius is opened through its opening to the cloaca and inspected (Gumboro disease; often associated with swelling and oedema of the organ or bleedings. Often tumours in case of Leucosis (not Marek!)).

31) The kidneys are subsequently examined (clostridial infections - pale due to toxins, nephropathies of other causes).

32) Plexus lumbo-sacralis is examined (Mareks disease).

33) Lungs are removed and examined. Always remember to investigate by floating on water in case of abnormalities.

34) The brachial plexus is examined (Mareks disease).

35) Nervus ischiadicus is examined on both sides (beneath Musculus gracilus) (Mareks disease).

36) Knees and hock joints are opened and inspected and tendons and

tendon sheets are examined during the same procedure. All other joints are palpated and opened in case of swellings or asymmetry. (*Staphylococcus aureus*, reoviruses, *Mycoplasma synoviae*, *E.coli*, *P.multocida*, and *S. gallinarum/pullorum*)

37) Ossification is examined in young animals. Attention is paid to the ribs and their junction to the spine and the cartilaginous part (swelling avitaminosis D).

38) Parallel incisions to the bones are made on tibia/tarsus to examine for TD.

39) The brain is examined upon indication. (Avian encephalitis, avitaminosis E).

40) Lesions found are summarized and the pathogenesis/connection between lesions is evaluated. Finally a tentative diagnosis of the animal is made based upon anamnesis (disease history), symptoms and lesions demonstrated.

Note: *post mortem* of more animals of the flock must be conducted if conclusions concerning the disease status of the flock are to be made.

4.6 Identification and preservation of helminths

Examination for parasites in the dead animal can start from point 24 through to 26 in the *post mortem* procedure (→ 4.5). But generally the examiner should carry out a full *post mortem* examination and incorporate the parasitic examination as a part of this.

Equipment

☞Tray measuring 40 x 30 cm (or equivalent) with some edge.

☞Pair of scissors

☞Pair of forceps

☞Laboratory sieve with an aperture diameter of 100 μm or less.

☞Small bottles for storage of parasites

☞Alcohol (70 %)

☞Lactic acid

☞Tap water preferably with some pressure

Procedure

☞A transverse incision cranially to the proventriculus is made and the whole intestinal tract is removed in a caudal direction. A transverse incision is made 1-2 cm cranial to the cloaca and the intestinal tract is removed completely. The oesophagus and the crop is removed by separation from the other tissue and pulled in a cranial direction.

surface for the presence of *Gongylonema ingluvicola* (→ 3.2.1) and *Tetrameres* spp. (→ 3.2.2).

☞After this examination the whole intestine is placed in the tray.

☞The gastrointestinal tract is opened in a longitudinal section starting from the proventriculus.

☞The ventriculus (gizzard) is opened and the keratinised layer is removed to look for *Acuaria hamulosa* (→ 3.2.4) or *Amidostomum anseris* (→ 3.2.5).

☞The intestinal contents are carefully washed through a test sieve with an aperture of 100μ or less. The contents of the sieve are transferred to a petri dish. Remember to cleanse the tray, scissors and forceps before opening a new intestine.

☞After washing the mucosa is carefully scraped in order to loosen parasites embedded in the mucosal layer. The intestine is washed again and the contents of the sieve transferred to a petri dish.

☞The petri dishes should be examined under a stereo microscope and **all** helminths should be grouped and counted before being stored in 70% ethanol.

☞For the final identification, a representative sample (not less than 20 from each group) of the helminths are cleared in lactic acid* and examined under a light microscope with 40 - 100 x magnification.

Note: the majority of the helminths can be identified by using the helminthological keys of Chabaud (1978), Anderson & Bain (1982), Soulsby (1982), Calnek *et al.* (1991), Anderson (1992) or Khalil *et al.* (1994) (→ 9). Unfortunately the nomenclature is not always consistent and the above mentioned reference books are not always satisfactory in a final identification of poultry helminths. In such cases the parasites can be sent to the FAO Collaborative Centre on taxonomi for further identification.

* The use of lactic acid may not be sufficient to distinguish between different cestodes species. Special staining procedures may be required.

5 Epidemiological disease investigation at flock or population level

This chapter will briefly describe the principles behind an epidemiological field study, how to design the study and what sample size to select. For more detailed explanations please refer to the section with references (→ 9.1).

5.1 Measures of disease occurrence

When investigating a disease in a flock or in a population it is necessary to count the numbers of affected animals so that the disease can be described quantitatively, and to relate the number of affected animals to the size of the flock or population at risk of developing the disease of interest. Furthermore, it is important to describe where and when the disease occurred. The relative number of affected animals is termed the **morbidity** and the relative number of deaths is termed **mortality**. **Case fatality** is the relative number of individuals dying from their disease

Measures of disease occurrence can be described in two ways:

- ☞ Prevalence
- ☞ Incidence

The prevalence, *P*, is the number of individuals with the disease or related attributes (e.g., infection or presence of antibodies) in a known population, at a designated time, without distinction between old and new cases. It can be expressed as follows:

$$P = \frac{\textit{Number of individuals having a disease at a particular point} \in \textit{time}}{\textit{Number of individuals} \in \textit{the population at risk at that point} \in \textit{time}}$$

Prevalence may vary from values between 0 and 1. If multiplied with 100 it is expressed as a percentage from 0 - 100%.

The incidence, *I*, is the relative number of new cases that occur in a known population over a specified period of time. The two essential components of an incidence value are:

a) the number of new cases; and
b) the period of time over which the new cases occur.

