COCCIDIA (PROTOZOA:SPOROZOASIDA) OF CATS AND DOGS

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ABSTRACT

Studies were undertaken to establish for the first time the identity and prevalence of coccidian parasites of New Zealand cats and dogs and to determine the effect of various factors on the activation of *Isospora felis* and *Isospora rivolta* sporozoites. The associations between these protozoa and such organisms as *Toxoplasma, Sarcocystis, Besnoitia* and other related genera are examined and the literature concerning their life cycles, nomenclature and general biology reviewed.

Examination of faecal samples from 508 cats and 481 dogs from North Island localities revealed that 155 (30.5%) and 307 (63.8%) respectively, contained coccidia. The majority of infected samples were found to contain a single coccidian but in total four valid coccidians from cats and four from dogs, as well as several pseudoparasitic coccidia, were recorded and described. The identities and prevalences of these valid coccidians were:

(a)	Cats :	*Isospora felis*	(17.5%)	*Isospora rivolta*	(2.2.%)
		Toxoplasma gondii	(0.98%)	*Sarcocystis* sp.	(16.9%)
(b)	Dogs :	*Isospora canis*	(4.0%)	*Isospora ohioensis*	(9.2%)
		Hammondia heydorni	(2.7%)	*Sarcocystis* sp.	(58.8%)

The sex of the host had no significant effect on the prevalence of infection. The effect of other factors, such as season, host age and host origin, however, was found to vary from coccidian to coccidian and appeared to be explicable in terms of differences in routes of transmission, host immunity and intermediate host specificity.

Levels of sporocyst shedding in cats and dogs naturally infected with *Sarcocystis* sp. tended to be low with the majority excreting 200 sporocysts per gram of faeces or less. The specific identities of such sporocysts are unknown but at least some from cats were demonstrated, by mouse infection, to be those of *S. muris*. Attempts to induce similar

Sarcocystis infection in mice, using isolates of *I. felis* recovered from the faeces of naturally infected cats were unsuccessful.

After completion of the main survey, a further coccidian showing similarities to *Besnoitia wallacei* was recovered from the faeces of one of five feral cats. The feeding of sporulated oocysts of this coccidian to mice, rats, rabbits and guinea pigs resulted in the formation of typical *Besnoitia* cysts in all hosts except the last.

Studies on the activation of *I. felis* and *I. rivolta* sporozoites revealed that, although some differences were apparent between the two, both were capable of activation over a wide range of conditions.

Activation of both species was observed to take place in trypsin and bile between temperatures of 21° and 43°C (the range tested) and to occur rapidly at 39°C. While the presence of bile appeared to be essential for this process that of trypsin did not. In general, neither the concentration of bile (above 5%) nor the type of bile was found to have any marked effect on the level of activation attained while hydrogen ion concentration (pH range 5.0 to 10.0) also appeared to have little influence.

Unlike many species of coccidia which have been studied, pretreatment of oocysts before exposure to trypsin and bile was found not to be an essential prerequisite for the activation of *I. felis* and *I. rivolta*. However, higher levels of activation were attained when pretreatment was used than when it was not although for *I. rivolta* at least, the level of activation appeared to be less dependent on pretreatment for oocysts stored in sulphuric acid than for those stored in potassium dichromate.

The process of activation and excystation of both species was observed to be essentially similar to that described for other species of coccidia which also lack either sporocyst stieda bodies or oocyst micropyles. Sporozoites escaped following the collapse of the sporocyst wall and were observed to complete excystation through identations and fractures at one or both ends of the oocyst.

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1.1 SYSTEMATICS OF THE COCCIDIA

Protozoans of the subphylum Apicomplexa, which are all parasitic, are characterized by the presence of an apical complex (Fig 1.1) at some stage or other in their life cycles. Of the two classes of this subphylum, the Sporozoasida and the Piroplasmasida, the Sporozoasida represent the spore or oocyst forming protozoa which are divided into two subclasses, the Gregarinasina and the Coccidiasina. Members of the former subclass are mostly extracellular parasites of invertebrates or lower chordates while members of the latter are typically intracellular parasites mainly of vertebrates. The Coccidiasina are divided into two orders; a primitive group containing only a few known species, the Protococcidiorida, and the main order, the Eucoccidiorida in which the coccidia proper are represented by the suborder Eimeriorina. The most well known coccidia are members of the family Eimeriidae. Members of this family usually parasitise the intestinal tract of a single host in which they undergo asexual and sexual reproduction leading to the production of an oocyst. The oocyst, as the only exogenous part of the life cycle, is the most widely recognised coccidian stage and in many cases, serves as the sole criterion for diagnosis and species identification.

1.2 COCCIDIA OF CATS AND DOGS

The coccidian parasites of cats and dogs belong to the family Eimeriidae (Levine, 1977a) and can be divided into two main morphological groups; - those producing eimerian-type oocysts and those producing isosporan-type oocysts. Sporulated oocysts of the former group are characterized by having four sporocysts each containing two sporozoites (Fig 1.2) and the latter by having two sporocysts each containing four sporozoites.

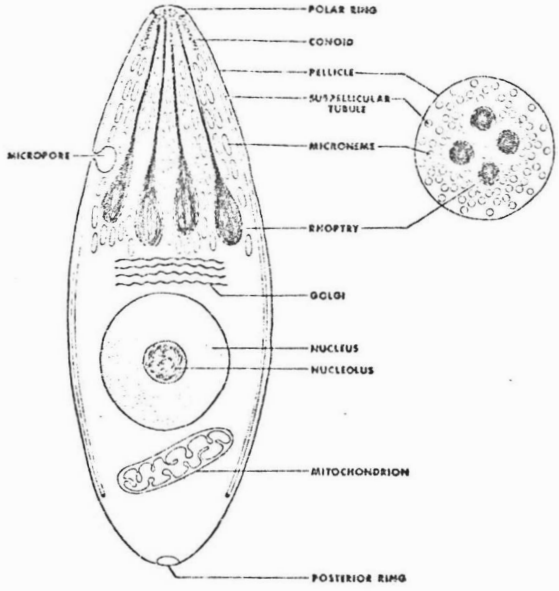

Fig.1.1 An apicomplexan sporozoite or merozoite, illustrating the apical complex (From Levine, 1973).

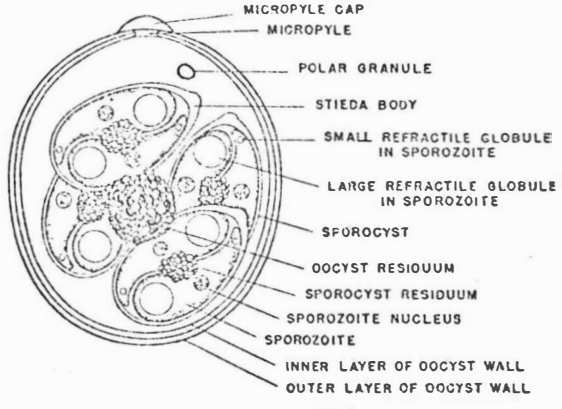

Fig. 1.2 A sporulated coccidian oocyst (*Eimeria* sp.) (from Levine, 1973).

1.2.1 Eimerian oocysts of cats and dogs

The morphological characteristics of eimerian oocysts recorded from cats and dogs are summarized in Table 1.1. In all cases they are known only from their oocysts and have been recorded infrequently since their original discoveries (Table 1.2). Reported attempts at infecting cats and dogs with these species are also few. Honess (1936) claimed to have infected a dog with *E. canis* but Streitel and Dubey (1976) and Christie *et al* ., (1976) failed to infect coccidia-free puppies and kittens respectively with eimerian oocysts obtained from naturally infected hosts.

At present eimerian oocysts found in cat and dog faeces are considered to be of 'accidental' or pseudoparasitic origin (Dubey, 1976b).

1.2.2 Isosporan oocysts of cats and dogs

(a) Historical

The first report of coccidia in the cat was given by Fink (1854) and that of the dog by Virchow (1860) both of whom observed oocysts in sections of intestine though neither recognised them as parasitic organisms. Wenyon (1923) reviewed the available literature and concluded that cats and dogs both haboured the same three species of *Isospora; I. felis, I. rivolta* and *I. bigemina.* The same author (Wenyon, 1926) later suggested that *I. bigemina* was represented by two races, one large and one small. The smaller race developed in the epithelial cells of the small intestine and was shed unsporulated; the larger race developed in the lamina propria where sporulation occurred.

TABLE 1.1 MORPHOLOGICAL CHARACTERISTICS OF EIMERIAN OOCYSTS RECORDED FROM CATS AND DOGS

				Oocyst characters					Sporocyst characters	
Author	Locality	Name	Host	Size (µm)	Wall	Micropyle	Polar granule	Residuum	Size (µm)	Residuum	Stieda body
Wenyon, 1923	England	E. canis	Dog	18-45 x 11-28	pink, ellipsoidal--ovoid, rough thick	+		+		+	+
Nieschulz, 1924	Holland	E. canis	Dog	17-32 x 12-20							
		E. felina	Cat	21-26 x 13-17 (24 x 13.5)	Colourless, ellipsoidal, smooth, 1µm thick	-	-	+		+	+
Yakimoff, 1933	U.S.S.R	E. cati	Cat	18-23 x 14-20 (20.8 x 17.1)	Colourless, ovoid to spherical	-	+	-	10.8 x 6.3	+	
Skidmore & McGrath, 1933	Nebraska	E. canis	Dog	24.4 - 33.8 x 16.9 - 18.8 (28.8 x 16.2)	Yellowish brown ellipsoidal, 0.75 - 1.0 µm thick	(+)		-	6.6 - 12.2 x 6.6 - 7.5 (9.4 - 7.3)		
Honess, 1936	Wyoming	E. canis	Dog	15.8 - 26.2 x 10.4 - 16.6 (17.8 x 12)	Colourless ellipsoidal			+	10.5 x 5.8	+	

(+) Questionable

TABLE 1.2 REPORTED PREVALENCES OF EIMERIAN OOCYSTS IN CAT AND DOG FAECES

Author	Locality	Name	Host	Number examined	Infected
Choquette & Gelinas 1950	Montreal	*E. canis*	Dog	95	16.5
Streitel & Dubey 1976	Ohio	*Eimeria* sp.	Dog	500	0.5
Christie *et al.*, 1976	Ohio	*Eimeria* sp.	Cat	1000	0.4

Apart from some questionable cross-transmission experiments (Andrews 1927, Lee 1934), the view that cats and dogs shared the same coccidial species was accepted by later authors with little experimental verification until Nemeseri (1959) found that he was unable to transmit *I. felis* from cats to dogs and vice-versa. He concluded that the form in the dog was a different species which he named *I. canis*. Support for the host-specificity of *I. felis* and *I. canis* was later provided by other workers (Shah 1970a; Dubey *et al.*, 1970a; Rocha and Lopes 1971; Dubey 1975a; Gutterbock and Levine, 1977).

The host specificity of *I. rivolta* was examined by Mahrt (1967) who found that he was unable to infect cats with *I. rivolta* from dogs. Other workers (Dubey *et al.*,1970a; Pellerdy 1974; Gutterbock and Levine, 1977) also failed to infect dogs with *I. rivolta* from cats although Rocha and Lopes (1971) claimed they could do so. Pellerdy (1974) concluded that the canine and feline forms of *I. rivolta* were different species and proposed the name *I. novocati* for the cat form on the assumption that *I. rivolta* was discovered first in the dog. Dubey (1975c) pointed out that *I. rivolta* was, in fact, first discovered in the cat and proposed therefore that *I. rivolta* be retained as the feline species and suggested the name *I. ohioensis* for the canine form.

Major progress in the elucidation of the identities of coccidial species of cats and dogs was made, however, when several investigators (Overdulve, 1970; Hutchinson *et al.*, 1970; Sheffield and Melton, 1970; Frenkel *et al.*, 1970; Weiland and Kuhn, 1970) independently discovered that the ubiquitous protozoan *Toxoplasma gondii* had a coccidian life cycle. These authors found that on being fed mice infected with *T. gondii*, cats produced in their faeces small isosporan oocysts identical to those previously described as the small race of *I. bigemina* . Similar attempts to induce *T. gondii* oocyst production in dogs were not successful (Miller *et al.*, 1972; Dubey *et al.*, 1970a) although Heydorn (1973) found that small unsporulated oocysts of *I. bigemina* were shed by

dogs fed raw beef. Although these oocysts were morphologically indistinguishable from those of *T. gondii* from cats, serological, host-specificity and life cycle differences pointed to the fact that they were distinct species (Heydorn, 1973). Later another oocyst resembling that of *T. gondii* was isolated from the faeces of a cat (Frenkel and Dubey, 1975). Although this coccidian produced weak cross-reacting antibodies to *T. gondii* in mice (but not in cats), life cycle and host-specificity differences were again able to distinguish between them. Frenkel and Dubey (1975) considered the differences sufficient to warrant the creation of a new genus, *Hammondia*. They named their species *H. hammondi* and suggested that Heydorn's (1973) small form of *I. bigemina* in the dog may also be a member of this genus. This suggestion has recently been accepted by Dubey (1977b) who has proposed the name *H. heydorni* for the dog form.

With the discovery of the coccidial nature of *T. gondii* , species of two other cyst-forming sporozoan genera, *Sarcocystis* and *Besnoitia*, also of previously uncertain taxonomic status, were examined to see if they too were transmitted by cats or dogs. Rommel *et al.*, (1972) fed sarcocysts isolated from the muscles of sheep to cats and found that fully sporulated isosporan sporocysts corresponding to those previously described as the large race of *I. bigemina* were passed in their faeces 12 days later. Subsequently similar sporocysts were found in the faeces of either cats or dogs or both when fed sarcocysts from cattle (Heydorn and Rommel, 1972a, b; Mahrt, 1973; Fayer, 1974; Dubey and Streitel, 1976c), pigs (Rommel *et al.*, 1974; Golubkovan and Kisliakova, 1974), horses (Rommel and Geisel, 1975; Dubey *et al.*, 1977c), gazelles (Janitschke *et al.*, 1976), mule deer (Hudkins-vivion *et al.*, 1976), rabbits (Fayer and Kradel, 1977; Crum and Prestwood, 1977) and mice (Ruiz and Frenkel, 1976). In addition to cats and dogs, man (Rommel *et al.*, 1974), baboons (Heydorn *et al.*, 1976), wolves (Rommel *et al.*, 1974), coyotes (Fayer and Johnston, 1975a), foxes (Golemansky, 1975a; Ashford, 1977), raccoons (Fayer, *et al.*, 1976a), owls (Munday, 1977) and snakes (Rzepczyk, 1974),

have also been shown to act as final hosts for species of *Sarcocystis*.

The fourth mammalian tissue parasite found to produce isosporan oocysts was *Besnoitia*. Peteshev *et al*., (1974) fed *B. besnoiti* cysts from cattle to a number of animals including dogs and cats. It was found that unsporulated oocysts were produced in the faeces of cats alone. Feeding these oocysts, after sporulation, to calves resulted in *Besnoitia* cyst formation once more.

Further confirmation of the coccidian nature of this protozoan genus was provided when cysts of *B. wallacei* of rodents and *B. darlingi*; of opossums were also shown to be transmitted as isosporan oocysts in the faeces of cats (Wallace and Frenkel, 1975; Smith and Frenkel, 1977).

(b) Current Species and Problems of Nomenclature

The association of many of the canine and feline coccidia with mammalian tissue cysts has raised a number of nomenclatural problems.

According to the established practice of naming coccidian species strictly on the basis of oocyst morphology, all these protozoans would be placed in the genus *Isospora*. Some of these organisms, however, were named long before their oocyst, or sporocyst stages were discovered. These names (e.g. *Toxoplasma gondii*) have been in use for many years now, as have the names of the diseases they cause (e.g. toxoplasmosis). Consequently any alteration of their generic names to *Isospora* would lead to considerable confusion. Similarly, adoption of different names for different stages of their life cycles (e.g. oocysts in definitive hosts and cysts in intermediate hosts) would also be confusing. Accordingly, the general practice has been to adopt the names previously assigned to the cyst stages of these protozoans for their oocyst stages also. Unfortunately, however, it is now apparent that in some genera, such as *Sarcocystis*, many of these so called 'species' of cysts are in fact composed of more

than one actual species (Munday and Rickard, 1974; Melhorn *et al.*, 1975a; Heydorn *et al.*, 1975c; Collins *et al.*, 1976). In addition new genera of previously unknown cyst-forming coccidia have been erected on the basis of life cycle and host specificity differences. It is not surprising, therefore, that the nomenclature of the canine and feline coccidia is in considerable disarray. Recent reviews by Dubey (1976b, 1977b), Tadros and Laarman (1976), Levine (1977a, b) and Frenkel (1977) which attempted to clarify the situation have added to the chaos.

Dubey (1976b) recognised five genera of canine and feline coccidia; *Isospora, Toxoplasma, Hammondia, Besnoitia* and *Sarcocystis*. Levine (1977a, b) however, synonymised the genus *Hammondia* with *Toxoplasma*. He considered that differences between *Toxoplasma gondii* and *Hammondia hammondi* from the cat were insufficient to warrant the creation of a new genus and accordingly renamed the latter species *Toxoplasma hammondi*. Unfortunately, Levine (1977a, b) appears not to have considered the position of the corresponding species *Hammondia heydorni* in the dog. This species has a very similar life cycle to that of *H. hammondi* in the cat. Logically, therefore, if *H. hammondi* is now to become *T. hammondi* then the dog species (*H. heydorni*) must be placed in the genus *Toxoplasma* also. This would lead to widespread confusion and misunderstanding concerning the aetiology of toxoplasmosis and it seem preferable, therefore, to leave the genus *Hammondia* as it is.

Tadros and Laarman (1976) proposed a more major reclassification of isosporan coccidia based on the occurrence of the sporulation of the oocysts within or outside the host. They placed *Toxoplasma, Besnoitia* and *Hammondia* within the genus *Isospora* because all four sporulate outside the host. *Sarcocystis* was placed in a new genus *Endorimospora* because sporulation occurs within the host. Dubey (1977b) pointed out that well-established genera such as *Toxoplasma* and *Besnoitia* should not be changed and that under the international rules of zoological nomenclature the name *Endorimospora* cannot be accepted

in place of *Sarcocystis*. He suggested, therefore, that the genera *Toxoplasma*, *Besnoitia*, *Hammondia* and *Sarcocystis* should be retained but proposed, as a compromise, a new nomenclature for the genus *Isospora*. He considered that this genus includes two biologically distinct types of organisms. One group contains isosporans that have a homoxenous (one host) life cycle (e.g. *I. canaria*, Box, 1977) in which infection may only be acquired by the ingestion of oocysts and in which development is confined to the intestinal tract. The other (e.g. *I. felis*, *I. rivolta*, *I. canis*, *I. ohioensis*) contains organisms which in addition to the transmission of infection by the ingestion of oocysts may also transmit infection by means of infected intermediate hosts (see section 1.4.1) and in which development is not confined to the intestine. He proposed a new genus, *Levineia*, for this group of isosporans retaining the genus *Isospora* for the former group. A similar system was proposed by Frenkel (1977) who suggested the name *Cystoisospora* for those isosporans with a heteroxenous (two host) life cycle. Logical as these proposals may appear, such a change is probably premature since the life cycles of species of *Isospora* other than those of the cat and dog have not been fully investigated.