Incidence can be calculated as **cumulative incidence (*CI*)** or as **incidence rate (*I*)**.

$$CI = \frac{\textit{Number of individuals that become diseased during a particular time}}{\textit{Number of healthy individuals} \in \textit{the population at the beginning of that period}}$$

The cumulative incidence is the proportion of non-diseased individuals at the beginning of a period of study that become diseased during the period. The cumulative incidence can take values between 0 and 1 (or between 0 and 100%). Cumulative incidence is an indication of the average risk of developing disease during a particular period, in the individual or in the population.

Incidence rate, *I*, measures the rapidity with wich new cases of disease develop over time and is calculated:

$$I = \frac{\textit{number of new cases of disease that occur} \in \textit{a population during a particular period}}{\textit{the summary, of all individuals, of the length of time at risk for developing disease}}$$

Incidence rate is calculated as per animal-week, animal-month or animal-year.

5.2 Observational studies

Observational studies are used to identify risk factors and to estimate the quantitative effects of the various components that contribute to the occurrence of disease. The investigations are based on analysis of natural disease occurrence in populations by comparing groups of individuals with respect to disease occurrence and exposure to risk factors.
Risk factors may be categorical, e.g., breed, sex or quantitative continuous measurements, e.g., weight, age, climate, agro-ecological zones etc.

There are three main types of observational studies:

- ☞ Cross-sectional studies
- ☞ Cohort studies
- ☞ Case-control studies[1]

5.3.1 Cross-sectional studies

The cross-sectional studies involve the random selection of a sub-sample of individuals from a larger population "N", and then the determination, for each individual, of the simultaneous presence or absence of disease. Prevalence is recorded.

5.3.2 Cohort studies

In a cohort study, a group (cohort) of animals exposed to a risk factor, and a group not exposed to the factor are randomly selected and observed for a given period to record the development of disease in each group. No animals are ill at the beginning of the study, therefore, incidence is measured.

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[1] Case-control studies will not be mentioned in further details in this text due to the difficulties of applying this type of studies in relation to parasitic diseases.

5.3.3 Design of a parasitic study

Diseases of long durations, such as parasitic diseases, are more likely to be detected during a cross-sectional prevalence survey than diseases of a short duration (i.e., viral infections). Whereas diseases of a short period in the population are often detected in cohort studies. It could thus seem logical to choose cross-sectional prevalence studies to measure the disease occurrence in a flock or in a population. But under the circumstances that the onset and spread of a parasitic disease is to be investigated, a cohort study may be more appropriate.

In general, the first step of an investigation is to estimate the helminth occurrence in a flock/population (→ 5.3.4), whereas long-term monitoring of the helminth status in a flock/population or of a control programme (→ 5.3.5) is a different way of illustrating the more dynamic epidemiology of the helminth species and may give information on the changes in prevalence and intensity of the infection in a poultry flock /population. The use of tracer animals (→ 5.3.6) may provide knowledge about the development, dissemination and survival of the infective free-living stages.

The unit of investigation is the flock or the population. Where flocks of poultry scavenge around during daytime or have close contact with each other, the entire population should ideally be the unit of investigation. Helminth infections generally involve the entire flock or population, and to be effective, diagnoses, treatments and control measures should be directed at the entire unit.

When investigating a poultry flock, one should be aware that many factors may exert an influence on the helminth infections (number of species, prevalence, infection intensities). One of the primary factors is the age of the birds combined with the immunogenicity of the helminth species concerned. Other important factors are management (housing facilities, hygiene) and local seasonal climatic variations (i.e., Agro-Ecological Zones), which may have an immense influence on the transmission rates. The nutritional status of the hosts and any control measures (management and anthelmintic routines, especially the time span since the latest anthelmintic treatment) may determine the infection rates.

5.3.4 Helminth occurrence in a flock/population

To reveal the significance of the helminth infections within a flock/population of poultry, faecal samples (→ 4.3) should be taken from a representative number of live animals, preferably belonging to selected age groups. Alternatively, as faecal examination may not be sufficiently sensitive, dead (moribund or sacrificed) animals may be examined using the post-mortem procedures (→ 4.6).

Live or dead (moribund or sacrificed) animals

It is necessary to collect faecal samples from at least three age groups of poultry, to increase the chances of 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 may cause the largest problems (Table 5.1, where only the most common parasites are mentioned).

Table 5.1 Suggested age groups of poultry to sample, together with some of their most prevalent parasites.

Age group to sample	Most prevalent parasite species
chicks (< 3 months of age)	*Eimeria* spp., *A.galli, Heterakis* spp., *Capillaria* spp.
growers (3 - 7 months of age)	*Eimeria* spp., *A.galli, Tetrameres* spp., *Raillietina* spp.
adults (> 7 months of age)	*Raillietina* spp., *Heterakis, Tetrameres* spp.

Most helminth infections in poultry are subclinical (no clinical signs are

seen), therefore samples from diseased animals should never replace the sampling from a representative number of randomly selected animals. Random sampling is necessary for a valid estimation of the flock/population problem.
It should be noted that the full truth is only found when all animals are sampled, but when the sample size is calculated and the animals are sampled randomly a valid estimation of the flock/population problem is achieved.

What sample size should be taken?

Appropriate sample size can be determined for cross-sectional studies. However, accurate calculations can be rather detailed and in complex studies a statistician is required. In simple surveys the sample size can be calculated by using the following formula:

$$n = Z^2PQ/L^2$$

where n=sample size, P=expected prevalence in the flock/population, Q=1-P and L=required precision, e.g., the largest acceptable difference between the true and the estimated prevalence. If the confidence level is 95% Z equals 2. The formula can then be expressed as:

$$n = 42PQ/L^2$$

In order to calculate the sample size **n**, needed to estimate the prevalence of a given disease in a population, the investigator must provide an educated guess of the probable prevalence P and must specify the required precision of the study. A P of 0.5 calculates the maximum sample size. Furthermore, the lower the L-value is, the higher the precision is and the bigger the sample size will be.

If it is supposed that 50 % (P= 0.5) of the population is infected with a certain parasite, a precision of 10 % is desirable (L=0.10) and the confidence level is 95%, then the sample size will be:

$$n = 4 \times 0.5 \times 0.5/0.10^2$$

$$n = 96$$

Thus 96 randomly selected animals are needed for the survey. If n is greater than the size of the flock or the population (N), then the required number to obtain the same precision is the reciprocal of (1/n + 1/N) (Table 5.2).

Table 5.2 Suggested sample sizes for given total numbers of birds in an age group when the prevalence is 50%, a precision of 10 % is desired and the confidence level is 95%.

Number of animals in the age group	Number of animals to sample
1-10	1 - 9
11-25	10 - 20
26-100	21 - 49
101-200	49 - 65
201-500	65 - 81
500-1000	81 - 96
>1000	96

More complicated studies may involve cluster or multistage sampling (→ 10.1).

5.3.5 Long-term monitoring (Cohort studies) of a flock/population

The estimation of helminth prevalence in a flock/population is important, but only provides a snapshot of the situation and does not provide a full understanding of the epidemiology. Therefore, if possible, a long-term monitoring (Cohort studies) of parasites in the flocks/populations should be carried out.

Faecal samples (→ 5.3) from living animals will provide the information (depending on what parasite is studied), but they may be replaced/supplemented with post-mortem examinations (→ 4.6) on dead (moribund or sacrificed) birds or tracer (sentinel) birds. Climatic data should be recorded.

Live animals

If the flock/population is large, it may only be possible to sample the age groups specified in Table 5.1. However, in most cases cohorts of chicks must be selected (preferably several cohorts, starting at different times during the year in order to reveal seasonal variation), and then selected animals should be sampled repeatedly at specified intervals (see below). With respect to the adults, 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.

Ideally, the sampling should be carried out every second week during periods with suspected high transmission rates (rainy seasons, summer seasons), continuing for approximately every month into the more unfavourable seasons (dry seasons, winter seasons). For the remaining dry/winter season, sampling every 4 to 6 weeks will be appropriate. The sampling 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 seasons (including the first month of the dry/winter seasons), and once every second month during the remaining part of the dry seasons/winters.

Dead (slaughtered, moribund or sacrificed) animals

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

What sample size should be taken?

To identify whether 2 groups differ in disease occurrence, samples can be selected from 2 populations. Based on an analysis it can be tested whether the groups differ statistically or not.

Appropriate sample sizes can be calculated for cohort studies. The following example is based on a two-tailed test.
Four values should be known and specified to calculate the sample size.

1) The desired level of significance (α : the probability of a Type I error, claiming that exposure to a factor is associated with disease when it is not). α is often put to 0.05, which implies a confidence level of 0.95 ($\approx$ 95%);

2) The power of the test ($1 - \beta$: the probability of claiming correctly that exposure to a factor is associated with disease, where β is the probability of a Type II error). β is often put to 0.2, which implies that the power of the test is 0.8 ($\approx$ 80%);

3) The anticipated incidence of disease in unexposed animals in the target population;

4) An hypothesized relative risk that is considered important enough, from the point of view of the health of the animal population.

The formula for a study in which exposed and unexposed cohorts are of equal size is:

$$n = \frac{(p1q1 + p2q2)K}{(p1 - p2)^2}$$

where:

n = number required in each cohort
p1 = anticipated incidence in unexposed animals
q1 = 1 - p1
p2 = minimum incidence to be detected in exposed animals

$q2 = 1 - p2$
$K = (M_{\alpha/2} + M_{\beta})^2$, where $M_{\alpha/2}$ and M_{β} are the respective multipliers associated with α and β.

As an example, if the relative risk of 3 is to be detected, the anticipated incidence in unexposed animals during the period of the study is 1 per 100, and the significance level and test power are set at 0.05 and 0.80 ($\beta = 0.20$), respectively, then:

$p1 = 0.01$ (because the relative risk is 1)
$q1 = 0.99$
$p2 = 0.03$ (because the relative risk is 3)
$q2 = 0.97$

$M_{\alpha/2} = 1.96$ (equivalent of the Z-value when $\alpha = 0.05$)

$M_{\beta} = 0.84$ (equivalent of $\div$ the Z-value when $\beta = 0.02$)

Therefore $K = 7.84$

Thus

$$n = (0.01x0.99 + 0.03x0.97) \times 7.84 / (0.01 - 0.03)^2 = 765$$

Two groups are needed in this cohort study, thus a total of 1530 animals are needed.

If the relative risk is 8, then $p1 = 0.01$, $q1 = 0.99$, $p2 = 0.08$ and $q2 = 0.92$. K is still 7.84. Inserted in the formula $n = 134$, thus 268 animals are needed for the study.

5.3.6 Tracer (sentinel) animals

Tracer birds are intended to provide direct information on the availability of infective eggs in the environment.

Tracers should be parasite-naïve before taken into use. The principle is to place these animals in the environment 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 - 8 weeks, during which the young parasites will develop into adults facilitating the identification after *post mortem* examinations. The animals are slaughtered and subjected to post-mortem examinations (→ 5.5). Ideally, 6 or more tracer animals should be introduced to the environment once a month over at period of at least one calendar year.