Thus while the present nomenclature of the canine and feline coccidia is far from satisfactory, it is also clear that it is unlikely to be improved by unilateral attempts to reclassification. In the following tables (Tables 1.3 and 1.4) the generally accepted coccidian species of cats and dogs are given although, no doubt, they will be subjected to future modification and change.[1] Synonyms of these species are presented in Appendix 1.

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1. Since completion of this thesis evidence that two further species of *Sarcocystis* may be transmitted by cats and dogs has been provided. The first, *S. cymruensis*, of the rat (*R. norvegicus*) is transmitted by the cat and has sporocysts of 10.5 x 7.9 µm (Ashford, 1978). The second (species unnamed), intermediate host domestic fowl, is apparently transmitted by the dog and has sporocysts of 11.5 x 8.3 µm (Munday *et al.*, 1977). Acceptance of the dog as the definitive host of the latter species, however, requires further investigation.

1.3 DIAGNOSIS AND IDENTIFICATION

Oocysts of all of the canine and feline coccidia are structurally very similar. All are of the isosporan type, lack micropyles, polar granules and oocyst residua and all contain sporocysts which lack stieda bodies but possess residua. Many of these oocysts and sporocysts cannot be differentiated from each other on the basis of size (Tables 1.3 and 1.4). *Hammondia* oocysts cannot be differentiated from those of *Toxoplasma* or *Besnoitia darlingi;* while sporocysts of most species of *Sarcocystis* cannot be distinguished from one another or from the closely related coccidian *Frenkelia*. Thus the traditional practice of identifying species strictly on the basis of oocyst morphology is no longer tenable as far as the canine and feline coccidia are concerned.

Similar problems are encountered when trying to identify these coccidia solely on the morphology of the cysts they produce in their intermediate hosts. Apart from *Isospora* which produces cysts that are difficult to detect (Frenkel and Dubey, 1972a; Melhorn and Markus, 1976) and *Besnoitia* in which the cyst wall surrounds the infected host cell (Scholtyseck *et al.*, 1974), all produce intracellular cysts containing a large number of bradyzoites. Bradyzoites of *Sarcocystis* may, in some cases, be distinguished from those of other genera by their greater size. However, ultrastructural studies are required to differentiate between those of the remainder (Scholtyseck, *et al.*, 1973). Both *Hammondia* and *Toxoplasma* produce thin-walled cysts while in *Besnoitia* the cyst wall is thick and fibrillar (Frenkel, 1974).

Cysts of *Sarcocystis* may be microscopic or macroscopic in size when fully mature (Munday and Rickard, 1974; Tadros and Laarman, 1976) and cyst structure may vary with the stage of development (Dubey, *et al.*, 1977c). The cyst wall may be thick or thin, often with septa extending into the cyst and sometimes with radial spines extending outwards (Frenkel, 1974). Although recent ultrastructural studies (Melhorn *et al.*, 1975a, b, 1976; Gestrich *et al.*, 1975b; Bergmann and Kinder,

TABLE 1.3 COCCIDIAN SPECIES OF CATS

Coccidian species	Natural Intermediate Hosts	Oocyst dimensions* (µm)	Sporocyst dimensions* (µm)	Reference+
Isospora felis Wenyon 1923	Not known	41.6 x 30.5 (38-51 x 27-39)	22.6 x 18.4 (20-26 x 17-22)	Shah, 1970a
Isospora rivolta (Grassi 1879) Wenyon 1923	Not known	25 x 21.1 (21-28 x 18-23)	15.2 x 11.6 (14-16 x 10-13)	Shah, 1970a
Besnoitia besnoiti (Marotel 1912) Henry 1913	Cattle	15 x 13 (14-16 x 12-14)		Peteshev et al., 1974
Besnoitia wallacei (Tadros and Laarman 1976) Dubey 1977	Rats, Mice	16 x 13 (15-18 x 12-15	11 x 8 (10-11 x 7-8)	Wallace and Frenkel, 1975
Besnoitia darlingi (Brumpt 1913) Schneider 1967	Opossum	12 x 10.3 (11-13 x 10-11)	7.9 x 5.4 (6-9 x 5-6)	Smith and Frenkel, 1977
Toxoplasma gondii (Nicolle and Manceaux 1908) Nicolle and Manceaux 1909	Mammals, Birds	12 x 10 (11-13 x 9-11)	8.5 x 6 (8-10 x 5-7)	Dubey et al., 1970a
Hammondia hammondi Frenkel and Dubey 1975	Rats, Mice	13.2 x 10.6 (13-14 x 10-11)	9.8 x 6.5 (8-11 x 6-8)	Frenkel and Dubey, 1975
Sarcocystis hirsuta Moule 1886	Cattle		12.5 x 7.8 (11-14 x 7-9)	Heydorn and Rommel, 1972a
Sarcocystis tenella Ralliet 1886	Sheep		12.4 x 8.1 (11-14 x 8-9)	Rommel et al., 1972
Sarcocystis porcifelis Dubey 1976	Pig		13 x 8 (13-14 x 7-8)	Golubkovan and Kisliakova, 1974
Sarcocystis muris (Blanchard 1885) Labbe 1899	Mouse		10.3 x 8.5 (9 -12 x 8-9)	Ruiz and Frenkel 1976
Sarcocystis leporum Crawley 1914	Cottontail rabbit		13.6 x 9.4 (13-17 x 9-11)	Fayer and Kradel, 1977
Sarcocystis spp Janitschke, Protz and Werner 1976	Gazelle		13.2 x 9.2 (11-16 x 8-12)	Janitschke et al., 1976

* Mean values, with range in brackets

\+ For oocyst and sporocyst dimensions

TABLE 1.4 COCCIDIAN SPECIES OF DOGS

Coccidian species	Natural Intermediate Hosts	Oocyst dimensions* (µm)	Sporocyst dimensions* (µm)	Reference+
Isospora canis Nemeseri 1959	Not known	38 x 30 (32-42 x 27-33)	21 x 16 (18-24 x 15-18)	Levine and Ivens, 1965
Isospora ohioensis Dubey 1975	Not known	24 x 20 (19-27 x 18-23)	17 x 12 (15-19 x 10-13)	Dubey, 1975c
Hammondia heydorni (Tadros and Laarman, 1976) Dubey 1977	Cattle	11.9 x 11.1 (10-15 x 9-13)	7.7 x 5.6 (5-9 x 5-6)	Heydorn, 1973
Sarcocystis cruzi Hasselman, 1926	Cattle		16.3 x 10.8 (14-17 x 9-13)	Heydorn et al., 1975b
Sarcocystis ovicanis Heydorn, Gestrich, Melhorn and Rommel, 1975	Sheep		14.8 x 9.9 (13-16 x 9-11)	Rommel et al., 1974
Sarcocystis miescheriana (Kuhn 1865) Labbe 1899	Pig		12.6 x 9.6 (11-14 x 9-11)	Rommel et al., 1974
Sarcocystis bertrami Doflein 1901	Horse		15.2 x 10 (15-16 x 9-11)	Rommel and Geisel, 1975
Sarcocystis fayeri Dubey, Streitel, Stromberg and Toussant 1977	Horse		12 x 8 (11-13 x 7-9)	Dubey et al., 1977c
Sarcocystis hemionilatrantis Hudkins and Kistner, 1977	Mule Deer		14.5 x 9.2 (14-16 x 9)	Hudkins-vivion et al., 1976
Sarcocystis spp Janitschke, Protz and Werner 1976	Gazelle		16.1 x 10.6 (13-18 x 8-12)	Janitschke et al., 1976

* Mean values, with range in brackets

\+ For oocyst and sporocyst dimensions

1975) have shown that several species of *Sarcocystis* have distinctive cyst wall structures, some do not (Melhorn *et al.*, 1976).

Serological idenfication of infections may also be less accurate than was once thought. In the Sabin Feldman dye test, *T. gondii* antigen cross reacts with antibody to *H. hammondi* in several intermediate hosts (Frenkel and Dubey, 1975) and there is evidence of cross-reactivity between *Toxoplasma, Besnoitia* and *Sarcocystis* in the indirect fluorescent antibody test (Frenkel, 1977).

At present, therefore, the identification of the canine and feline coccidia may only be achieved by the use of a combination of criteria including morphological, life cycle, host-specificity and serological differences combined in various ways. Even this approach however, may in some instances, permit identification only to the generic level. One such system of identification is presented by Frenkel (1974) and another by Tadros and Laarman (1976). An alternative approach is presented in Table 1.5. It is to be hoped that as our knowledge of the canine and feline coccidia increases better means of identification will be forthcoming.

1.4 LIFE CYCLES

Traditionally the coccidia were considered to be highly host-specific and to parasitise only the intestinal tract. Their common features were thought to include; life cycle in one host (homoxenous), asexual reproduction within host cells (schizogony or merogony), followed by sexual differentiation (gametogony) into male microgametes and female macrogametes that give rise to oocysts which are shed in the faeces. By a process of sporogony or sporulation a variable number of sporocysts containing one or more sporozoites are formed within the oocyst which typically serves as the sole source of infection for all potential hosts.

The successful completion of the homoxenous cycle is largely dependent on the regular contamination of host territory and food

TABLE 1.5 TENTATIVE KEY AND INVESTIGATIONAL SEQUENCE FOR THE IDENTIFICATION OF ISOSPORAN COCCIDIA IN CAT AND DOG FAECES

			Cats	Dogs
1.	a)	Oocysts shed as sporulated sporocysts in fresh faeces	*Sarcocystis* sp.	*Sarcocystis* sp.
	b)	Oocyst shed unsporulated in fresh faeces.....	2	2
2.	a)	Oocysts greater than 30 µm long.....	*I. felis*	*I. canis*
	b)	Oocysts less than 30 µm long.....	3	3
3.	a)	Oocysts 18 µm or more in both length and width.....	*I. rivolta*	*I. ohioensis*
	b)	Oocyst less than 18 µm in both length and width	4	*H. heydorni*
4.*	a)	Oocysts produce thick fibrillar-walled cysts enclosing host cell nuclei.....	*Besnoitia* sp **	
	b)	Oocysts produce thin-walled cysts excluding host cell nuclei.....	5	
5.	a)	Cysts predominantly in neural tissue, infective for other mice.....	*T. gondii*	
	b)	Cysts predominatly in striated muscle, not infective for other mice.....	*H. hammondi*	

* In mice orally inoculated with sporulated oocysts, examined 30-40 days post-infection.

** Although it does not appear to have been investigated, it is assumed that *B. besnoiti* oocysts are infective for mice since cysts from cattle tissues are (Neuman, 1962).

with infective oocysts. Although these conditions are readily met in respect of grazing herbivores, carnivores have comparatively less oral contact with soil and generally do not contaminate their living quarters with faeces. Thus in these hosts, the chances of direct oocyst infection are more limited. Canine and feline coccidia appear therefore, to have evolved away from the classical homoxenous cycle towards one involving an intermediate host stage. The advantages of such a two-host (heteroxenous) cycle are obvious. Intermediate hosts may assist in the distribution of these coccidia by their mobility and by serving as reservoirs of infection in conditions deleterious to oocyst survival.

Within the coccidian parasites of cats and dogs there is a gradation of heteroxenicity. For some genera direct transmission by the ingestion of oocysts still appears to be the most important means of infection. For others, however, the ability of oocysts to initiate the gametogonic cycle in the definitive host has been lost and the completion of the life cycle is totally dependent on the ingestion of infected intermediate hosts. In the section which follows the life cycle of each genus is reviewed; differences between genera are summarised schematically (Fig 1.3) and data relating to sporulation times, periods of patency and prepatency of each species are tabulated (Tables 1.6 to 1.9).

1.4.1 Genus *Isospora*

Of all the canine and feline coccidia, the genus *Isospora* departs least from the classical homoxenous cycle. It has long been known that it develops typical asexual, sexual and oocyst stages in the intestine. Detailed descriptions of the endogenous stages have been provided for *I. felis* (Hitchcock 1955; Lickfeld 1959; Shah, 1971), *I. canis* (Lepp and Todd, 1974) and *I. ohioensis* (Mahrt, 1967) but not for *I. rivolta* in the cat.

The unsporulated oocysts are shed in the faeces and after sporulation are infective for the same host species. Recently it has been shown that these oocysts are also infective for such additional hosts as rats, mice and hamsters (Frenkel and Dubey, 1972a). In these hosts, only limited multiplication takes place leading to the development of extra-intestinal unizoic cysts. Ingestion of these cysts by cats and dogs results in the production of oocysts once more. The life cycle can therefore be described as facultatively heteroxenous with cats and dogs, in which sexual reproduction takes place, being the final or definitive hosts and rats, mice and hamsters, intermediate hosts.

Extra-intestinal stages may also develop in the cat and dog. Thus dogs may act as intermediate hosts for the feline species *I. felis* and *I. rivolta* and cats as intermediate hosts for the canine species *I. canis* and *I. ohioensis* (Dubey, 1975 a,c). In addition cats may also act as both intermediate and final hosts for *I. felis* and *I. rivolta* (Dubey and Frenkel, 1972b). The extra-intestinal stages of the latter two species may persist in feline tissues for at least three months (Dubey and Frenkel, 1972b). This has led to speculation that they might be transmitted congenitally, but attempts to demonstrate this have been unsuccessful (Dubey, 1977a).

The relative importance of the direct or indirect means of infection of these coccidia under natural conditions is unknown. Frenkel and Dubey (1972a) consider it probable that the direct faecal-oral route is of greater importance than carnivorism although Dubey and Streitel (1976b) found that cats may acquire infections of *I. felis* and *I. rivolta* with equal efficiency whether ingesting infected mice or oocysts.

1.4.2 Genus *Toxoplasma*

Like the canine and feline species of *Isospora*, *T. gondii* is a facultatively heteroxenous coccidian although its routes of

transmission are more numerous.

Only domestic cats and certain other felids have been shown to produce oocysts and thus act as definitive hosts for this species (Jewel *et al.*, 1972; Miller *et al.*,1972). It appears that any warm blooded animal may act as an intermediate host and infection has been recorded in over 200 species of mammals and birds, including man (Levine 1977a).

Cats can acquire *Toxoplasma* by ingesting infected intermediate hosts or sporulated oocysts. The former means of infection appears more efficient than the latter since patent infections are more frequently produced and pre-patent periods are shorter in cats infected with intermediate-host-stages than in those infected with oocysts (Dubey *et al.*, 1970a; Dubey and Frenkel, 1972a; Frenkel *et al.*, 1970; Hutchinson *et al.*, 1971; Wallace, 1973b).

In cats the intestinal cycle is generally similar to that of other coccidia with a period of asexual reproduction followed by gametogony and oocyst production. There is, however, a series of asexual multiplicative generations that do not equate precisely with schizogony as it occurs with other coccidia in *T. gondii* infections. These multiplicative phases of *Toxoplasma* have been arbitrarily designated types A to E (Dubey and Frenkel, 1972a). Simultaneous with the enteric development of *Toxoplasma*, invasion of the extra-intestinal organs of the cat occurs. The extra-intestinal phase in cats is similar to that in non-feline intermediate hosts, which like the definitive host, may acquire infection by ingestion of oocysts or infected tissues. In carnivores it is likely that the latter mode of infection is most common while herbivores are assumed to become infected largely by ingestion of oocysts (Hartley and Munday, 1974).

Unlike the situation with the canine and feline species of *Isospora*, extensive multiplication of the extra-intestinal stages of *T. gondii*

TABLE 1.6 REPORTED SPORULATION TIMES OF FELINE COCCIDIAN SPECIES

Species	Sporulation Time (hrs)	Temperature (^{o}C)	Reference
I. felis	14	28	Tomimura, 1957
	24	?	Lickfeld, 1959
	40	20	Shah, 1970b
	25	25	Shah, 1970b
	12	30	Shah, 1970b
	8	38	Shah, 1970b
	24	30	Rocha and Lopes, 1971
I. rivolta	36	30	Rocha and Lopes, 1971
	24 - 48	'room'	Pellerdy, 1974
T. gondii	24 - 48	25	Dubey et al., 1970b
	48 - 120	15	Dubey et al., 1970b
	21 days	11	Dubey et al., 1970b
	48 - 72	24	Frenkel et al., 1970
	5 - 8 days	15	Frenkel et al., 1970
	14 - 21 days	11	Frenkel et al., 1970
	48	?	Zaman, 1970
	72 - 96	21	Munday and Rickard, 1974
H. Hammondi	72	20 - 23	Frenkel and Dubey, 1975
	48 - 72	?	Rommel and Seyerl, 1976
B. wallacei	48 - 96	24	Wallace and Frenkel, 1975
B. darlingi	48 - 72	'room'	Smith and Frenkel, 1977

Table 1.7 REPORTED SPORULATION TIMES OF CANINE COCCIDIAN SPECIES

Species	Sporulation Time (hrs)	Temperature (°C)	Reference
I. canis	96	'room'	Nemeseri, 1959
	24	30	Rocha and Lopes, 1971
I. ohioensis	8	38	Mahrt, 1968
	16	30	Mahrt, 1968
	24	25	Mahrt, 1968
	48	20	Mahrt, 1968
	24	22 - 26	Dubey, 1975c
	36	30	Rocha and Lopes, 1971
H. heydorni	72	21	Heydorn, 1973
	36 - 48	23	Dubey and Fayer, 1976
	12	30 - 37	Dubey and Fayer, 1976

can occur in both intermediate and definitive hosts. The proliferative form, which multiplies intracellularly during acute infection, is termed the trophozoite or tachyzoite. Tachyzoites spread from cell to cell and are disseminated throughout the body in macrophages, lymphocytes, granulocytes and as free organisms in the circulation (Frenkel, 1973).
Transplancental infection of foetuses by tachyzoites has been recorded in many intermediate hosts, including man (Jones, 1973) but not in cats (Dubey and Hoover, 1977). *Toxoplasma* tachyzoites have also been isolated from the milk of cows, goats, pigs, dogs, cats, rabbits, guinea pigs and mice with naturally occurring or experimentally induced infections (Frenkel , 1973; Jones, 1973).

Within one to two weeks of infection, as immunity develops, tachyzoites enter host cells in both definitive and intermediate hosts and induce the development of a cyst wall around them. Within these cysts a large number of slowly replicating bradyzoites are formed by endodyogeny (internal budding). The cysts, which are found mainly in the brain, heart and skeletal muscle (Jones, 1973) may persist for the life of the host (Turner, 1976) and in intermediate hosts represent the end-point of development. Both bradyzoites and tachyzoites are infective for other hosts. However, in cats, ingestion of the former results in shorter prepatent periods than does ingestion of the latter (Frenkel *et al.*, 1970; Dubey *et al.*, 1970a; Wallace, 1973b).

1.4.3 Genus *Besnoitia*

Cats are definitive hosts of *Besnoitia besnoiti* of cattle, *B. wallacei* of rodents and *B. darlingi*; of opossums (*Didelphis marsupialis*) (Peteshev *el al.*, 1974; Wallace and Frenkel, 1975; Smith and Frenkel, 1977). Cats infected with *B. wallacei* shed unsporulated oocysts in their faeces. After sporulation the oocysts are infective for the intermediate hosts, rats and mice, but not for cats. Tachyzoites develop in several organs of the intermediate host and subsequently

TABLE 1.8 REPORTED PREPATENT AND PATENT PERIODS OF FELINE COCCIDIAN SPECIES

Species	Infective Agent	Prepatent Period (days)	Patent Period (days)	Reference
I. felis	Oocyst	7 - 8		Hitchcock, 1955
	"	8	16 - 20	Tomimura, 1957
	"	10	6	Lickfeld, 1959
	"	7 - 8		Nemeseri, 1959
	"	7 - 8	10 - 11	Shah, 1971
	"	3 - 7		Rocha and Lopes, 1971
	"	7 - 11		Dubey and Frenkel, 1972b
	"	8 - 10		Frenkel and Dubey, 1972a
	Extraint. Stages	5 - 6		Frenkel and Dubey, 1972a
	" "	4 - 8		Dubey and Frenkel, 1972b
I. rivolta	Oocyst	3 - 7		Rocha and Lopes, 1971
	"	5 - 7		Dubey and Frenkel, 1972b
	"	5 - 6		Frenkel and Dubey, 1972a
	"	5	35 - 42	Pellerdy, 1974
	Extraint. Stages	5 - 6		Frenkel and Dubey, 1972a
	" "	5 - 7		Dubey and Frenkel, 1972b
	" "	6		Dubey, 1975c
T. gondii	Oocyst	20 - 24		Dubey et al., 1970b
	"	21 - 24		Frenkel et al., 1970
	"	9		Hutchinson et al., 1971
	"	21 - 49		Wallace, 1973b
	Bradyzoites	3 - 5	7 - 14	Frenkel et al., 1970
	"	3 - 5		Dubey et al., 1970b
	"	3	4 - 7	Sheffield and Melton, 1970
	"	3 - 6	7	Overdulve, 1970
	"	3 - 6	10 - 20	Zaman, 1970
	"	5 - 10		Hutchinson et al., 1971
	"	4 - 5	9 - 20	Wallace, 1973b
	"	4 - 5	5 - 8	Ito et al., 1974
	"	5 - 12	8 - 12	Munday and Rickard, 1974
	Tachyzoites	8 - 10		Frenkel et al., 1970
	"	7 - 9		Dubey et al., 1970b
	"	5 - 18		Wallace, 1973b
H. hammondi	Bradyzoites	5 - 8	10 - 28	Frenkel and Dubey, 1975
	"	6 - 9	1 - 14*	Dubey, 1975b
B. wallacei	Bradyzoites	11 - 13	5 - 12	Wallace and Frenkel, 1975
B. besnoiti	"	13 - 16	3 - 5	Peteshev et al., 1974
B. darlingi	"	11 - 14	5 - 10	Smith and Frenkel, 1977
S. hirsuta	"	7 - 9	42 - 63	Heydorn and Rommel, 1972a
S. tenella	"	12 - 13	8 - 53	Rommel et al., 1972
	"	11 - 12	14	Melhorn and Scholtyseck, 1974
S. porcifelis	"	5 - 10		Golubkovan and Kisliakova, 1974
S. muris	"	8 - 11 **	3 - 81***	Ruiz and Frenkel, 1976
S. leporum	"	10 - 25	3 - 46	Fayer and Kradel, 1977
	"	14	14 - 69	Crum and Prestwood, 1977

* spontaneous oocyst shedding up to 120 days after infection may occur

** up to 27 days

*** usually 5 - 10 days

TABLE 1.9 REPORTED PREPATENT AND PATENT PERIODS OF CANINE COCCIDIAN SPECIES

Species	Infective Agent	Prepatent Period (days)	Patent Period (days)	Reference
I. canis	Oocyst	11	28	Nemeseri, 1959
	"	3 - 7		Rocha and Lopes, 1971
	"	7 - 10	9 - 11	Lepp and Todd, 1974
	"	9 - 11		Dubey 1975a
	Extraint.Stages	8 - 9		Dubey 1975a
I. ohioensis	Oocyst	6	13 - 23	Mahrt, 1967
	"	3 - 7		Rocha and Lopes, 1971
	"	6	2	Dubey, 1975c
	Extraint.Stages	4 - 6		Dubey, 1975c
H. heydorni	Bradyzoites	7 - 10	4 - 8	Heydorn, 1973
	"	7 - 15	1 - 3	Dubey and Fayer, 1976
S. cruzi	Bradyzoites	9 - 10	57 - 71	Heydorn and Rommel, 1972a
	"	9 - 22	3 - 16	Fayer, 1974
S. ovicanis	Bradyzoites	8 - 9		Rommel et al., 1974
	"	15	45 - 75	Ford, 1974
	"	14 - 30	7 - 50	Munday & Corbould, 1974
	"	13 - 15		Munday et al., 1975
S. miescheriana	Bradyzoites	9 - 10		Rommel et al., 1974
S. bertrami	Bradyzoites	8		Rommel and Geisel, 1975
S. fayeri	Bradyzoites	12 - 15		Dubey et al., 1977c
S. hemionilatrantis	Bradyzoites	13		Hudkins - Vivion et al., 1976

form cysts containing bradyzoites in the mesentery, omentum and intestinal wall (Wallace and Frenkel, 1975). Bradyzoites of *B. besnoiti* and *B. darlingi* are experimentally infective for other intermediate hosts but this does not appear to be the case with *B. wallacei* (Wallace and Frenkel, 1975). Cats become infected only by ingestion of stages in intermediate hosts and the life cycle is therefore obligatorily heteroxenous. Schizonts, gametocytes and oocysts are formed in the intestine of the cat but apparently extra-intestinal organs are not invaded (Wallace and Frenkel, 1975).

Although congenital transmission has been shown to occur in cattle infected with *B. besnoiti* (Bwangamoi, 1968) it is not known whether this occurs in rodents infected with *B. wallacei* or in opossums infected with *B. darlingi*.

1.4.4 Genus *Hammondia*

Like *Besnoitia*, *Hammondia* has an obligatory two-host cycle. Two species are known: *H. hammondi* for which the cat is the definitive host and *H. heydorni* for which the final host is the dog. The intermediate hosts of *H. hammondi* are mice, rats, guinea pigs, hamsters and dogs (Frenkel and Dubey, 1975; Wallace, 1975; Dubey, 1975d), and of *H. heydorni*, cattle (Heydorn, 1973, Dubey and Fayer, 1976).

Cats can only acquire *H. hammondi* infection by the ingestion of mature cysts in tissues of intermediate hosts. Schizonts and gametocytes develop in the intestine and unsporulated oocysts are shed in the faeces. The extra-intestinal tissues of the cat are not invaded (Frenkel and Dubey, 1975). After sporulation the oocysts are infective for the intermediate hosts. In the mouse intermediate host the sporozoites released from the oocyst penetrate the intestinal cells and tachyzoites, which are not infective to cats (Dubey and Streitel, 1976a), multiply in the intestinal lamina propria, in muscles, Peyer's patches and mesenteric lymph nodes. During the second week of infection cysts containing bradyzoites appear in

other tissues, primarily in skeletal muscle (Frenkel and Dubey, 1975). Cats become infected by ingesting these cysts which, unlike those of *T. gondii* and some species of *Besnoitia*, are not infective for other intermediate hosts (Frenkel, and Dubey, 1975; Wallace 1975). Congenital infection does not occur in either the definitive host (Dubey, 1977a) or in intermediate hosts (Dubey and Streitel, 1976a).

The life cycle of *H. Heydorni* appears to be essentially similar to that of *H. hammondi* but differs to the extent that an extra-intestinal phase may occur in the final host. Thus dogs may serve as both definitive and intermediate hosts for this species (Heydorn, 1973; Dubey and Fayer 1976; Hedydorn *et al.*, 1975a).

1.4.5. Genus *Sarcocystis*

Like the previous two genera , *Sarcocystis* species have an obligatorily heteroxenous life cycle. They differ, however, from all the other canine and feline coccidia in two important respects. Firstly, asexual multiplicative stages have not been found in the intestines of the final host (Heydorn and Rommel, 1972b; Fayer 1974; Munday *et al.*, 1975). Secondly, the oocysts develop and sporulate in the lamina propria (Melhorn and Scholtyseck, 1974; Munday *et al.*, 1975; Ruiz and Frenkel, 1976) and are usually shed in the faeces as free sporocysts (Ford, 1974; Rommel *et al.*, 1972, 1974; Mahrt, 1973; Munday and Corbould, 1974).

Cats and dogs have each been shown to be final hosts for at least seven species of *Sarcocystis*. These species appear to be highly host-specific infecting only a single intermediate host species (Gestrich *et al.*, 1974, 1975a; Munday and Rickard, 1974; Munday, 1976; Rickard and Munday, 1976). Of these species only the development of *S. hirsuta* (Heydorn and Rommel, 1972b) and *S. muris* (Ruiz and Frenkel, 1976) in the cat and *S. cruzi* (Fayer, 1974; Fayer and Johnston 1973, 1974) and *S. ovicanis* (Munday *et al.*,

1975) in the dog, have been studied in any great detail.

Intermediate hosts acquire infection only by the ingestion of sporocysts shed in the faeces of the definitive host. Such sporocysts are not infective for the final host (Fayer, 1974; Rommel *et al.*, 1974). Sporozoites are released from the sporocysts in the intestine of the intermediate host and invade many tissues. Schizogony occurs in endothelial cells of blood vessels in various organs and tissues (Fayer and Johnston, 1973, 1974; Munday *et al.*, 1975) before the development of typical cysts in striated and cardiac muscle and, occasionally, central nervous tissue as well (Hartley and Blakemore, 1974). Young cysts contain globular peripheral multiplicative stages, the metrocytes. Two daughter cells are formed by endodyogeny within each metrocyte which, after several further divisions, give rise to the bradyzcites. Schizonts and metrocytes are not infective for definitive hosts which can only acquire infection by the ingestion of mature cysts containing fully developed bradyzoites (Ruiz and Frenkel, 1976). In the small intestine of the definitive host the bradyzoites penetrate the lamina propria, form gametes and eventually oocysts without undergoing schizogony (Heydorn and Rommel, 1972b; Fayer, 1974; Munday *et al.*, 1975). The oocysts, which sporulate in the lamina propria, have a thin, fragile wall (Melhorn and Scholtyseck, 1974) which usually ruptures releasing the sporocysts.

There is some evidence to suggest that *Sarcocystis* infection may be acquired congenitally by intermediate hosts (Cunningham, 1973; Munday and Black 1976). However, attempts to demonstrate such transmission experimentally have failed (Fayer *et al.*, 1976b).

1.5 DISSEMINATION OF INFECTION AND TRANSPORT HOSTS

The role of transport hosts (which are distinguished from intermediate hosts by the absence of any multiplication within them) in the dissemination of infection is largely unknown. Early recognition

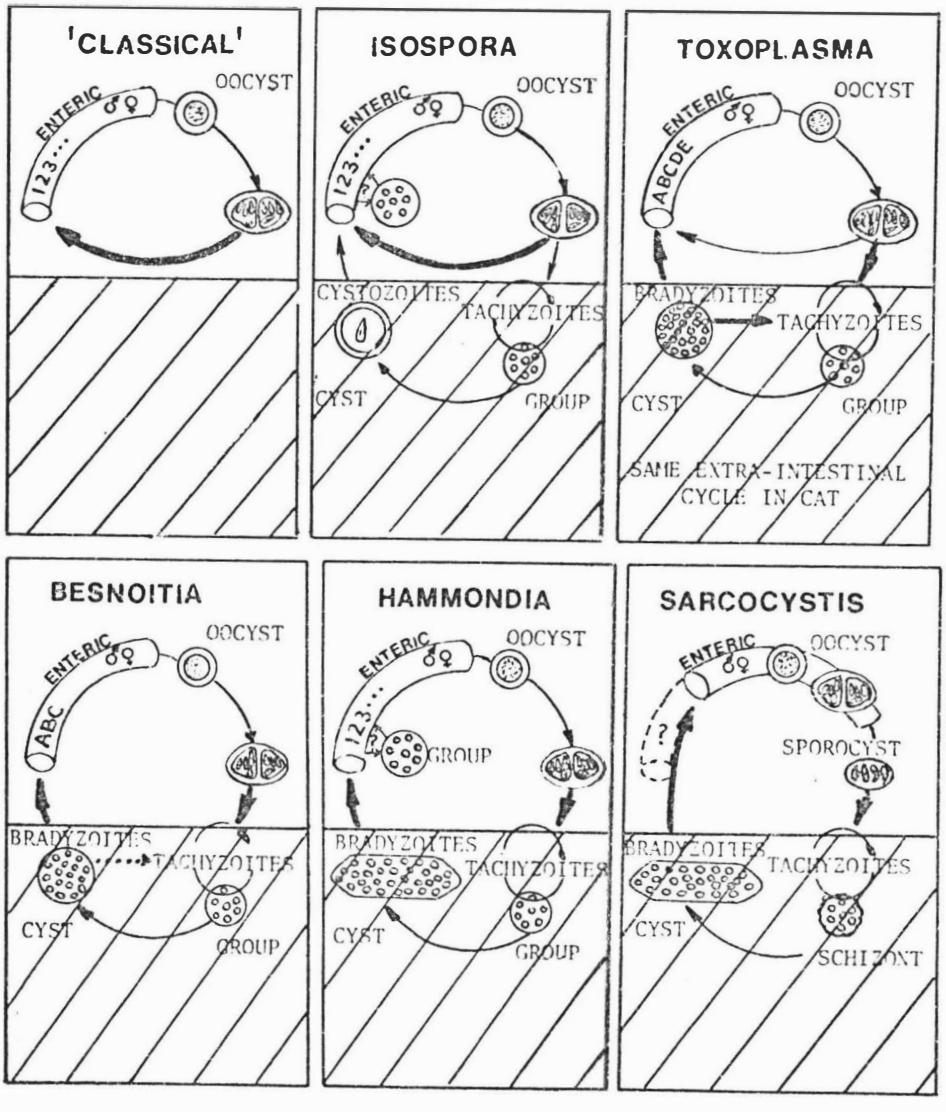


Fig. 1.3 Schematic comparison of the life cycles of the canine and feline coccidia. Clear areas represent cycles in final hosts, cross-hatched areas cycles in intermediate hosts. The importance of transmission routes is indicated by the thickness of the arrows between the top and bottom halves. (Adapted from Frenkel, 1974)

that a parasitaemia occurs in *Toxoplasma* and *Besnoitia* infections led several workers to investigate the potential role of blood-sucking arthropods in the mechanical transmission of these parasites. Such transmission has been demonstrated experimentally with *B. besnoiti* (Bigalke, 1967) while with *T. gondii* similar studies have generally produced negative or inconclusive results (Frenkel, 1973).

With the discovery of the coccidial nature of these protozoa, recent studies have examined the possible role of vectors in the dissemination of oocysts. Cockroaches (Wallace, 1972; Chinchilla and Ruiz 1976), flies (Wallace, 1971) and earthworms (Frenkel *et al.*, 1975) have been shown experimentally to act as carriers of *Toxoplasma* oocysts. Evidence that sheep may distribute infection by passing in their faeces oocysts that have been ingested but which have failed to excyst has also been provided (Beverley *et al* ., 1975).

Although comparable studies have not, as yet, been carried out with other canine and feline coccidia, similar means of oocyst dissemination by transport hosts appears possible.

1.6 RESISTANCE AND SURVIVAL

1.6.1 Oocysts

From an epidemiological point of view, the oocyst, as the only exogenous stage, is of central importance in the transmission of infection. Generally it is a hardy, long-lived resting phase capable of withstanding the action of many physical and chemical agents. The resistance of the canine and feline coccidial oocysts to such agents, however, is poorly documented. Apart from brief observations on the susceptibility of the oocysts of *I. felis* to desiccation (Lickfeld, 1959) and studies on the survival of unsporulated mixed *Isospora* oocysts from dogs (Lee, 1934) only

the resistance of those of *T. gondii* have been studied in any detail.

Toxoplasma oocysts resist 24 hours exposure to undiluted sodium hypochlorite and concentrated sulphuric acid but are destroyed by 30 minutes exposure to 10% aqueous ammonia and 4 days contact with 10% formalin (Dubey *et al.*, 1970b; Ito *et al.*, 1975a). In faeces and water they retain their infectivity for 17 months in the laboratory at 4°C (Hutchinson 1967), 410 days outdoors at a mean temperature of 19.5°C (Yilmaz and Hopkins, 1972) and for 12 to 18 months when buried under soil in Kansas (Frenkel *et al.*, 1975). Although desiccation is injurious they survive at least two days at 0% and 19% relative humidity and 11 days at 37% and 58% relative humidity (Frenkel and Dubey 1972b). Like other coccidia, sporulated oocysts of *T. gondii* appear more resistant than unsporulated ones. Sporulated oocysts survive freezing at -21°C for 28 days while unsporulated oocysts are killed after 1 day at 15--21°C and 14 days at -6°C (Frenkel and Dubey, 1973). Similarly, exposure to 45° to 50°C for one hour is lethal for unsporulated oocysts as is 37°C for over 24 hours (Dubey *et al.*, 1970a; Ito *et al.*, 1975c). Sporulated oocysts, however, remain infective at 37°C for 306 days (Yilmaz and Hopkins, 1972).

Studies on the resistance of *Sarcocystis* species should prove particularly interesting. Faecal stages of these coccidians are passed as fully sporulated, free sporocysts and are thus independent of climatic conditions unfavourable for sporulation. Whether or not their mature state also confers any long-term survival benefits is unknown although it appears likely that the absence of a protective oocyst wall may be disadvantageous. Tadros and Laarman (1976) have speculated that their prolonged maintenance at high body temperature in a sporulated state may, in fact, diminish their viability and infectivity.

1.6.2 Intermediate host stages

Intermediate host stages are less resistant than oocysts although information regarding their survival is limited and restricted to two genera - *Toxoplasma* and *Sarcocystis*. Both tachyzoites and bradyzoites in infected intermediate hosts may serve as a source of *Toxoplasma* infection. Bradyzoites, however, are more resistant to pepsin and trypsin digestion than are tachyzoites (Jacobs, *et al.*, 1960) and survive for longer after the death of the host (Hartley and Munday, 1974). Bradyzoites of *Toxoplasma* survive as long as 68 days at 4°C (Jacobs *et al.*, 1960) while those of *Sarcocystis* in beef remain infective for at least 18 and 3 days at 2° and 4°C respectively (Gestrich and Heydorn, 1974; Fayer, 1975).