The tracer principle has proven to be very valuable in epidemiological research on poultry helminths. However, there are several practical problems associated with this principle in poultry flocks/populations. First, it may be very difficult or impossible to rear helminth-free and non-immune animals unless very intensively managed indoor flocks are available. Secondly, good animal housing facilities are required for the tracer animals 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 animals are introduced into a flock/population 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 scavenge normally. The latter situation may, however, be eliminated either at very low stocking rates, or by having the tracer animals fenced off in a separate area.

6 General control and prevention of parasitic diseases in poultry

In commercial indoor production systems management practices largely determine the extent of parasitosis: e.g., total enclosure principles, improvement of cleaning, disinfection procedures and production according to the "all in - all out" principle have apparently decreased the significance of parasitic infections (→ 3.1). With the ban on battery cages in a number of countries, new intensive free-range systems have developed in which the prevention of parasitic infections has proven to be difficult. The use of outdoor areas, where parasite eggs may persist in the environment for years have increased the risk of infections. The contact with wild birds also increases the chances of attracting parasitic diseases.
In backyard systems the birds are in permanent contact with soil, a range of intermediate hosts and wild birds. Parasitic diseases are thus difficult to avoid in such systems, but may be controlled by the use of management and treatment strategies.

6.1 General principles of control

The purpose of a parasitic control strategy is to keep the parasitic challenge (especially in young birds) 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 enormous numbers of eggs passed with the faeces 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 parasitic infections in the population(s), i.e. helminth species present, haemoparasites and ectoparasites prevalence rates and transmission patterns. These characteristics may differ between geographical regions, local management traditions etc. If this knowledge is missing or is only scarce, an investigation of the parasitic occurrence and epidemiology (→ 5.1) should be initiated prior to starting any control programme.

The success of parasitic transmission depends to a large extent on management systems. This is visualized in Table 6.1, where a rough overview of parasitic occurrence in different production systems is presented for a number of the most important helminths.

Table 6.1 An overview of anticipated parasitic occurrence in different production systems

Helminth species	Summary of transmission characteristics	Management system		
		backyard	com./ free-range	com.
Capillaria spp.	direct or indirect	+	+	+
Ascaridia galli	direct	+	+	+
Ascaridia dissimilis	direct	+	+	+
Heterakis spp.	direct	+	+	+
Syngamus trachea	direct or indirect	+	+	(+)
Eimeria spp.	direct	+	+	(+)
Choanotaenia	indirect	+	+	(+)
infundibulum	indirect	+	+	(+)
Raillietina spp.	indirect	+	(+)	-
Gongylonema	indirect	+	(+)	-
ingluvicola	indirect	+	(+)	-
Dispharynx nasuta	indirect	+	(+)	-
Tetrameres spp.	direct	+	(+)	-
Acuaria hamulosa	direct	+	(+)	-
Amidostomum anseris	indirect	+	(+)	-
Allodapa suctoria	indirect	+	(+)	-
Amoebotaenia cuneata	indirect	+	(+)	-
Davainea proglottina	indirect	+	(+)	-
Hymenolepis spp.	indirect	+	(+)	-
Prosthogonimus spp.	indirect	+	(+)	-
Plasmodium spp.	indirect	+	(+)	-
Leucocytozoon spp	indirect	+	(+)	-

Com. = commercial

It is seen that while backyard poultry may theoretically harbour all existing parasite species, commercial free-range management may eliminate those

parasites whose transmission depends on intermediate hosts. All helminths with an indirect life cycle are almost eradicated in commercial production systems, perhaps with the exception of *Raillietina* spp. (can be transmitted through flies). When production develops from backyard to commercial management systems, the number of parasites with direct life cycles will gradually decrease, i.e., *Ascaridia* spp., *Heterakis* spp., *Capillaria* spp. and *Eimeria* spp..

From this simple table it is evident that the most efficient way to control poultry parasites is to improve the management and hygiene of the flock. First of all, such improvements will eliminate some parasitic species, but additionally the burdens of the remaining parasites may be reduced to more acceptable levels. The eradication of parasites by routine parasitic treatment programmes has been shown to be impossible. Furthermore, it is often practically impossible to improve management sufficiently, and therefore parasitic control programmes normally include both management and antiparasitic drugs.

6.1.1 Stocking rate

The density of birds (stocking rate) in any poultry production system should not be too high. Overstocking will force the birds to come in a closer contact with material contaminated by faeces and may result in the consumption of a higher number of infective parasitic eggs.

6.1.2 Flock structure

Studies have shown that the susceptibility and occurrence of parasitic diseases vary between different age groups of poultry. Older animals may be carriers of a range of parasitic diseases without showing clinical signs, e.g, *Eimeria* spp., *Ascaridia* spp, *Tetrameres* spp. etc.. Therefore, it may be beneficial to separate different age groups vis-a-vis the "all in - all out" principle.

6.1.3 Alternate use of pens

As poultry have few parasites in common with other livestock, management may include mixed use of pens (i.e., poultry scavenging together with other livestock) or alternate use of pens (poultry alternating with other livestock in the same pen). However, when chickens scavenge in a pig parasite contaminated area, there is the risk of liver and lung lesions caused by migrating *Ascaris suum* larvae. Some parasitic species with indirect life cycles (tapeworms (→ 3.4) and flukes (→ 3.5)) may be controlled simply by avoiding contact with freshwater where the intermediate hosts live.