Freezing reduces the infectivity of bradyzoites of both genera. *Sarcocystis* infected beef is not infective for cats after freezing at -20°C for 3 days (Gestrich and Heydorn, 1974) or for dogs after freezing for 7 days (Fayer, 1975). Similarly, Dubey (1974) found that *Toxoplasma* -infected mice stored at -9°C and -20°C for as little as three hours were not infective to cats although others (Dubey and Frenkel, 1973) found that *Toxoplasma* in the skeletal muscle of a squirrel monkey frozen at -20°C for 16 days was still infective. The reasons for such anomalies are not clear but may possibly relate to the rate of freezing. Obviously there is a need for a more critical evaluation of these effects.

High temperatures also appear lethal for these genera. *Toxoplasma* tissue cysts are killed after 30 minutes exposure to temperatures of 50 to 55°C (Jacobs *et al.*, 1960; Dubey *et al.*, 1970b) while in beef *Sarcocystis* infectivity may be eliminated if a temperature of 65 - 70°C is attained throughout the meat (Gestrich and Heydorn, 1974).

1.7 PATHOGENICITY

1.7.1 For definitive hosts

There is little information regarding the pathogenicity of coccidial infections to cats and dogs and much of what there is, is contradictory. Andrews (1926) reported that 100,000 oocysts of *I. felis* killed young cats within one week of administration. Infected animals showed signs of anaemia, emaciation, weakness, depression and diarrhoea. Similar symptoms were observed by others (Tomimura, 1957, Lickfeld, 1959) who further noted that the severity of infection varied directly with the number of oocysts given. Hitchcock(1955) infected susceptible nine-week old kittens with 100,000 *I. felis* but did not observe any clinical signs. She suggested that Andrew's (1926) cats may have suffered from a concurrent viral infection. More recent reports tend to support Hitchcock's(1955) observations. Shah(1971) found *I. felis* to be only mildly pathogenic even after feeding as many as 150,000 oocysts while Dubey and Streitel (1976b) concluded that neither *I. felis* nor *I. rivolta* were serious pathogens. Pellerdy (1974) however, found that the latter species induced a transient diarrhoea in two-month old kittens.

Lee (1934) described the predominent symptoms of canine coccidiosis as bloody diarrhoea, emaciation, anaemia and weakness. Mahrt (1966) on the other hand, considered *I. ohioensis* to be only mildly pathogenic while Nemeseri (1959) found no indications of disease in young dogs infected with approximately 5,000 *I. canis* oocysts. Following infections with 50,000 to 80,000 *I. canis* oocysts, however, the animals showed the general symptoms described by Lee (1934) and had temperatures of 39.5 to 40.3^{o}C. Post-mortem examination revealed severe and often haemorrhagic inflammation of the small intestine (Nemeseri, 1959).

Less information is available concerning the more recently discovered coccidia of cats and dogs. Although none have been studied with any thoroughness, *Besnoitia*, *Hammondia* and *Sarcocystis*

are generally considered to be non-pathogenic for their definitive hosts (Dubey 1976b). Craige (1976), however, states that the presence of sarcocystan sporocysts in the faeces of dogs is invariably associated with chronic intestinal disorders, serious but obscure signs of toxicosis, anorexia, nausea, nervous disorders and even fever, but has provided no evidence. Gorbov (1975) has also observed enteritis in 8-to-10 week-old puppies fed *Sarcocystis* infected beef, mutton and pork. The degree of morbidity and mortality in experimental toxoplasmosis is influenced by the strain of *Toxoplasma* , the size of infective dose, the route of inoculation and the age of the cat (Dubey and Frenkel, 1974). In adult cats *Toxoplasma* infections are not usually clinically evident; newborn kittens however, are very susceptible (Dubey and Frenkel, 1972a) and may develop a foul smelling diarrhoea, hepatitis, pancreatitis, myositis or encephalitis. Deaths of kittens up to 12 weeks of age (Dubey and Frenkel 1972a) may also occur.

1.7.2 For intermediate hosts

The major importance of the canine and feline coccidia lies in their role as agents of infection for intermediate hosts. *Toxoplasma* is particularly well known in this regard (for reviews see Boughton, 1970; Quin and McCraw, 1972; Jones, 1973; Hartley and Munday 1974; Turner, 1976; Beverley, 1976). Retinochoroiditis and encephalitis in man and abortion in sheep, both resulting from congenital infections, are the most important clinical entities. *Toxoplasma* infection may also cause pyrexia, lymphadenopathy, pneumonia, hepatitis, myocarditis and a variety of other symptoms depending on the organs parasitised.(Dubey 1976b; Turner 1976). However, although infection is common, disease is relatively rare. A partial explanation for this may relate to the fact that strains of *Toxoplasma* vary in their pathogenicity for any one host species (Dubey and Frenkel, 1973; Ito *et al.*, 1975b). The virulence of any one strain,however, may be enhanced by frequent passage through a given host (Dubey and Frenkel, 1973).

Like toxoplasmosis, besnoitiosis is also a well known disease. *B. besnoiti* parasitises the skin and cornea of cattle in Europe and cattle and other ungulates in Africa (Tadros and Laarman, 1976). In the acute stages of infection it can cause pyrexia, photophobia, anasarca, diarrhoea and swelling of the lymph nodes. During the chronic seborrhoeic phase, wrinkling of the skin, hair loss and death may occur (Levine 1973). The pathogenicity of *B. wallacei* and *B. darlingi* for their natural intermediate hosts is largely unknown. However, infections with bradyzoites of the latter species have been found to be fatal for mice and hamsters (Smith and Frenkel, 1977).

Unlike the previous two genera, *Sarcocystis* has, until recently, been regarded as an innocuous protozoan. Its main importance lay in the disfigurement of meat intended for human consumption. Following the discovery of the coccidian nature of this parasite, however, some species have been shown to be potentially highly pathogenic. In calves experimentally infected with very large numbers of sporocysts of *S. cruzi*, clinical signs include anorexia, pyrexia, anaemia, cachexia and weight loss, frequently resulting in death within 23 days. A generalized lymphadenopathy and petechiation of the serosal membranes with numerous schizonts in the endothelial cells of blood vessels are found post-mortem (Fayer and Johnston, 1973, 1974; Mahrt and Fayer, 1975; Johnston *et al.*, 1975; Gestrich *et al.*, 1975a). Similar clinical symptoms and death may occur in lambs experimentally infected with *S. ovicanis* (Gestrich *et al.*, 1974, 1975a; Munday *et al.*, 1975; Heydorn and Gestrich 1976) and both this species and *S. cruzi* have been shown to be capable of inducing abortion in their intermediate hosts (Leek and Fayer, 1978; Fayer *et al.*, 1976b). Although experimentally infected animals have been inoculated with greater numbers of sporocysts than they might reasonably be expected to encounter under natural conditions, field outbreaks of suspected bovine sarcosporidioisis have been reported (Corner *et al.*, 1963; Meads, 1976; Frelier *et al.*, 1977).

The pathogenicity of *Isospora* species or *H. heydorni* has not been studied and while *H. hammondi* generally appears to be of low pathogenicity for mice and non-pathogenic for other hosts tested (Dubey 1975b) 30% mortalities have been recorded in mice infected with 10^5 to 10^6 *H.hammondi* oocysts (Frenkel and Dubey, 1975).

1.8 IMMUNITY

1.8.1 Definitive hosts

The immunity of definitive hosts to coccidia can be assessed by the pattern and degree of oocyst or sporocyst shedding following challenge infection. It has long been recognised that both cats (Andrews,1926) and dogs (Lee, 1934) that have been infected with species of *Isospora* are, to varying degrees, resistant to challenge infections. There have been few careful studies of this resistance but it appears that dogs can develop a very high level of resistance both to *I. ohioensis*(Mahrt, 1966) and to *I. canis* (Lepp and Todd, 1974). Studies on cats, however, indicate that neither *I. felis* (Lickfeld, 1959) nor *I. rivolta* (Pellerdy 1974) produce such a high level of resistance. There is scope for much more work on this subject. It is to be expected that the level of resistance produced will be affected by many factors such as host age, numbers of oocysts given and the interval between primary and challenge infection: very little is known of the immune systems involved in resistance to these coccidia or how they operate.

Cats are considered to develop a more solid and lasting acquired immunity to *Toxoplasma* than to *I. felis* or *I. rivolta* (Wallace, 1973b; Dubey and Frenkel, 1974). This, it has been suggested, may be due to the persistence of *Toxoplasma* bradyzoites in tissue cysts providing prolonged antigenic stimulation (Wallace, 1973b).

In *Toxoplasma* infections, oocyst production and periods of patency have been found to be similar whether cats infected for the first time are given single or multiple infective doses (Dubey and Frenkel, 1974). Young cats shed more oocysts than older cats and males shed more oocysts than females (Dubey *et al.*, 1977b); the latter finding, however, has not been examined statistically. In general, cats excrete *Toxoplasma* oocysts for a period of only one to three weeks (Table 1.8) and do not re-excrete them following reinfection within one to five months (Dubey *et al.*, 1970b). There are, however, exceptions to this generalisation. Latent infections of *T. gondii* may be reactivated by subsequent infections with *I. felis* (Chessum 1972 ; Dubey 1976a) while experimentally, immunity has been shown to depend on such factors as the strain of parasite, the age of cats at primary infection and the interval between primary infection and challenge. Cats first infected as suckling or weanling kittens have been shown to reshed *Toxoplasma* oocysts following challenge. In such cats the stage of *Toxoplasma* inoculated, the route of infection and serum antibody titres do not appear to correlate with the degree of immunity. However, prepatent periods are longer, patent periods are shorter and fewer oocysts are produced than after primary infection (Dubey and Frenkel, 1974). Cats first infected as adults, on the other hand, generally do not re-excrete oocysts after challenge and the presence of circulating serum antibodies usually indicates a high degree of immunity (Dubey and Frenkel 1974).

There appears to be little immunity to *Besnoitia* infection in cats (Frenkel, 1977) while data on *Hammondia* and *Sarcocystis* infections is very limited. Neither age nor sex of cats has any apparent effect on acquired immunity to *H. hammondi* infection, which is not absolute and may permit spontaneous reshedding of oocysts in some cases up to 120 days after initial infection (Dubey, 1975b). With repeated infections prepatent periods are longer, patent periods are shorter and fewer oocysts are shed (Dubey 1975b). A similar lack of complete immunity to *H. heydorni* in dogs is also apparent (Heydorn, 1973).

Sarcccystis infections in definitive hosts are characterized by long periods of sporadic sporocyst shedding - a fact which Tadros and Laarman (1976) attribute to their subepithelial location in intestinal tissues and their gradual release into the lumen. Several studies have demonstrated that reinfection is easily achieved (Rommel *et al.*, 1972; Heydorn and Rommel 1972a; Fayer 1974; Ruiz and Frenkel 1976). These findings, and the observations of Fayer (1974) that these parasites induce little cellular reaction suggest that only a low level of acquired immunity is induced. Whether or not partial immunity is conferred by previous infection is unknown since quantitative estimations of sporocyst production have not been made. In some species of *Eimeria* it appears that schizogonic stages stimulate protective immunity while gametogonic stages have little, if any, immunizing effect (Rose, 1973). It is tempting, therefore, to link the apparent lack of protective immunity to *Sarcocystis* infections in cats and dogs with the absence schizogony.

Although it has been little studied, cross-immunity between the coccidian genera of cats and dogs appears not to occur. Piekarski and Witte (1971) concluded that no immunity to *Toxoplasma* infection was conferred in cats by previous experience to *Isospora* species while Chessum (1972) found a similar lack of cross-immunity between *I. felis* , *I. rivolta* and *T. gondii*. Ruiz and Frenkel (1976) found that cats immune to *Toxoplasma* or previously infected with *Hammondia* or *Besnoitia* were still susceptible to *Sarcocystis muris*. In addition prior experience of the feline coccidia *I. felis* and *I. rivolta* was found not to confer immunity to subsequent infections with *I. canis* and *I. ohioensis* in dogs (Dubey *et al.*, 1970a).

1.8.2 Intermediate hosts

Other than to *Toxoplasma*, immunity to reinfection in intermediate hosts has been little studied. Survivors of bovine besnoitiosis develop a durable premunity (Pols, 1960) and mice show evidence

of resistance to challenge with *B. wallacei* (Frenkel, 1977). No information is available for *Isospora* and *Hammondia* and although data on the development of protective immunity to *Sarcocystis* infections is similarly lacking it is conceivable that, because of the intimate contact between schizogonic stages and the prolonged persistence of viable sarcocysts in muscle, intermediate hosts may be more resistant to reinfection with this parasite than are definitive hosts. Gestrich *et al.*, (1975a) has stated, without providing any supporting evidence, that the immunizing effect of *S. cruzi* sporocysts in cattle is fairly strong. Others, however, have found that mice may be reinfected repeatedly with *S. muris* (Ruiz and Frenkel, 1976).

Immunity to toxoplasmosis has been discussed in detail by Quin and McCraw (1972) Frenkel (1973) and Jacobs (1973) and will be mentioned only briefly. In general, although reinfection is possible, immunity to clinical disease is usually good. However, this may vary with the species and age of the host and perhaps also the strain of parasite.

Like *Besnoitia* immunity to *Toxoplasma* is thought to depend on the persistence of infection (premunity) while the suppression of established immunity by means of corticosteroids may lead to a reactivation of latent toxoplasmosis with the production of clinical disease (Frenkel, 1973).

1.9 TREATMENT

1.9.1 Definitive hosts

Treatments reported to be effective in the control of canine and feline coccidiosis in general are presented in Table 1.10. However, claims of the efficacy of these must be regarded with scepticism since, with the exception of those of Smith and Edmonds (1959) and

Rachman and Pollock (1961), the studies on which they are based have given little consideration to the self-limiting nature of coccidial infections or the need for non-medicated control groups. It is likely, therefore, that many of the cures apparently achieved following therapy may, in fact, have resulted from natural recover.

Treatments for specific coccidian infections are few and are restricted to those directed toward the prevention of *Toxoplasma* excretion in cats. Both SDDS (2-Sulfamoyl-4, 4-diaminodipheny-sulfone) administered at a daily dose rate of 10 mg/kg starting five days before infection (Ohshima and Kumada, 1974) and daily intramuscular injections of 2 mg/kg pyrimethamine and 100 mg/kg sulfadiązine (Sheffield and Melton, 1976) have been reported to inhibit *Toxoplasma* cocyst excretion. Other workers (Dubey and Yeary, 1977), however, have found that although these two treatments may reduce oocyst shedding they do not completely prevent it.

1.9.2 Intermediate hosts

Pyrimethamine and sulfadiazine are commonly used in the treatment of human toxoplasmosis and are known to reduce the severity of disease in experimental animals (Sheffield and Melton, 1976). SDDS has also been shown to be of value both as a therapeutic and as a prophylactic agent against swine toxoplasmosis (Ohshima *et al.*, 1969, 1970) while sulfamonomethoxine and 1, 3 bis (n-chlorobenzylidinamino), guanidine have recently been found to be effective in the treatment of *Toxoplasma* infections in chickens, rabbits, mice and rats (Sokolov, 1976).

Little information is available concerning treatment of other coccidial infections in intermediate hosts but *Besnoitia* like *Toxoplasma* , is sensitive to pyrimethamine and sulfadiazine (Frenkel, 1973) while amprolium, administered at a daily dose rate of 100 mg/kg, appears to reduce the severity of sarcosporidiosis in experimentally infected calves (Fayer and Johnston, 1975b).

TABLE 1.10 REPORTED "EFFECTIVE" TREATMENTS FOR GENERAL CANINE AND FELINE COCCIDIOSIS

Drug	Host	Dose Rate/Day	Route	Treatment Period (Days)	Reference
Sodium Sulphanilate	Dog and cat	10cc of 1% solution/kg	Enema	1-3	Parkin, 1943
Aureomycin hydrochloride	Dog	28-220 mg/kg	Oral	2-34	Altman, 1951
Nitrofurazone	Dog	9-22 mg/kg	Oral	5-20	Smith and Edmonds, 1959; Duberman, 1960; Rachman and Pollock, 1961
Combined Sulfonamides (Sulfamethazine, Sulfathiazole, Sulfamerazine)	Dog	143 mg/kg	Oral	5-20	Duberman, 1960
Sulfadimethoxine	Dog and cat	26-55 mg/kg	Oral	14	Fish, 1964
Amprolium	Dog	220 mg/kg	Oral	10-12	Smart, 1971
Trimethoprim and Sulfadiazine	Dog and cat	30-60 mg/kg	Oral	6	Durr, 1976

2. THE IDENTITY AND PREVALENCE OF COCCIDIAN PARASITES IN NEW ZEALAND DOGS AND CATS

2.1 INTRODUCTION

Surveys of the prevalence of coccidia in cats and dogs have been reported from many parts of the world (Tables 2.1 and 2.2). However, few have been made since the role of feline and canine coccidia as aetiological agents for such diseases as toxoplasmosis and sarcosporidiosis were discovered and none have been conducted here.

In New Zealand clinical toxoplasmosis appears to be rare in man. Serological evidence, however, indicates a high prevalence of subclinical infection with 50% of adult over 40 years of age showing positive titres (Manning and Reid, 1956). Both *Toxoplasma* and *Sarcocystis* also cause important animal health problems in this country. The former protozoan is recognised as the most commonly diagnosed cause of ovine abortion (Hartley and Boyes, 1964) while the latter is estimated to cost the meat industry about $3 million annually through the trimming, downgrading and condemnation of infected carcases (Collins, 1974).

In view of the economic importance of these infections and the epidemiological importance of the cat and dog in their transmission, the present study was undertaken to provide information concerning the identity, morphology and prevalence of canine and feline coccidia in New Zealand.

2.2 MATERIALS AND METHODS

2.2.1 Faecal Samples:

Over the period November 1974 to August 1976, faecal samples from 508 North Island cats and 481 North Island dogs were obtained from the following sources:

TABLE 2.1. REPORTED PREVALENCES OF COCCIDIAN SPECIES IN CATS

Author	Locality	No. cats examined	Percent Infected					
			I.felis	I.rivolta	Toxoplasma/ Hammondia -like*	T.gondii**	Besnoitia sp.	Sarcocystis sp.
Reinhardt, 1934	Germany	75	2.3	5.3	2.3			
Machulskii and Timofeev 1940	Leningrad	106	5.5	3.7	2.9			
Alves da Cruz et al., 1952	Lisbon	40	10					
Hitchcock, 1953	Michigan	147	75	13	1			
Tomimura, 1957	Osaka	200	9.5	1.5				
Bearup, 1960	Sydney	50	18	16				
Mata and Briecno, 1960	Mexico	11	27					
Dubey, 1966	London	110	9.1	0.9				
Shah, 1970a	Illinois	130	13	3	1.5			
Burrows and Hunt, 1970	New Jersey	757	26.7	16.2	1.5			
Niak, 1972	Liverpool	18	11.1					
Janitschke and Kuhn, 1972	Berlin	520			1.4	1		
Werner and Walton, 1972	Tokyo	90				1.1		
Lodal, 1973	Copenhagen	210	8	7	1			
Vanparijis & Thienpont, 1973	Belgium	500	38.6d					
Wallace, 1973b	Hawaii	1604	18	8	1.4e	0.7	(0.2)a	
Knoch et al., 1974	Berlin	200	1	3	2		(13.5)b	
Pampiglione et al., 1973	Italy	250				0.4		
Dubey, 1973	Kansas	516	17.2	9.5				0.2
Ito et al., 1974	Tokyo	446	8.7	0.4		0.9	(0.7)c	
Gregory and Munday, 1976	Tasmania	55	12.7	5.4				1.8
Christie, et al., 1976	Ohio	1000	6.2	3.2	0.9f	0.7		0.2
Guterbock and Levine, 1977	Illinois	217	23	24	1			

* specific identity undetermined

** confirmed by mouse inoculation

a. Described as an oocyst between 15 -18 µm in length
b. Described as an oocyst between 15 - 20 µm in length
c. Cocysts 14 to 18 by 12 to 15 (16 x 13 µm)
d. I. felis and/or I. rivolta
e. 0.7% proved to be T. gondii, 0.2% H. hammondi, 0.5% unidentified } Dubey et al., 1977a
f. 0.7% proved to be T. gondii, 0.2% H. hammondi.