Management of the pens may also include alternating plant crops with poultry production as this will reduce the contamination in a field considerably, although it should be recognized that infective eggs of especially the nematodes may survive for years under favourable conditions. The development of such alternating 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 *Eimeria* spp., *Ascaridia* spp., *Heterakis* spp. and *Capillaria* spp. cannot embryonate and develop to infectivity during the winter (i.e. below 10-15 °C).

6.1.4 Hygiene of pens

When poultry are kept in concrete pens (outdoor or indoor), the litter should be removed frequently (i.e., weekly or more often) to reduce the large majority of parasitic eggs before they become infective. Furthermore, the floor should be kept as dry as possible, as external stages of all parasites 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 intensive systems seem to be rather effective in reducing parasitic 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 it may help spreading the infective stages from developmental foci (sheltered crevices etc.) all over the pens. Another possibility is to use high temperatures, i.e., flame-thrower (gas), but be aware of the fire hazard!
Disinfectants are generally not active against parasite eggs, but should be

incorporated into the general action in order to minimize viral and bacterial infections.

After mechanical removal of the litter and disinfection (steam, burning and chemical disinfectants), lime-wash should be applied and allowed to dry. The effects achieved by this procedure are: 1) The drying effect of lime decreases the survival of parasite eggs, and 2) the pH - level exceeds 8, which also decreases the survival of parasite eggs. After application of lime, the house should be left empty for 2 - 4 weeks before new animals are introduced.

6.1.5 Dose and move

As mentioned before a general management practice in poultry production is the "all in - all out" principle. 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 unfortunately it also increases the risk of development of anthelmintic resistance (→ 6.7).

6.1.6 Routine deworming

Routine deworming programmes often appeal to farmers for reasons of convenience, and as a result worm treatments are generally the only control measure carried out. However, the effect of each treatment will be rather transitory if the poultry 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 in the helminth population for development of anthelmintic resistance (→ 6.6), and therefore parasitic control programmes should reduce the number of treatments to a minimum and rather increase other control measures. Nevertheless, some kinds of routine anthelmintic treatments are relevant in the control of nematodes in most management systems.

Several programmes for routine deworming of poultry have been worked out, and most are adjusted to the age or the reproduction cycle of the

poultry. The standard procedure is treatment of hens shortly before the commencement of laying, followed by a move to a clean stable unit. The objective is to eliminate the worms from the hens thus reducing production losses and to prevent contamination of the environment.

The choice of drug should partly depend on the worm species present. Some drugs have a broader spectrum of activity than others and some nematodes may be controlled only by certain drugs; some drugs are more expensive than others etc.. Furthermore, it is important to alternate between drugs with different modes of action in order to reduce the risk of developing anthelmintic resistance (→ 6.6), and to avoid drugs against which resistance has already developed.

6.1.7 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. It seems though that protein levels above 14 - 16 % may favour the establishment of certain nematodes in the intestine. But generally scarce information is available on this subject.

6.1.8 Genetic resistance

Little is known about genetic resistance to parasitic infections in poultry, although a 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 poultry as well.

6.2 Control of nematodes

Nematodes are the most common helminths in poultry. Some may have a direct life cycle and others have an indirect life cycle. Apart from the

beneficial results of improving management (→ 6.1), control of nematodes may also be achieved by using anthelmintic drugs (→ 6.6).

6.3 Control of cestodes

Poultry kept in free-range systems are often infected with tapeworms (→ 3.4). The development of poultry production systems, from being free-range backyard systems to commercial indoor production systems, has thus reduced the prevalence of tapeworm infections by not having access to the intermediate hosts. Prevention of contact with the intermediate host is therefore one of the first steps in controlling tapeworm infections (→ 3.4). After an identification of the species present in a flock, recommendations on specific preventive measurements may be given. So if for instance *Raillietina* spp. is diagnosed in a cage system, beetle, ant or housefly control (→ 3.4) may reduce the infection. Insecticides may be used against flies and ants in the stable. Beetles are difficult to control, but the use of alternating pens may be useful (→ 6.1.3).

Control through anthelmintic treatment is also possible but it should be emphasised that without control of the intermediate hosts, treatment is of little value.

6.4 Control of trematodes

Flukes which infect poultry may include freshwater snails or dragonflies in their life cycles (*Echinostoma revolutum, Prosthogonimus* spp. (→ 4.5), and these infections may be controlled simply by avoiding contact between poultry and freshwater reservoirs (even small ponds and temporary pools). Anthelmintic drugs for prevention or control of trematodes in poultry are not available.

6.5 Anthelmintics

6.5.1 Definition

An anthelmintic is a compound which destroys helminths or causes them 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 is available, some with broad spectrum activity and others with activity against specific helminth infections. Many modern anthelmintics are effective against both adults and larval stages, 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 (see Section 6.6), 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.

6.5.2 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 bird often harbours 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 scheme, 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 animals slaughtered for human consumption, and thus reduce the slaughter withdrawal period. Furthermore, the long-lasting presence of sub-therapeutic concentrations of a drug may constitute a severe risk factor for the development of anthelmintic resistance (→ 6.6).

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

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

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

6.5.3 Dosing methods

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

Anthelmintics are so far not available in a formulation for external application ("pour on" preparations) or as injections to birds.

6.5.4 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 albendazole, 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 milbymicins.* The compounds act on the nervous system of the worms, causing flaccid paralysis and removal by gut motility. Class III consists of two distinct types of drugs, i.e. the piperazines and the avermectins (ivermectin, doramectin, moxidectin), the latter having effects against some ectoparasites, e.g. mange mites.

***Class IV anthelmintics**: Salicylanilids and substituted nitrophenols.* These drugs are typically used against bloodsucking parasites.

***Class V anthelmintics**: Acetylcholine esterase antagonists.* 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.

6.6.5 What drug to use ?

It is important first to identify the nature of the parasitic problem in order to select the appropriate drug. Recent studies have shown that even in commercial systems the range of helminth infections in poultry includes several species (→ 3.2). Broad-spectrum drugs are in such cases appropriate

(Table 6.2).

Table 6.2 List of most common drugs for anthelmintic treatment of poultry

Active ingredient	Administration	Indication
Fenbendazole	In feed	*Ascaridia galli*, *Capillaria* spp., *Syngamus trachea*
Flubendazole	In feed	Intestinal nematodes, cestodes in chickens
Hygromycin	In feed	Intestinal nematodes (mainly *Ascaridia galli*)
Levamisole	In feed	*Ascaridia galli*, *Heterakis gallinarum*, *Capillaria* spp., *Syngamus trachea*
Mebendazole	In feed	Intestinal nematodes, *Syngamus trachea*, cestodes
Piperazine	In feed or drinking water	*Ascaridia galli*, (*Heterakis gallinarum*), *Tetrameres* spp.
Thiabendazole	In feed	*Syngamus trachea* (pheasants)

6.6 Anthelmintic resistance

6.6.1 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 lose 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 is now so widespread that several farmers are left without any means of drug control of helminths of grazing animals. Anthelmintic resistance has not yet been observed in poultry, but it is important to establish proper control programmes in order to avoid such development.

6.6.2 Detection of anthelmintic resistance

Many cases of anthelmintic resistance 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:

Procedure

☞Collect faecal samples from 20-30 identified animals which have not been treated for at least 2-3 months.

☞In the laboratory, the faecal samples are subjected to a McMaster egg count procedure (→ 5.3.8 or 5.3.9).

☞Distribute the animals by faecal egg counts, if possible, 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 animals of the other group are left untreated).

☞10-14 days post treatment, new faecal samples are collected from the same individuals.

☞The faecal samples are subjected to a McMaster egg count procedure.

☞Calculate the post-treatment arithmetic 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.

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 confirm the presence of anthelmintic resistance, groups of poultry 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).

6.6.3 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:

Frequency of anthelmintic treatment. A number of surveys on anthelmintic resistance unanimously conclude that the more frequent 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, poultry are normally treated 2-4 times a year, or even less.

Use of the same class of drugs for extended periods. As pointed out in Section 6.5.4, 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 (*cross 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 obviously 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 poultry is treated while they remain in a heavily contaminated environment (e.g. permanent pens), resistant worms surviving the treatment will produce resistant progeny. 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 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 the few worms which are able to survive a treatment constitute the basis for the development of anthelmintic resistance. Similarly, under dosing has been shown to be a potential risk factor. Common reasons for under dosing 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 feed 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.

6.6.4 Prevention of anthelmintic resistance

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

Even though anthelmintic resistance in poultry helminths is apparently not a widespread problem at 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 poultry.

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 management (time delay between change of flocks - "all in - all out-principle", cleaning, disinfection etc.), pen hygiene and rotation 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. A special problem arises when the animals are treated flockwise with a drug mixed up in the fodder or the drinking water.

Here it may be suspected that some animals will be under dosed if the drug is administered for only one day, while the risk of under dosing 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 3rd 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. If new animals, e.g., breeding animals, are to be introduced to the flock, they should be kept separate from the rest of the flock for the first 2-3 weeks. 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.

6.7 Control of coccidia

Coccidiosis is the most common parasitic disease in poultry. The life cycle is direct and it is characterized by a very short prepatent time. Control of coccidiosis may be achieved by

1) Improvement of management (→ 6.1)

2) Prevention and control of coccidiosis by vaccination programmes

3) Control of coccidiosis by chemotherapy

Ad 1) Management strategies as described in section 6.2 are important for the prevention of coccidiosis in poultry flocks, but are not considered fully valid in commercial systems due to frequent failures in management programmes and the extreme resistance of oocysts towards disinfectants.

Ad 2) The research on and recent development of vaccines have lead to a product which is now commercially available. The vaccines are based on live but attenuated strains of various coccidia. The use of vaccines is convenient for commercial production, but may be of limited value for backyard production.

Ad 3) Different programmes have been developed for chemotherapy of poultry against coccidiosis. These are either based on treatment based on clinical outbreaks of coccidiosis or they are based on preventive medication.

Clinical outbreak of coccidiosis: Infections with coccidiosis produce a strong immunity against new infections. Naturally immunity is usually achieved by controlled exposure using a commercial product or by natural exposure assuming that the most common species are present in the environment. A broad spectrum drug is then used to treat the clinical disease. Management, climatic and seasonal differences may increase outbreaks of disease. This method is recommended in backyard and free-range systems.

Programmes for use of anticoccidial drugs in broilers: In broilers the aim is to produce growth at lowest cost. Several approaches are used:

Continuos feeding: a single drug is used from time of hatching to a week before slaughter.

Shuttle programmes: One drug is used in the starter feed for the first 2 - 3 weeks and another in the grower feed. This approach is thought to reduce development of resistant coccidia strains.

Rotation programmes: A continuous change of drugs may

improve productivity because of the buildup of resistant strains in the environment.