TABLE 2.2. REPORTED PREVALENCES OF COCCIDIAN SPECIES IN DOGS

Author	Locality	No. Dogs examined	Percent Infected			
			I.canis	I.ohioensis	H. heydorni	Sarcocystis sp.
Gassner, 1940	Colorado	320	6	20	71a	3
Catcott, 1946	Ohio	113	3.5	4.4	2.6	
Choquette and Gelinas, 1950	Montreal	155	9	13.5	1.9	
Ehrenford, 1953	Indiana	377		71.8	0.7	
Nemeseri, 1959	Budapest	220	8.2			
Mimioglu et al., 1960	Turkey	50		2	6	
Laarman, 1962	Netherlands	80				50b
Levine and Ivens, 1965	Illinois	139	16	18	1	3
Burrows and Lillis, 1967	New Jersey	660	13.6	10.6		0.8b
Vanparijis and Thienpont, 1973	Belgium	1832	13c			
Lepp and Todd, 1974	Illinois	308	1.9			
Torres et al., 1974	Chile	59	1.6	3.3	19.6d	
Streitel and Dubey, 1976	Ohio	500	1.8	3.6		1.8

a. "Oocysts" described as 10 to 12 by 8 to 10 µm. The identity of this species is uncertain and Gassner may have been referring to sporocysts (Sarcocystis?) when he wrote about oocysts.

b. "I. hominis -like" i.e. free sporocysts = Sarcocystis?

c. Dogs infected with I. canis and/or "I. bigemina".

d. "I. bigemina", may be either H. heydorni or Sarcocystis sp.

(i) Diagnostic submissions to the Palmerston North Animal Health Laboratory and the Parasitology Section of the Department of Veterinary Pathology and Public Health, Massey University.

(ii) Animal boarding establishments and the S.P.C.A. in Palmerston North.

(iii) Animals presented at the Small Animal Clinic, Massey University.

(iv) The pets of friends and colleagues.

Data relating to the number of samples obtained from each of these sources are presented in Table 2.3, while information concerning the geographical distribution of donor animals is presented in Figures 2.1 and 2.2. Wherever possible information pertaining to the age, sex, and whether living in a town or country environment was obtained for each host.

2.2.2 Detection of coccidia :

Samples were either examined immediately on receipt or after storage at 4°C. One to 2 grams of each faecal sample was suspended in water, broken up with an electric beater and strained through a sieve (aperture 250 µm). The filtrate was placed in a centrifuge tube and centrifuged at 400 g for 5 minutes. The supernatant was discarded, the sediment resuspended in sucrose solution (specific gravity 1.2) and centrifuged once more. Following centrifugation more sucrose solution was carefully added to form a convex meniscus and a coverlsip placed over it. After 1 to 2 minutes the coverslip was removed, placed on a microscope slide and examined at a magnification of x 400.

2.2.3 Sporulation of coccidia :

Samples found to contain unsporulated oocysts were mixed with 2.5%

potassium dichromate and placed in petri dishes to a depth of 0.5cm. These were incubated at 22° - 26°C for 7 days or more until sporulation was completed. The sporulated oocysts were then floated onto a coverslip as previously described, and examined.

2.2.4 Morphological characteristics :

Sporulated oocysts were examined for morphological characteristics and measurements were made using an ocular micrometer at a magnification of x 1000 Photomicrographs were taken using a x 1000 apochromatic oil immersion objective on a Leitz photomicroscope.

2.2.5 Identification of *Toxoplasma* - like oocysts :

Each *Toxoplasma* - like isolate recovered from cat faeces was sporulated and administered orally to three laboratory mice. The mice were killed 30 to 40 days after infection and fresh spreads of brain, diaphragm and abdominal muscle examined for tissue cysts. Samples of muscle and brain were also fixed in 10% formalin and sections examined microscopically for cysts after staining with haematoxylin and eosin. Brains and small portions of skeletal muscle from mice found to contain thin-walled cysts in the spread preparations were macerated in physiological saline, sieved and concentrated. Aliquots of these concentrates were administered orally to another three mice which were examined 30 to 40 days later.

2.2.6 Oocyst and sporocyst counts :

Where sufficient material was available estimates of the numbers per gram of faeces of coccidial oocysts and/or *Sarcocystis* sporocysts were made using the modified McMaster method. For this purpose 1.7g of faeces was diluted in 51ml of saturated aqueous NaCl and the number of coccidia in both chambers of a McMaster slide counted at a magnification of x 200. Each oocyst and/or sporocyst counted represented 100 oocysts and/or sporocysts per gram of faeces.

TABLE 2.3 SOURCES OF FAECAL SAMPLES

Source	Dogs		Cats	
	No.	%	No.	%
"Diagnostic"	333	69.2	47	9.3
Massey Clinic	90	13.7	52	10.2
S.P.C.A.	22	4.6	185	36.4
Animal boarding estabs.	-	-	183	36.0
"Pets"	36	7.5	41	8.1
All Sources	481	100	508	100

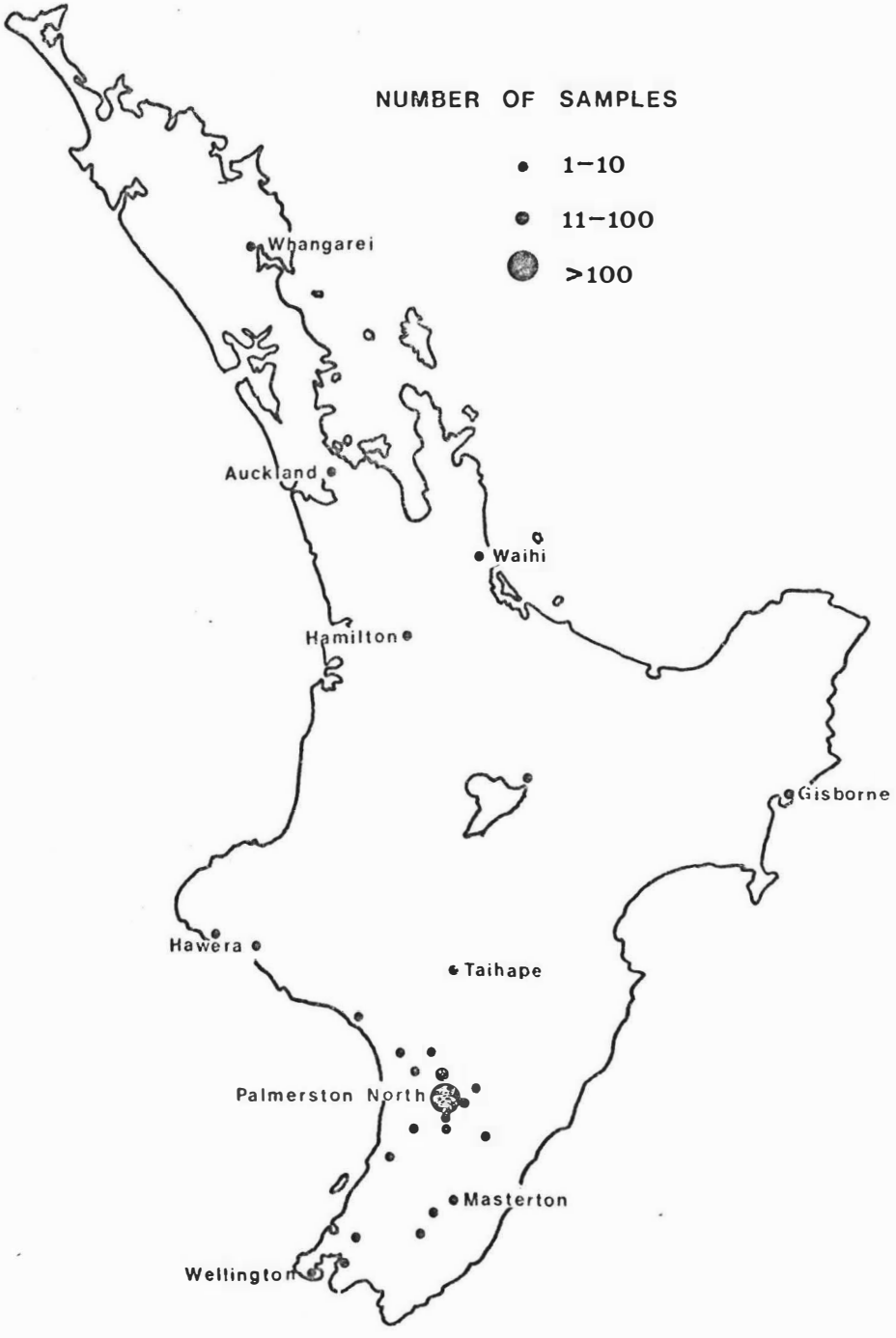


Fig. 2.1 Geographical distribution of sampled cats.

Fig. 2.2 Geographical distribution of sampled dogs.

2.3 RESULTS

Oocysts or sporocysts of four valid feline and four valid canine coccidia as well as several pseudoparasitic forms were found. Descriptions of these coccidia are given in section 2.3.1. In these descriptions all dimensions are given in micrometres while the figures in parenthesis represent the mean ± the standard error.

In order to illustrate the oocyst and sporocyst dimensions of the valid coccidia more clearly their frequency distributions are shown in figures 2.3 to 2.6. In addition, the significance of the differences of the oocyst and sporocyst dimensions between similar canine and feline coccidia were determined using a 2-level nested analysis of variance (Tables 2.4 and 2.5).

Information relating to the prevalence of the valid coccidia is presented in section 2.3.2 Where appropriate all data in this section were examined for statistical significance using the chi-squared test incorporating Yates correction. Contingency tables for these tests are given in Appendices 2 to 17. The use of one, two or three asterisks after the results of an analysis indicates a probability of less than 0.05, 0.01 and 0.001 respectively.

2.3.1 Identity and morphology :

(a) Feline coccidia

(i) *Isospora felis* Wenyon, 1923

Description:

Oocysts ovoid (Plate 2.1A, B). Oocyst wall smooth, colourless 1.6 thick, single layered. A tiny blob adheres to the inside of the wall at the broad end. Micropyles polar granule and oocyst residuum absent. One hundred sporulated oocysts from eight cats

measured 35.8 to 47.3 (41.5 ± 0.2) by 27.7 to 37.4 (31.8 ± 0.2) with length-width ratios of 1.2 to 1.42 (1.30 ± 0.02).

Sporocysts ellipsoidal with smooth, colourless walls. Stieda body absent. Granular residuuum, containing one or more large waxy globules, present. Fifty sporocysts measured 19.5 to 26.0 (23.2 ± 0.2) by 16.3 to 21.0 (18.9 ± 0.1) with length-width ratios of 1.1 to 1.4 (1.23 ± 0.01)

Remarks:

The oocysts described above are morphologically indistinguishable from those described by Shah (1970a).

(ii) *Isospora rivolta* (Grassi, 1879) Wenyon 1923

Description:

Oocysts broadly ovoid to spherical (Plate 2.1 C,D) Oocyst wall smooth, colourless about 0.8 thick, composed of a single layer. Micropyle, polar granule and oocyst residuum absent. One hundred sporulated oocysts from two cats measured 22.8 to 27.7 (25.0 ± 0.1) by 19.5 to 24.4 (22.8 ± 0.1) with length-width ratios of 1.0 to 1.25 (1.10 ± 0.01).

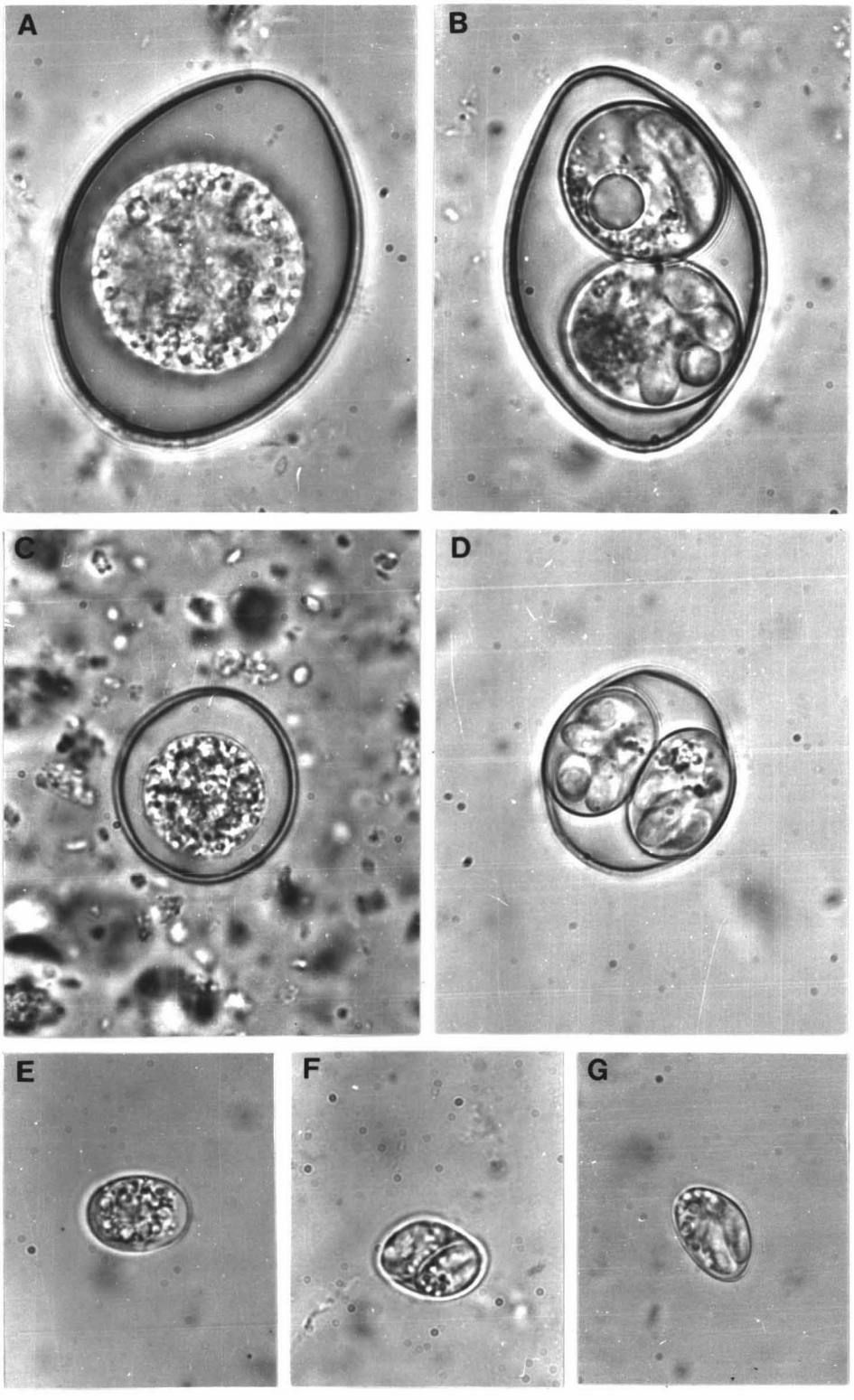
Sporocysts broadly ellipsoidal with smooth, colourless walls. Stieda body absent. Sporocysts residuum present, either compact or as scattered granules. Fifty sporocysts measured 14.7 to 19.5 (16.5 ± 0.2) by 11.4 to 14.7 (13.0 ± 0.1) with length-width ratios of 1.0 to 1.43 (1.27 ± 0.02).

Remarks:

Apart from having slightly larger sporocysts, the oocysts described above are indistinguishable from those described by Shah (1970a),

Plate 2.1 Photomicrographs of the valid coccidia recovered from the faeces of naturally infected cats (x 3000).

A. and B. Unsporulated and sporulated oocysts of *I. felis*
C. and D. Unsporulated and sporulated oocysts of *I. rivolta*
E. and F. Unsporulated and sporulated oocysts of *T. gondii*
G. Free sporocyst of *Sarcocystis* sp.


A
B
C
D
E
F
G

(iii) *Toxoplasma gondii* (Nicolle and Manceaux, 1908) Nicolle and Manceaux, 1909

Description:

Oocysts spherical to subspherical (Plate 2.1 E,F). Oocyst wall smooth, colourless about 0.4 thick, composed of a single layer. Micropyle, polar granule and oocyst residuum absent. One hundred sporulated oocysts from four cats measured 9.8 to 14.7 (12.2 ± 0.1) by 9.8 to 13.0 (10.4 ± 0.1) with length-width ratios of 1.0 to 1.5 (1.17 ± 0.01)

Sporocysts broadly ellipsoidal with smooth, colourless walls. Stieda body absent. Scattered sporocyst residuum present. Fifty sporocysts measured 6.5 to 8.2 (7.9 ± 0.1) by 4.9 to 6.5 (6.0 ± 0.1) with length-width ratios of 1.0 to 1.7 (1.34 ± 0.03).

Remarks:

The oocysts described above are indistinguishable from those described by Dubey *et al.*, (1970a) and their identity was confirmed by infecting mice. Isolates recovered in the present study appeared to be of low virulence for mice and no deaths were recorded. However, this may have been due to the small numbers of oocysts available for administration.

(iv) *Sarcocystis* sp.

Description:

Free sporocysts, asymmetrically ellipsoidal to ellipsoidal (Plate 2.1G). Sporocyst wall smooth, colourless, without a stieda body. Residuum present either as a compact mass at one end of sporocyst or as scattered granules. One hundred sporocysts from fifteen cats measured 11.4 to 14.7 (13.3 ± 0.1) by 8.2 to 9.8 (8.4 ± 0.1) with length-width ratios of 1.33 to 1.8 (1.59 ± 0.01).

Remarks:

The mean dimensions of these sporocysts most nearly approximate to those of *S. porcifelis* of the pig (Table 1.3). It was later found however, that at least some of these sporocysts were of *S. muris* (Chapter 3) and it is likely that they represent those of a number of other species as well (see section 2.4).