Programmes for use of anticoccidial drugs in breeders and layers: Animals that will be kept on floor or free-range should have an immunity to coccidiosis. Naturally immunity is usually achieved by controlled exposure using a commercial product or by natural exposure assuming that the most common species are present in the environment. A broad spectrum drug is then used to treat the clinical disease. Management, climatic and seasonal differences may cause outbreaks of disease. This method is thus also recommended for backyard and free-range systems.

The development of resistant coccidia strains after use of anticoccidial drug is the most serious limitation to the effectiveness of such products. Avoidance of drug resistance may be achieved through the use of less intensive production systems, use of shuttle treatment programmes and frequent changes of drugs.

Table 6.2 Some common drugs for treatment of coccidiosis in polultry for application in drinking water or feed.

Name	Feed or water
Amprolium	water
Chlortetracycline	feed
Furazolidone*	feed
Nitrofurazone*	water
Oxytetracycline	feed
Pyrimethamine + sulfaquinoxaline	water
Sodium sulfachloropyrazine monohydrate	water
Sulfadimethoxine	water
Sulfamethazine	water
Sulfaquinoxaline	feed

* only for chickens

Table 6.3 Some common prophylactic anticoccidial drugs for application in feed.

Name of drug
Amprolium
Amprolium + Ethopabate
Chlortetracykline
Metichlorpindol, Methylbenzoquat
Aklomide Sulfanitran
Amprolium + Sulfaquinoxaline + Ethopabate
Pancoccin + Pyrimethamin
Sulfadimethoxine + Ormetoprim
Bithionol + Methiotriaziamin
Nitromide + Sulfanitran + Roxarsone

6. 8 Control of ectoparasites

To minimize the risk of transmitting ectoparasites from wild to domesticated bird, the former should never be allowed to have contact with poultry flocks. Fencing is thus of crucial importance in order to keep other birds away.

6.8.1 Ticks

The nymphal and adult stages of fowl ticks feed on their hosts for a limited period. The control of ticks therefore requires treatment of the environment

(indoor and outdoor) in which the poultry are. After mechanical cleaning of the stable the entire building (walls, ceilings, cracks and crevices) is sprayed with a high pressure sprayer using either carbaryl, coumaphos, malathion or stirofos (→ Table 6.3). The outdoor facilities (feed troughs, woodpiles, tree trunks etc.) may be treated using approved insecticides, but this is not recommended due to environmental concerns.

6.8.2 Mites

Infections with the chicken mite, tropical fowl mite and northern fowl mite may be controlled by using acaricides such as carbaryl, coumaphos, malathion, stirofos or a pyrethroid. Each bird should be sprayed as well as the stable. Specially all hiding places for the mites should be treated.

Prophylactic measures such as cleaning and disinfection of the house are advisable. Spraying of all surfaces with insecticides followed by lime wash is recommended. Furthermore, it should be avoided to insert new animals in a house straight after cleaning and disinfection.

Animals should not be introduced into an existing flock without examination first.

Control of scaly leg mites should begin by isolation or culling of affected birds and thereafter the house should be cleaned as recommended for other mites. Individual animals are treated by dipping the affected legs in kerosene, linseed or mineral oil or coated with Vaseline. Treatments should be given twice with a 10 day interval. Infections may also be treated using acaricides.

6.8.3 Fleas

Infections with fleas are best controlled by removing litter followed by treatment of the house with permethrin (Table 6.3). Prophylactic measures as mentioned under section 6.8.2 are advisable.

Table 6.3 Some insecticides for ectoparasitic control on poultry.

Fowl ticks	
Malathion	0.3% spray
Tetrachlorvinphos	1.0% spray (not direct)
Carbaryl	0.2% spray
Mites, lice and fleas	
Malathion (4 - 5%)	dust
Permethrin (0.25%)	dust
Rabon	dust
Carbaryl (5%)	dust
Malathion	0.5% spray
Permethrin	0.05% spray
Tetrachlorvinphos	0.5% spray
Stirofos (23%) with dichlorfos (5.7%)	0.5% spray

6. 9 Control of haemoparasites

Haemoparasites are transmitted by mosquitoes, flies, biting midges etc. Control of the arthropods is thus of crucial importance. Screening of the poultry houses may avoid transmission of the haemoparasites. Also insecticides may be used to minimize the vectors (Table 6.4).

Table 6.4 Control of flies in poultry houses.

Name	Formulation
Bomyl (1%)	Bait
Dibrom	Bait
Dibrom (0.25%)	spray
Dichlorvos (0.5%)	spray
Dichlorvos (1%)	bait
Methomyl (1%)	bait
Permetrin (0.25%)	spray
Pyrethrin (0.5%) & piperonylbutoxide (3.75%)	spray
Stirofos (1%)	spray
Tetrachlorvinphos (1%)	spray
Trichlorfon (1%)	bait

A number of drugs are available for treatment against Avian malaria, Leucocytozoonosis and *Aegyptinella* spp., although treatment against Leucocytozoonosis is not effective.

Table 6.5 Some common drug for use against haemoparasites.

Drug and mode of application	effective against
Chloroquine (1 mg/kg intra muscular (im) for 5 days)	Avian malaria
Chloroquine (2000 mg/l in water for 1 day)	Avian malaria
Quinacrine (1.6 mg/kg im. for 5 days)	Avian malaria
Primaquine (100 mg/kg per os (po) for 1 day)	Avian malaria
Sulfonamids + trimethroprim (im)	Avian malaria
Pyrimethamine (1 ppm) + sulfadimethoxine (10 ppm) po.	Prevention of Leucocytozonosis
Pyrimethamine (25 ppm po.)	Cure of Leucocytozonosis
Tetracycline (15 - 30 mg/kg po.)	*Aegyptinella* spp.