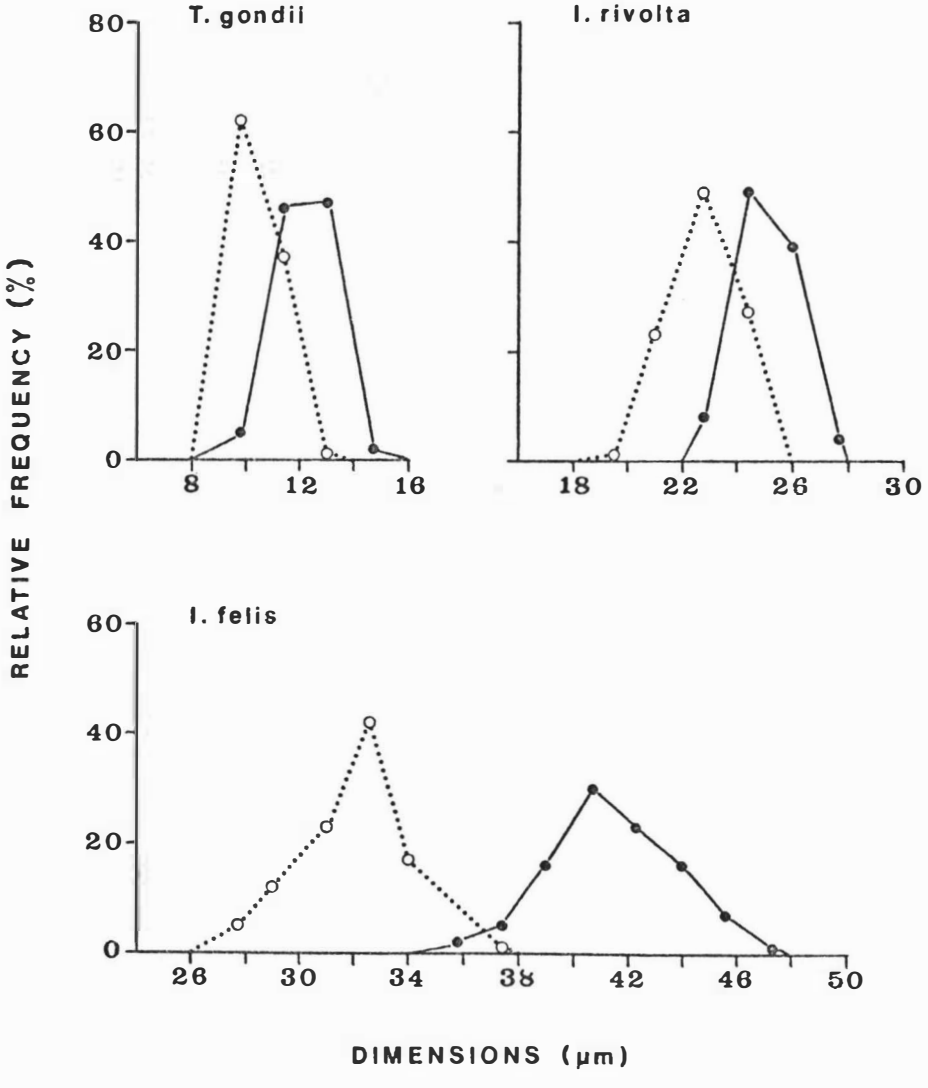


Fig. 2.3 Frequency distribution of oocyst lengths (solid lines) and widths (broken lines) of feline coccidia (n = 100 in all cases).

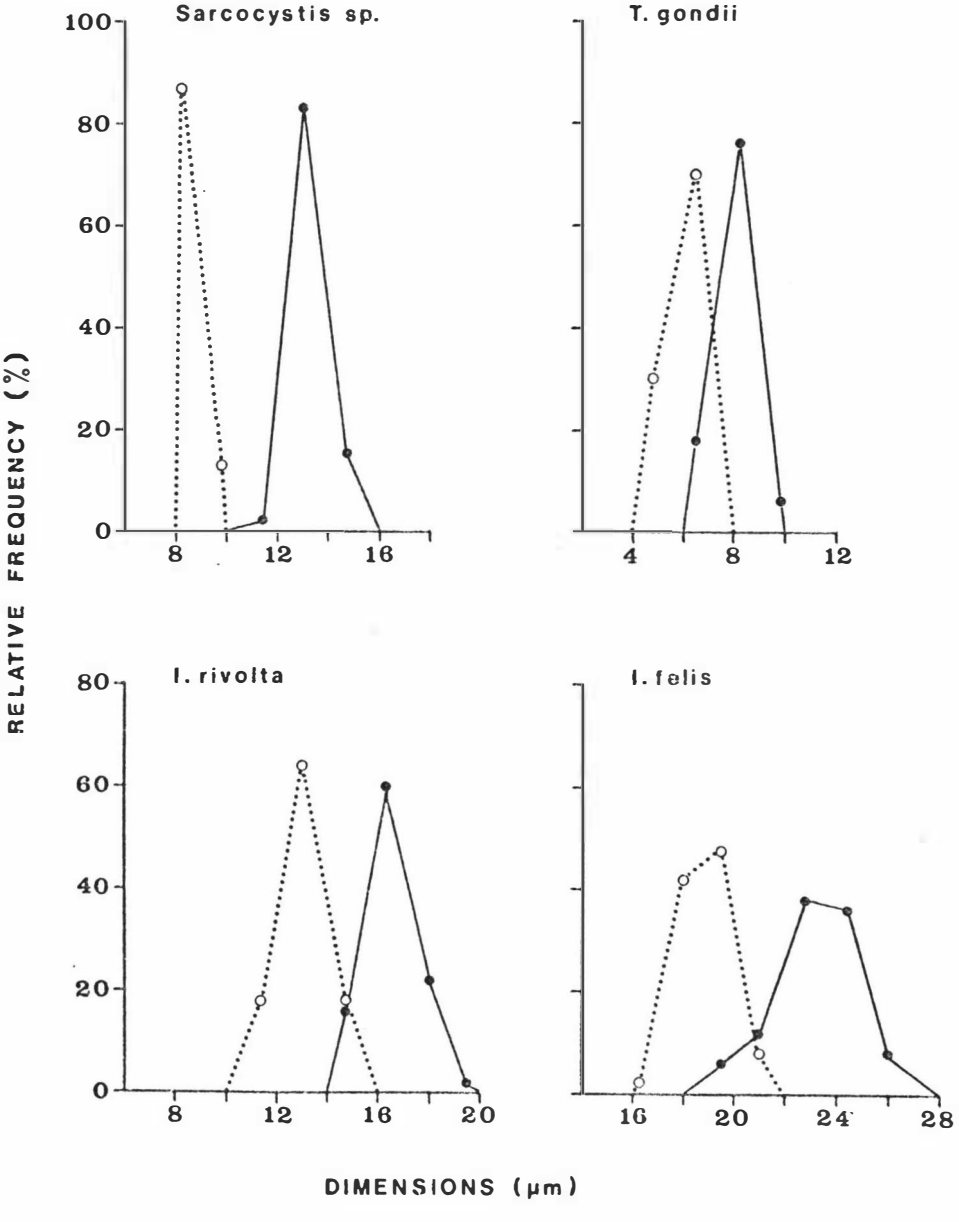


Fig. 2.4 Frequency distributions of sporocyst lengths (solid lines) and widths (broken lines) of feline coccidia (n = 50 in all cases except for *Sarcocystis* sp. where n = 100).

(b) Canine coccidia

(i) *Isospora canis* Nemeseri, 1959

Description:

Oocysts broadly ellipsoidal to ovoid (Plate 2.2. A,B). Oocyst wall single-layered, smooth, colourless, about 1.6 thick. A tiny blob adheres to the inside of the oocyst wall at the broad end. Micropyle, polar granule and oocyst residuum absent. One hundred sporulated oocysts from six dogs measured 37.4 to 45.6 (40.2 ± 0.2) by 31.0 to 35.8 (33.0 ± 0.1) with length-width ratios of 1.14 to 1.47 (1.22 ± 0.01).

Sporocysts ellipsoidal with smooth, colourless walls. Stieda body absent. Granular residuum, often containing one or more large waxy globules, present. Fifty sporocysts measured 19.5 to 24.4 (22.8 ± 0.2) by 16.3 to 19.5 (17.1 ± 0.1) with length-width ratios of 1.17 to 1.50 (1.34 ± 0.01).

Remarks:

The oocysts described above are indistinguishable from those described by Levin and Ivens (1965) although they are slightly larger. *I. canis* is also very similar to *I. felis* of the cat. However, the oocysts of the former species differs from those of the latter by their significantly greater mean widths and shorter mean lengths (Table 2.4).

(ii) *Isospora ohioensis* Dubey, 1975

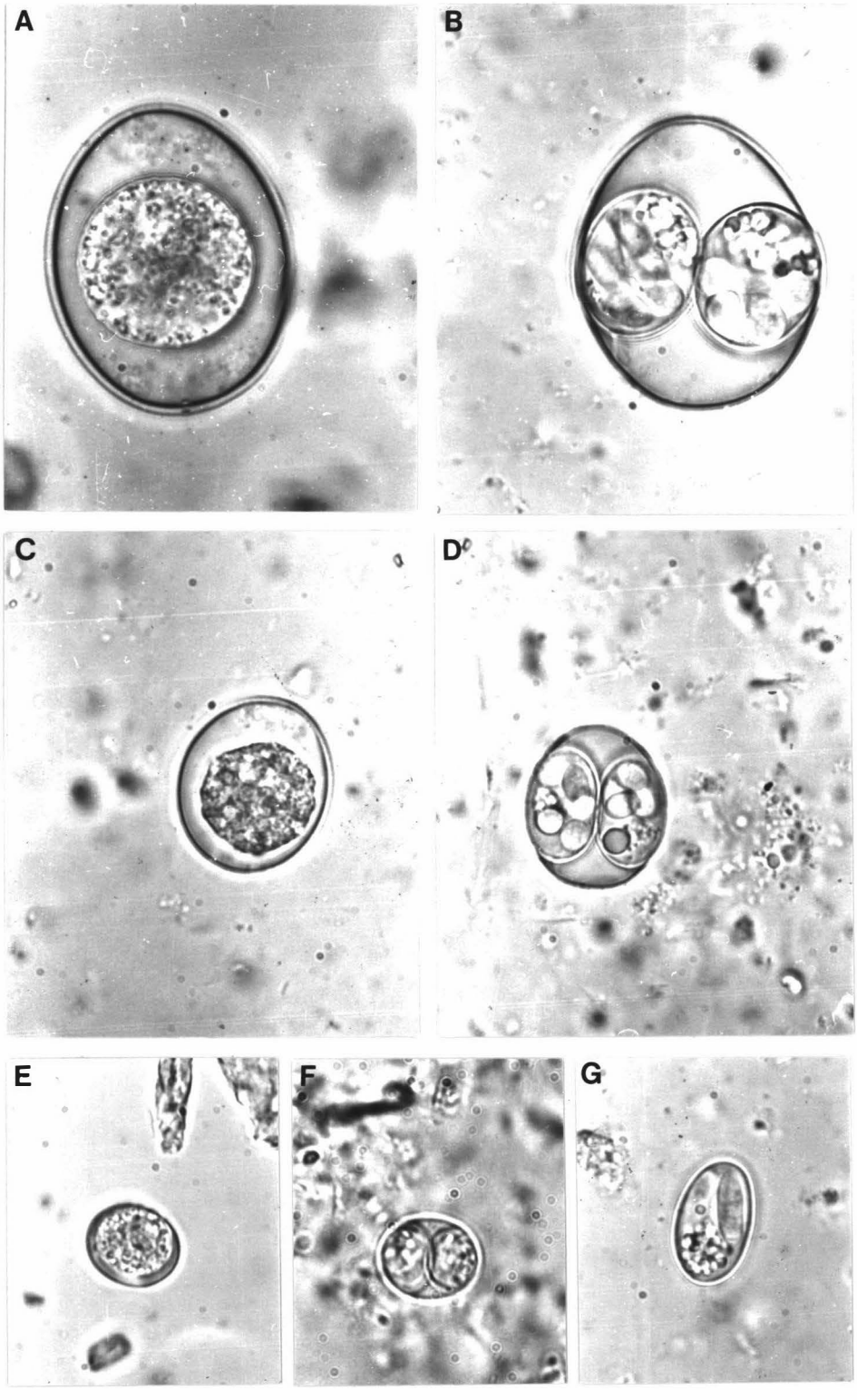
Description:

Oocysts broadly ovoid to spherical (Plate 2.2. C,D). Oocyst wall smooth colourless, about 0.8 thick, composed of a single layer. Micropyle, polar granule and oocyst residuum absent. One hundred oocysts from six dogs measured 21.0 to 27.7 (24.9 ± 0.2) by 18.0 to 24.4 (21.5 ± 0.2) with length-width ratios of 1.0 to 1.33 (1.16 ± 0.01).

Sporocysts ellipsoidal with smooth colourless walls. Stieda body absent. Granular sporocyst residuum present. Fifty sporocysts measured 13.0 to 16.3 (15.7 ± 0.1) by 9.8 to 11.4 (10.8 ± 0.1) with length-width ratios of 1.14 to 1.67 (1.47 ± 0.02).

Plate 2.2 Photomicrographs of the valid coccidia recovered from the faeces of naturally infected dogs (x 3000).

A. and B. Unsporulated and sporulated oocysts of *I. canis*. C. and D. Unsporulated and sporulated oocysts of *I. ohioensis*. E. and F. Unsporulated and sporulated oocysts of *H. heydorni*. G. Free sporocyst of *Sarcocystis* sp.


A
B
C
D
E
F
G

Remarks:

These oocysts are indistinguishable from those described as *I. ohioensis* from the dog (Dubey, 1975). *I. ohioensis* is also very similar to *I. rivolta* from the cat. *I. rivolta* , however, has statistically significant greater mean oocyst and sporocyst widths than *I. ohioensis* (Tables 2.4 and 2.5).

(iii) *Hammondia heydorni* (Tadros and Laarman, 1976) Dubey, 1977

Description:

Oocyst sperical to subspherical (Plate 2.2. E,F). Oocyst wall smooth, colourless, about 0.4 thick, composed of a single layer. Micropyle, polar granule and occyst residuum absent. One hundred oocysts from four dogs measured 9.8 to 13.0 (11.6 ± 0.1) by 9.8 to 11.4 (10.2 ± 0.1) with length-width ratios of 1.0 to 1.33 (1.14 ± 0.01).

Sporocysts broadly ellipsoidal with smooth colourless walls. Stieda body absent. Scattered sporocyst residuum present. Fifty sporocysts measured 6.5 to 8.2 (7.9 ± 0.1) by 4.9 to 6.5 (6.3 ± 0.1) with length-width ratios of 1.0 to 1.67 (1.29 ± 0.02).

Remarks:

Except for very minor differences in size, the oocysts described above are identical to those described by Heydorn (1973). *H. heydorni* is also almost identical to the feline coccidian *T. gondii*. The latter species, however has a satistically significant greater mean oocyst length than *H. heydorni* (Table 2.4).

(Iv) *Sarcocystis* sp.

Description:

Free sporocysts, ellipsoidal in shape (Plate 2.2G). Sporocyst wall smooth, colourless, without stieda body. Sporocyst residuum present either as compact mass at one end or as scattered granules. One hundred sporocysts from 18 dogs measured 14.7 to 18.0 (16.2 ± 0.1) by 8.2 to 11.4 (10.3 ± 0.1) with length-width ratios of 1.29 to 1.84 (1.59 ± 0.01).

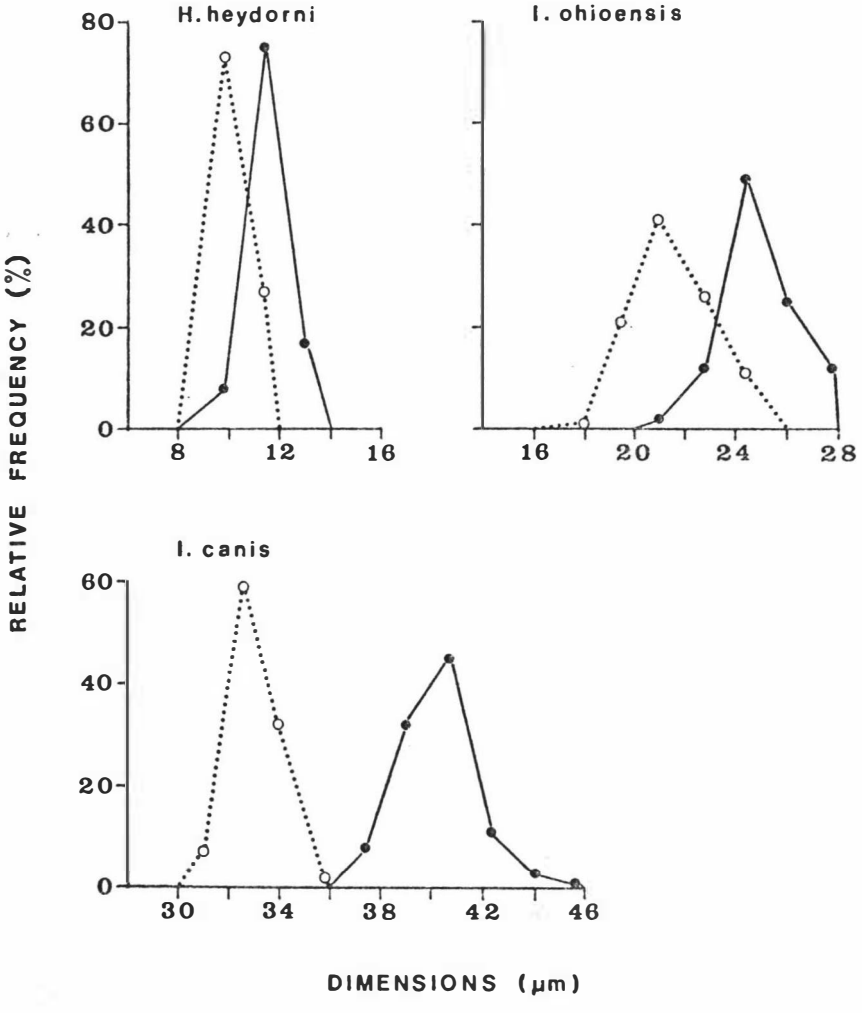


Fig. 2.5 Frequency distributions of oocyst lengths (solid lines) and widths (broken lines) of canine coccidia (n = 100 in all cases).

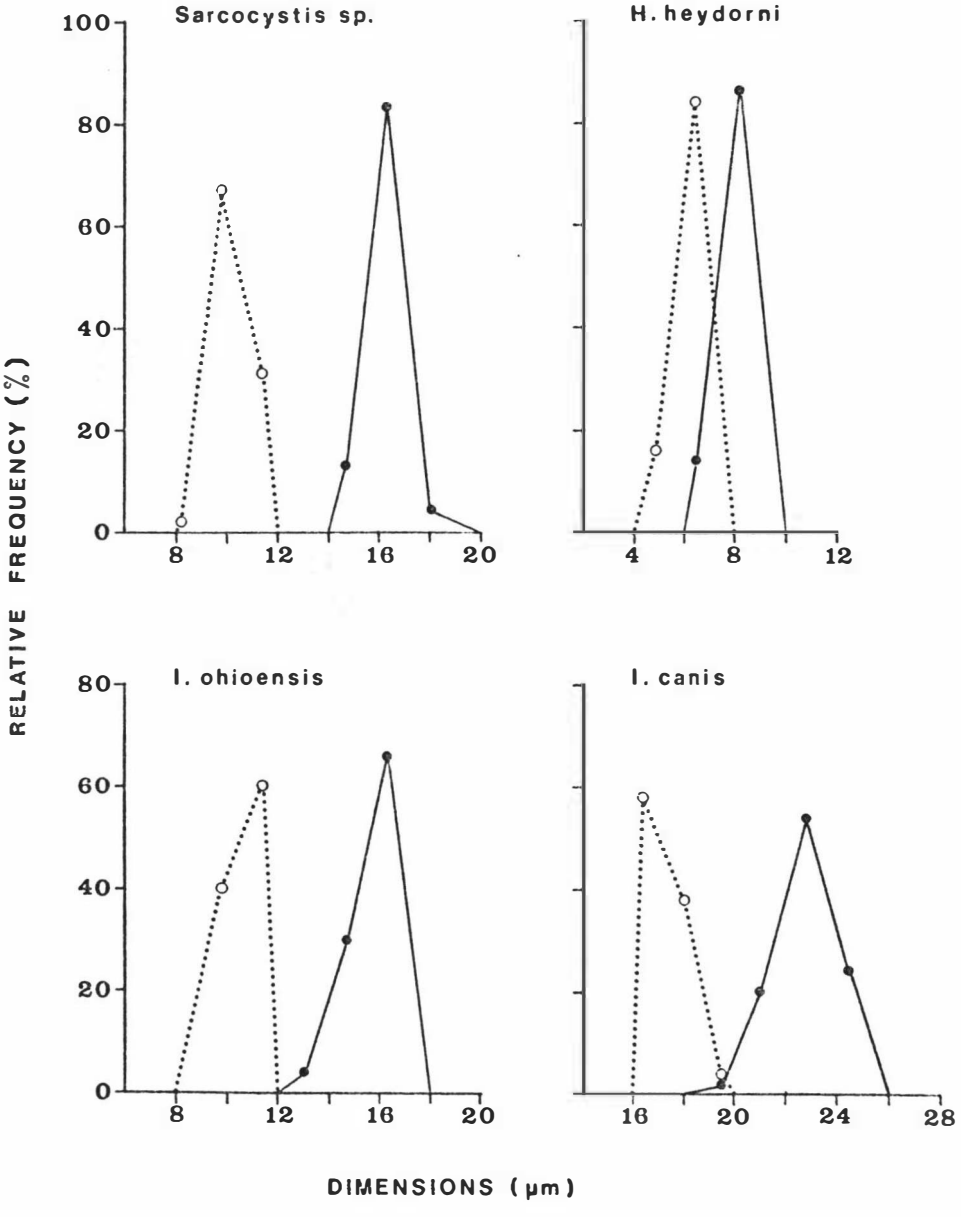


Fig. 2.6 Frequency distributions of sporocyst lengths (solid lines) and widths (broken lines) of canine coccidia (n = 50 in all cases except for *Sarcocystis* sp. where n = 100).

Remarks:

The above sporocysts have statistically significant greater mean lengths and widths than those recovered from cat faeces (Table 2.5) and most nearly approximate to the dimensions of those of *S. cruzi*. As in cats, however, it is likely that these sporocysts represent those of several species of *Sarcocystis* (see section 2.4).

(c) Pseudoparasitic coccidia of cats and dogs

(i) *Klossia* sp?

Description:

Oocysts spherical to subspherical (Plate 2.3A). Oocyst wall smooth, colourless, about 1.6 thick. Micropyle polar granule and oocyst residuum absent. Two oocysts measured 37.4 to 40.7 by 34.0. One oocyst appeared to contain 8 sporocysts and the other 12, although they were difficult to count.

Sporocysts smooth-walled, spherical to subspherical in shape. Two sporocysts measured 14.7 and 13.0 in diameter and contained 4 sporozoites and a coarse residuum. Stieda body absent.

Remarks:

Found in one (0.2%) of cats examined. These coccidia are tentatively identified as members of the genus *Klossia* (Sporozoa: Adeleidae) on the basis of the apparently variable number and tetrazoic nature of their sporocysts. Members of this genus are parasites of invertebrates, including land snails of the genus *Helix*. They have also occasionally been recovered from the faeces of rodents (Levine *et al.*, 1955; Mullins and Colley, 1972), and more recently, foxes (Golemansky, 1975b). Like the present record these latter findings are considered to be spurious infections.

(ii) *Eimeria (perforans?)*

Description:

Oocysts ellipsoidal with smooth walls (Plate 2.3B). Micropyle and polar granule absent. Oocyst residuum present. Ten oocysts measured 22.8 to 29.3 (25.7 ± 0.7) by 14.7 to 19.5 (16.6 ± 0.5) with length-width ratios of 1.42 to 1.70 (1.55 ± 0.03).

Sporocysts ellipsoidal. Stieda body and fine granular residuum present. Six sporocysts measured 13.04 to 16.3 (14.1 ± 0.5) by 6.5 to 8.2 (7.1 ± 0.3) with length-width ratios of 1.8 to 2.3 (2.0 ± 0.1).

Remarks:

Found in one (0.2%) of cats examined. Apart from minor differences in the size of the sporocysts this coccidian closely resembles *E. perforans* which has been found to be very common in New Zealand rabbits (Bull, 1953). The above oocysts were fed to a 12 week-old coccidia-free kitten. Daily faecal examination for 24 days, however, failed to detect any oocyst excretion.

(iii) *Isospora (lacazei?)*

Description:

Oocysts spherical to subspherical (Plate 2.3C). Oocyst wall smooth, thick without micropyle. Residuum absent, polar granules frequently present. Five oocysts measured 21.0 to 27.7 (25.0 ± 1.1.) by 21.0 to 26.0 (24.0 ± 0.8) with length-width ratios of 1.0 to 1.07 (1.04 ± 0.02).

Sporocysts lemon-shaped with button-like stieda body and finely granular residuum. Residuum and sporozoites enclosed in a membrane. Five sporocysts measured 16.3 to 19.5 (17.9 ± 0.7) by 9.8 to 11.4 (10.8 ± 0.4) with length-width ratios of 1.58 to 1.71 (1.67 ± 0.02).

Remarks:

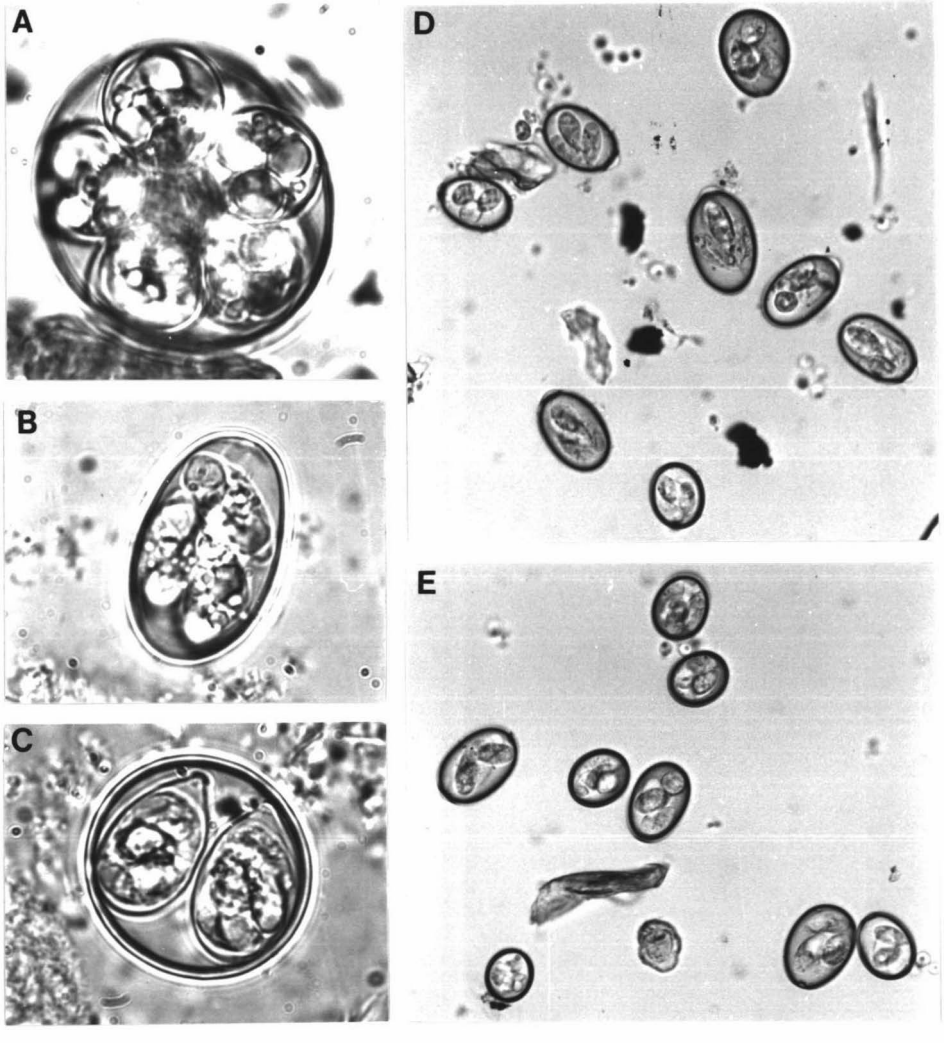
Found in one (0.2%) of cats and ten (2.1%) of dogs examined. These oocysts very cosely resemble those of *I. lacazei* from the house sparrow *(Passer domesticus)*. It is likely that the observed 'infections' resulted from the ingestion of food contaminated with sparrow droppings.

(iv) *Eimeria* spp

Infections of mixed *Eimeria* species, often in a sporulated state, were found in 47 (9.8%) of dogs examined (Plate 2.3D,E). Infections were more common in country dogs (23.7% infected) than town dogs (4.1% infected). These oocysts were identified as sheep coccidia with almost all the species reported to occur in New Zealand (McKenna, 1972) being recovered at one time or another. They were probably acquired by

Plate 2.3 Photomicrographs of the sporulated oocysts of pseudoparasitic coccidia recovered from the faeces of naturally infected cats and dogs (x 3000).

A. *Klossia* sp? (cat). B. *Eimeria (perforans?)* (cat).
C. *Isospora (lacazei?)* (cat and dog).
D and E. Mixed *Eimeria* sp. (dog).


A
B
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E

TABLE 2.4 RESULTS OF TWO-LEVEL NESTED ANALYSES OF VARIANCE TO DETERMINE THE SIGNIFICANCE OF THE DIFFERENCES IN OOCYST DIMENSIONS BETWEEN SIMILAR DOG AND CAT COCCIDIA

Species	Measurement	Source of Variation	df	SS	MS	F
I. canis / I felis	Length	Between species	1	78.70	78.70	5.52*
		Within species	12	171.20	14.27	4.39***
		Error	186	604.48	3.25	
		Total	199	854.38		
	Width	Between species	1	67.17	67.17	5.65*
		Within species	12	142.73	11.89	8.03***
		Error	186	275.59	1.48	
		Total	199	485.49		
I. ohioensis / I. rivolta	Length	Between species	1	0.43	0.43	0.22 ns
		Within species	6	11.88	1.98	1.14 ns
		Error	192	332.25	1.73	
		Total	199	344.65		
	Width	Between species	1	82.95	82.95	8.05*
		Within species	6	61.88	10.31	5.89***
		Error	192	335.57	1.75	
		Total	199	480.40		
H.heydorni/T.gondii	Length	Between species	1	18.19	18.19	25.26**
		Within species	6	4.30	0.72	0.85 ns
		Error	192	161.16	0.85	
		Total	199	185.65		
	Width	Between species	1	1.91	1.91	0.78 ns
		Within species	6	14.73	2.46	4.45***
		Error	192	106.16	0.55	
		Total	199	122.80		

F.05 (1,12) = 4.75 F.001 (12,∞) = 2.74 F.05 (6,200) = 2.14

F.05 (1, 6) = 5.99 F.001 (6,∞) = 4.04 F.01 (1, 6) = 13.74

TABLE 2.5 RESULTS OF TWO-LEVEL NESTED ANALYSES OF VARIANCE TO DETERMINE THE SIGNIFICANCE OF DIFFERENCES IN SPOROCYST DIMENSIONS BETWEEN SIMILAR DOG AND CAT COCCIDIA

Species	Measurement	Source of Variation	df	SS	MS	F
I. canis/*I. felis*	Length	Between species	1	5.30	5.30	0.62 ns
		Within species	4	34.34	8.59	4.75**
		Error	94	169.99	1.81	
		Total	99	209.63		
	Width	Between species	1	85.74	85.74	49.28**
		Within species	4	6.97	1.74	1.85 ns
		Error	94	88.45	0.94	
		Total	99	181.16		
I. ohioensis/ *I. rivolta*	Length	Between species	1	15.87	15.87	9.02 ns
		Within species	3	5.28	1.76	1.62 ns
		Error	95	98.36	1.04	
		Total	99	119.51		
	Width	Between species	1	130.19	130.19	67.11**
		Within species	3	5.81	1.94	2.49 ns
		Error	95	73.89	0.78	
		Total	99	209.89		
H. heydorni/*T.gondii*	Length	Between species	1	0.03	0.03	0.04 ns
		Within species	2	1.5	0.75	1.63 ns
		Error	96	44.46	0.46	
		Total	99	45.99		
	Width	Between species	1	1.3	1.3	6.19 ns
		Within species	2	0.42	0.21	0.45 ns
		Error	96	45.33	0.47	
		Total	99	47.05		
Sarcocystis sp. (dog)/ *Sarcocystis* sp. (cat)	Length	Between species	1	421.72	421.72	593.97***
		Within species	31	22.09	0.71	1.92**
		Error	167	62.62	0.37	
		Total	199	506.43		
	Width	Between species	1	178.76	178.76	212.81***
		Within species	31	26.10	0.84	2.05**
		Error	167	69.28	0.41	
		Total	199	274.14		

F.05 (1,4) = 7.71 F.01 (4,100) = 3.51 F.01 (1,4) = 21.20 F.05 (4,100) = 2.46
F.05 (1,3) = 10.13 F.05 (3,100) = 2.70 F.01 (1,3) = 34.12 F.05 (1,2) =18.51
F.05 (2,100) = 3.09 F.001 (1,30) =13.29 F.01 (30,150)= 1.83

coprophagy. Support for this contention was provided by the frequent concurrent presence of eggs of the trichostronglid nematode *Nematodirus* and the cestode *Moniezia*.

2.3.2 Prevalence:

a) General prevalence

Examination of 508 feline and 481 canine faecal samples revealed that 155 (30.5%) and 307 (63.8%) respectively, contained valid coccidial species. Data relating to the prevalence of each of these cocidia are presented in Table 2.6 while information concerning the number and composition of coccidian infections identified in individual faecal samples are recorded in Tables 2.7 and 2.8. In both hosts the majority of infected samples contained only a single coccidian with *I. felis* in cats and *Sarcocystis* sp. in dogs being the most commonly encountered.

b) Age prevalence

(i) Cats :

There was a tendency for all species to show a decreasing prevalence with increasing host age although some differences between species were apparent. (Table 2.9) *Toxoplasma* infections were not found in any cat over 6 months of age while *Sarcocystis* sp. tended to be most common in the > 3 months to 12 months age groups. A sharp decline in the occurrence of the latter species in animals > 2 years of age was observed. With the exception of *I. rivolta* all coccidia were significantly more common in 'kittens' than in 'adults' (Table 2.10).

(ii) Dogs :

As in cats there was a general tendency for the prevalence of coccidial infections in dogs to decrease with age (Table 2.11). All coccidians were more common in 'puppies' than 'adults' but only for *I. canis* and *I. ohioensis*, however, were these differences statistically significant.

TABLE 2.6 PREVALENCE OF COCCIDIA IN CANINE AND FELINE FAECAL SAMPLES

Host	No. Examined	Species	No. infected	% Infected
Cat	508	*I. felis*	89	17.5
		I. rivolta	11	2.2
		T. gondii	5	0.98
		Sarcocystis sp	86	16.9
Dog	481	*I. canis*	19	4.0
		I. ohioensis	44	9.2
		H. heydorni	13	2.7
		Sarcocystis sp.	283	58.8

TABLE 2.7

NUMBER AND COMPOSITION OF COCCIDIAL INFECTIONS OCCURRING IN 508 INDIVIDUAL FELINE FAECAL SAMPLES

No. of species present	No. of samples	% of total +ve samples	Species composition	No.	%
0	353	-	-	-	-
1	121	78.1	*I. felis*	59	48.8
			Sarcocystis sp.	59	48.8
			I. rivolta	3	2.4
2	32	20.6	*I. felis* + *Sarcocystis* sp.	21	65.6
			I. felis + *I. rivolta*	4	12.5
			I. felis + *T. gondii*	3	9.4
			I. rivolta + *Sarcocystis* sp.	2	6.2
			T. gondii + *Sarcocystis* sp.	2	6.2
3	2	1.3	*I. felis* + *I. rivolta* + *Sarcocystis* sp	2	100
4	0	-	-	-	-

TABLE 2.8

NUMBER AND COMPOSITION OF COCCIDIAL INFECTIONS OCCURRING IN 481 INDIVIDUAL CANINE FAECAL SAMPLES

No. of species present	No. of samples	% of total +ve samples	Species composition	No.	%
0	174	-	-	-	-
1	268	87.3	*Sarcocystis* sp.	248	92.5
			I. ohioensis	14	5.2
			H. heydorni	4	1.5
			I. canis	2	0.8
2	26	8.5	*Sarcocystis* sp. + *I. ohioensis*	13	50.0
			Sarcocystis sp. + *H. heydorni*	7	26.9
			I. canis + *I. ohioensis*	4	15.4
			Sarcocystis sp + *I. canis*	2	7.7
3	13	4.2	*Sarcocystis* sp + *I. canis* + *I. ohioensis*	11	84.6
			Sarcocystis sp. + *I. ohioensis* + *H. heydorni*	2	15.4
4	0	-	-	-	-

Sarcocystis infections appeared to be less influenced by host age in dogs than cats; infection with this coccidian in dogs tended to be most common in the > 2 months to 2 years age groups with prevalence declining less sharply in animals beyond this age in dogs than in cats.

c) Sex prevalence

No significant differences in the prevalence of any coccidian species between male and female cats (Table 2.13) or male and female dogs (Table 2.14) were found.

d) Town and country prevalence

(i) Cats :

Data relating to the prevalence of coccidia species in town and country cats are presented in Table 2.15. *I. felis* and *I. rivolta* were more common in country cats than town cats while *T. gondii* and *Sarcocystis* sp. were more common in town cats than country cats. With the exception of *I. felis* , however, no statistically significant differences could be demonstrated. The population of country cats examined was small and contained a greater proportion of 'kittens' (68%) than did the town cat population (32.8%). It was suspected, therefore, that the apparently significantly higher prevalence of *I. felis* infection in country cats may merely have been a result of this. The significantly higher prevalence of this species in country 'kittens' than town 'kittens' (Table 2.16), however, suggested this was not the case.