7 Fluids and reagents

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

7.1 Flotation fluids

The preparation of three different flotation fluids is described below. Any one of them can be used, depending on the availability of reagents. However, the salt/sugar solution gives the best results due to its high specific gravity and is thus recommended in order to standardize the techniques for examination of faeces.

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

Saturated salt solution:

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

Saturated sugar solution:

White sugar	Q.S.
Water	1000 ml
Specific gravity:	1.120 - 1.200

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

Salt/sugar solution:

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

Dissolve the salt in water (saturated solution) and then add the sugar to the solution. Stir until the sugar is dissolved.

7.2 Other reagents for use in diagnostic tests

Giemsa stain: A 10 % Giemsa solution is prepared.

Distilled water (or tap water) buffered to pH 7.2	900 ml
Giemsa stock	100 ml

The solution is poured in to a staining jar and may be used 4 -5 times, staining 50 - 100 smears.
If buffered water is not available tap water can be used temporarily, but the staining might not give good results.

Berlese's fluid: Conservation and identification of ectoparasites.

Gum Acacia	30 g.
Chloral hydrate	200 g.
Glycerine	20 g.
Distilled water	50 g.

Preparation:

The Gum Acacia is soaked in water for 12 hours, add glycerine and stir while heating (do not boil) until the gum has dissolved. The chlorate hydrate is added and dissolved. Bubbles will appear and the fluid must be set aside and kept warm until they disappear. The medium is then stored for use.

8 Conclusion

Poultry production is increasing rapidly partly due to the low establishment cost and efficiency of poultry to convert nutrients into animal protein. At present 30 % of the world animal protein for human consumption comes from poultry products. Two systems, with intermediates, exist at present: the backyard system and the commercial system. Approximately 80% of the world poultry population is found in backyard systems. These systems are characterized by a low input in terms of management and investment and a low output in terms of products. Among disease constraints parasitic diseases constitute a limiting factor to the effectiveness and continuing development of poultry production in developing countries. Furthermore, commercial poultry production is developing into free-range systems, where parasitic diseases are highly prevalent. The present knowledge on the epidemiology and impact of parasitic diseases on productivity and health is scarce. There is a need to conduct a range of studies in this area in order to fill the gaps in the knowledge base. To date *Eimeria* spp., *Ascaridia galli* and *Heterakis gallinarum* are the best studied poultry parasites. But as indicated in Chapters 2 and 3 a number of parasites are found in poultry. These include endo-, ecto- and haemoparasites. A number of these are known to be highly pathogenic, causing not only heavy production losses, but also death.

It is thus the hope of the authors that this manual will facilitate the isolation and identification of parasitic problems in backyard and industrial poultry production systems. The precise knowledge on which parasites are involved might in the long term lead to a better understanding of the mechanisms involved in poultry parasitism and may lead to higher productivity.

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9.1 Books

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9.2 Articles

(1,2)), Central African Republic ((3)), Egypt (Gaafar, 1952; Ahmed et al., 1995; Ibrahim et al., 1995), Ethiopia ((4); Woldemeskel &Gebreab, 1996), Kenya ((5)), Morocco (Dakkak & Houadfi, 1992; Kichou et al., 1996), Niger ((6)), Nigeria ((7); (8); (9); Akinboade &Ogunji, 1984; (10); (11); Sa'idu *et al.*, 1994), South Africa ((12)), Sudan ((13)), Tanzania ((14); (15); (16); (17); Permin *et al.*, 1997), Uganda ((18); (19); (20) and Zimbabwe ((21); Chabra &Donora, 1994).

Bangladesh (Samad & Chakraborty, 1993; Karim et al., 1994; Islam et al., 1995), India (Kaushik & Deorani, 1968; Matta & Ahluwalia, 1981; Hemalatha *et al.*, 1987; (22); (23); (24); Dhanakkodi & Logaswamy, 1993; Mir et al., 1993; Kalra et al., 1994; Basith & Karunamoorthy, 1995; Basith et al., 1995; Choudhury et al. 1995; Saxena et al., 1995; Panda et al., 1997), Indonesia ((25); Salfina *et al.*, 1992), Iran (Ghorbani et al., 1990), Nepal ((26)), Phillipines, Malaysia and Taiwan ((27); Abella et al., 1994), Pakistan (Hussain, 1967; Qureshi & Sheikh, 1978; Anjum, 1990; Buriro et al., 1992; Khan *et al.*, 1994; Shahjehan & Iqbal, 1995) and Thailand (Kunjara & Sangvar, 1993).

USA ((28); (29)); (30); Worley *et al.*, 1991; (31); Willoughby *et al.*, 1995)

Denmark ((32,33); Permin *et al.*, 1998), Germany ((34); Zeller, 1990), Hungary (Rozsa, 1990), Sweden (Hoglund *et al.*, 1995), Switzerland ((35)), United Kingdom ((36)).

Poultry products are among the most important protein sources for humans throughout the world, and the poultry industry has experienced continuing growth during the past 20 to 30 years. The extensive traditional rural scavenging systems have not, however, seen the same growth as commercial production systems and are faced with serious management, nutritional and disease constraints. These include a number of parasites that are widely distributed in developing countries and contribute significantly to the low productivity of backyard flocks. This handbook provides an overview of the parasites of major pathogenic and economic importance and presents procedures and techniques for their diagnosis, epidemiological study, 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 the distribution of the handbook will facilitate the standardization and improvement of diagnostic capabilities as well as stimulate the collection and use of epidemiological data, the foundation for effective disease control programmes.

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