(ii) Dogs :

I. canis and *I. ohioensis* were more common in town dogs than country dogs while *H. heydorni* and *Sarcocystis* sp. were more common in country dogs than town dogs (Table 2.17). With the exception of *I. canis* these differences were not statistically significant. A comparison between the prevalence of this species in town 'puppies' and country

TABLE 2.9 PREVALENCE OF COCCIDIA IN CATS ACCORDING TO AGE

Age of cats	No. examined	No. infected (%)			
		I. felis	I. rivolta	T. gondii	Sarcocystis sp.
< 2 mths	51	23(45.1)	3(5.9)	1(2.0)	10(19.6)
2-3 mths	68	31(45.6)	1(1.5)	3(4.4)	15(22.1)
> 3-6 mths	52	15(28.8)	3(5.8)	1(1.9)	16(30.8)
> 6-12 mths	59	7(11.9)	3(5.1)	-	19(32.2)
>12mths-2 yrs	48	4(8.3)	-	-	11(22.9)
> 2-5 yrs	98	5(6.1)	1(1.0)	-	4(4.1)
> 5-10 yrs	82	3(3.7)	-	-	3(3.7)
>10 yrs	37	-	-	-	3(8.1)
Unknown	13	-	-	-	5
Total	508	89(17.5)	11(2.2)	5(0.98)	86(16.9)

TABLE 2.10 PREVALENCE OF COCCIDIA IN 'KITTEN' (UP TO 6 MONTHS OF AGE) AND 'ADULT' (OVER 6 MONTHS OF AGE) CATS

Age	No. examined	No. infected (%)			
		I. felis	I. rivolta	T. gondii	Sarcocystis sp.
'Kitten'	171	69(40.4)***	7(4.1)ns	5(2.9)**	41(24.0)**
'Adult'	324	20(6.2)	4(1.2)	-	40(12.3)
Unknown	13	-	-	-	5
Total	508	89	11	5	283

(Asterisks indicate significant differences, ns = not significant; read vertically).

TABLE 2.11 PREVALANCE OF COCCIDIA IN DOGS ACCORDING TO AGE

Age of Dogs	No. examined	No. infected (%)			
		I. canis	I. ohioensis	H. heydorni	Sarcocystis sp.
< 2 mths	28	7(25.0)	21(75.0)	2(7.1)	13(46.4)
2-3 mths	38	5(13.2)	8(21.1)	4(10.5)	23(60.5)
> 3-6 mths	41	6(14.6)	7(17.1)	-	27(65.9)
> 6-12 mths	69	-	4(5.8)	5(7.3)	46(66.7)
>12mths-2 yrs	100	-	-	-	66(66.0)
> 2-5 yrs	95	1(1.1)	2(2.1)	1(1.1)	49(51.6)
> 5-10 yrs	58	-	-	1(1.7)	28(48.3)
>10 yrs	11	-	-	-	3(27.3)
Unknown	41	-	2	-	28
Total	481	19(4.0)	44(9.2)	13(2.7)	283(58.8)

TABLE 2.12 PREVALENCE OF COCCIDIA IN 'PUPPY' (UP TO SIX MONTHS OF AGE) AND 'ADULT' (OVER SIX MONTHS OF AGE) DOGS

Age	No. examined	No. infected (%)			
		I. canis	I. ohioensis	H. heydorni	Sarcocystis sp.
'Puppy'	107	18(16.8)***	36(33.6)***	6(5.6)ns	63(58.9)ns
'Adult'	333	1(0.3)	6(1.8)	7(2.1)	192(57.7)
Unknown	41	-	2(4.9)	-	28
Total	481	19	44	13	283

(Asterisks indicate significant differences, ns = not significant, read vertically).

TABLE 2.13 PREVALENCE OF COCCIDIA IN CATS ACCORDING TO SEX

Sex	No. examined	No. infected (%)			
		I. felis	I. rivolta	T. gondii	Sarcocystis sp.
Male	152	31(20.4)ns	5(3.3)ns	1(0.7)ns	35(23.0)ns
Female	182	30(16.5)	6(3.3)	4(2.2)	38(20.9)
Unknown	174	28(16.1)	-	-	13(7.5)
Total	508	89	11	5	86

TABLE 2.14 PREVALENCE OF COCCIDIA IN DOGS ACCORDING TO SEX

Sex	No. examined	No. infected (%)			
		I. canis	I. ohioensis	H. heydorni	Sarcocystis sp.
Male	233	2(0.9)ns	7(3.0)ns	7(3.0)ns	135(57.9)ns
Female	178	2(1.1)	10(5.6)	5(2.8)	104(58.4)
Unknown	70	15	27	1	44
Total	481	19	44	13	283

(ns = not significant, read vertically).

TABLE 2.15 PREVALENCE OF COCCIDIA IN TOWN AND COUNTRY CATS

	No. examined	No. infected (%)			
		I. felis	I. rivolta	T. gondii	Sarcocystis sp.
Town	483	75(15.5)***	10(2.1)†	5(1.0)†	82(17.0) ns
Country	25	14(56.0)	1(4.0)	-	4(16.0)
Total	508	89	11	5	86

TABLE 2.16 PREVALENCE OF COCCIDIA IN 'KITTEN' (UP TO 6 MONTHS OF AGE) AND 'ADULT' (OVER 6 MONTHS OF AGE) TOWN AND COUNTRY CATS

	No. examined	No. infected (%)			
		I felis	I. rivolta	T. gondii	Sarcocystis sp.
Town 'Kittens'	154	55(35.7)***	6(3.9)†	5(3.2)†	39(25.3) ns
Country	17	14(82.4)	1(5.9)	-	2(11.8)
Town 'Adults'	316	20(6.3)†	4(1.3)†	- †	38(12.0) ns
Country	8	-	-	-	2(25.0)
Unknown age	13	-	-	-	5
Total	508	89	11	5	86

(Asterisks indicate significant differences, ns = not significant, † = insufficient numbers for statistical analysis. Read vertically).

TABLE 2.17 PREVALENCE OF COCCIDIA IN TOWN AND COUNTRY DOGS

	No. examined	No. infected (%)			
		I. canis	I. ohioensis	H. heydorni	Sarcocystis sp.
Town	342	18(5.3)*	36(10.5)ns	8(2.3)ns	196(57.3)ns
Country	139	1(0.7)	8(5.8)	5(3.6)	87(62.6)
Total	481	19	44	13	283

TABLE 2.18 PREVALENCE OF COCCIDIA IN 'PUPPY' (UP TO 6 MONTHS OF AGE) AND 'ADULT' (OVER 6 MONTHS OF AGE) TOWN AND COUNTRY DOGS

	No. examined	No. infected (%)			
		I. canis	I. ohioensis	H. heydorni	Sarcocystis sp.
Town 'Puppies'	89	18(20.2)ns	31(34.8)ns	5(5.6) ns	48(53.9)*
Country	18	-	5(62.5)	1(5.6)	15(83.3)
Town 'Adults'	224	- †	4(1.8) ns	3(1.3) ns	130(58.0) ns
Country	109	1(0.9)	2(1.8)	4(3.7)	62(56.9)
Unknown age	41	-	2	-	28
Total	481	19	44	13	283

(Asterisks indicate significant differences, ns = not significant, † = insufficient numbers for statistical analysis. Read vertically).

'puppies' showed no significant difference between the two (Table 2.18). This suggests, therefore, that the difference in prevalence of *I. canis* in town and country dogs was attributable to the greater proportion of 'puppies' in the former population (28.4%) than the latter (14.2%). *Sarcocystis* sp. were found to be significantly more common in country 'pups' than town 'pups' although significant differences were not found between 'adult' dogs from these two populations.

e) Seasonal prevalence

Data relating to the seasonal prevalence of coccidia in cats and dogs are presented in Figures 2.7 and 2.8. For the purposes of this study Spring was considered to include the months of September, October, November; Summer: - December, January, February; Autumn:- March, April, May and Winter:- June, July and August.

(i) Cats :

Although all four coccidians were more common in the Autumn-Winter period than in the Spring-Summer season (Table 2.19) only for two of them, *I. felis* and *Sarcocystis* sp. was there a statistically significant association between season and prevalence.

Cats examined during Autumn and Winter contained a greater proportion of 'kittens' (72.3% and 57.5%) than did those examined during Spring (12.5%) and Summer (19.5%). It was suspected, therefore, that the apparently significant association between season and prevalence for *I. felis* and *Sarcocystis Sp.* may have been due to this fact. Accordingly, tests to determine the significance of the association between season and prevalence in both 'kitten' and 'adult' cats were undertaken. Results of these tests, which are summarised in Table 2.20, revealed a significant association between season and prevalence in 'kitten' and 'adult' cats for *I. felis* and in 'adult' cats for *Sarcocystis* sp. and *I. rivolta*.

(ii) Dogs :

In contrast to cats, all species of coccidia, apart from *Sarcocystis* sp., were more common in the Spring-Summer period than in the Autumn-Winter season (Table 2.21). However, only for *I. ohioensis* and *Sarcocystis* sp. was a statistically significant association between season and prevalence demonstrated. Tests undertaken to determine the significance of the association between season and prevalence in 'puppy' and 'adult' dogs revealed a significant association for *I. ohioensis* in 'puppies' only (Table 2.22).

f) Levels of infection :

Information regarding the numbers of *Sarcocystis* sp. sporocysts found in faecal samples from 69 out of 86 infected cats and 236 out of 283 infected dogs are presented in figure 2.9. In both cats and dogs, counts in these samples were generally low with the majority containing 200 sporocysts/g of faeces or less. However, sporocyst numbers tended to be greater in samples from dogs than cats. In dogs the highest count recorded was 144,600/g with a mean of 2,270/g and in cats 11,000/g with a mean of 597/g.

With the exception of *I. felis* , in which faecal samples from 43 out of 86 infected cats had a mean count of 17,379 oocysts/g , insufficient material was available to enable any worthwhile information concerning oocysts counts of the other species to be obtained.

2.4 DISCUSSION

For many years it was erroneously assumed, primarily on the morphology of the oocysts that cats and dogs shared the same species of coccidia (see section 1.2.2). Although this assumption has since been disproved by careful cross-transmission experiments, the view

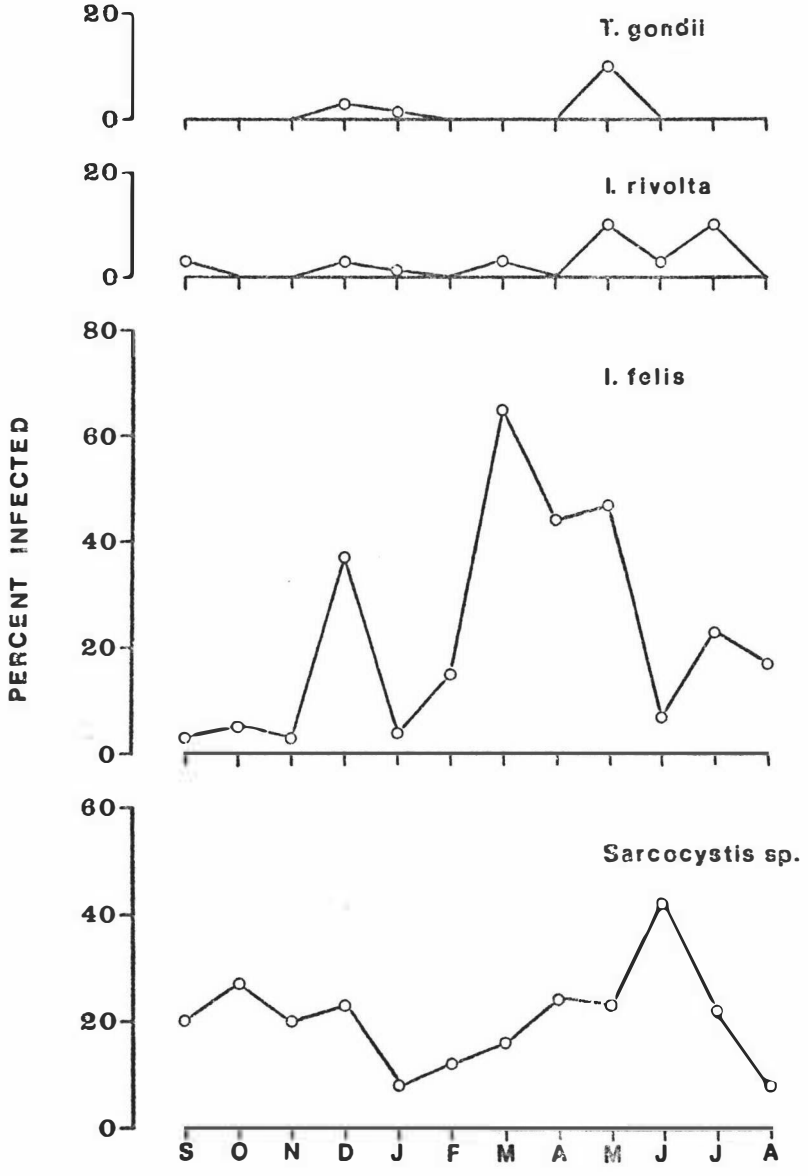


Fig. 2.7 Seasonal prevalence of coccidia in cats.

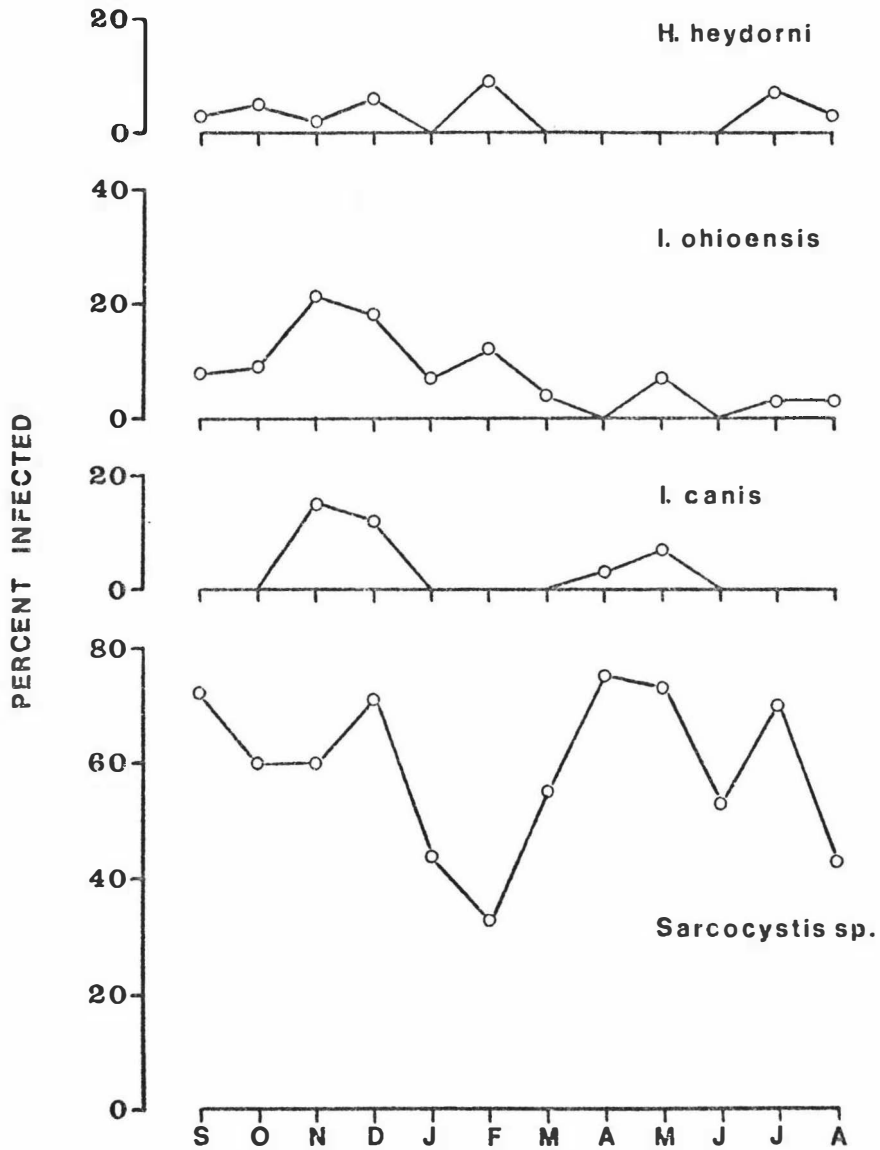


Fig 2.8 Seasonal prevalence of coccidia in dogs.

TABLE 2.19 SEASONAL PREVALENCE OF COCCIDIA IN CATS

Season	No. Examined	No. infected (%)			
		I. felis	I. rivolta	T. gondii	Sarcocystis sp.
Spring + Summer	328	27(8.2)***	3(0.9)ns	2(0.6)ns	44(13.4)**
Autumn + Winter	180	62(34.4)	8(4.4)	3(1.7)	42(23.3)
Total	508	89	11	5	86

Table 2.20 SEASONAL PREVALENCE OF COCCIDIA IN 'KITTEN' (UP TO SIX MONTHS OF AGE) AND 'ADULT" (OVER SIX MONTHS OF AGE) CATS

	Season	No. Examined	No. infected (%)			
			I. felis	I. rivolta	T. gondii	Sarcocystis sp.
'Kittens'	Spring + Summer	57	17(29.8)***	2(3.5)ns	2(3.5)ns	14(24.6)ns
	Autumn + Winter	114	52(45.6)	5(4.5)	3(2.6)	27(23.7)
'Adults'	Spring + Summer	264	10(3.8)***	1(0.4)*	- †	28(10.6)***
	Autumn + Winter	60	10(16.7)	3(5.0)	-	12(20.0)
Unknown Age		13	-	-	-	5
TOTAL		508	89	11	5	86

(Asterisks indicate significant association between prevalence and season,
ns = no significant association,
† = insufficient numbers for statistical analysis).

TABLE 2.21 SEASONAL PREVALENCE OF COCCIDIA IN DOGS

Season	No. examined	No. infected (%)			
		I. canis	*I. ohioensis*	*H. heydorni*	*Sarcocystis* sp.
Spring + Summer	300	16(5.3)ns	39(13.0)**	10(3.3)ns	171(57.0)*
Autumn + Winter	181	3(1.7)	5(2.8)	3(1.7)	112(61.9)
Total	481	19	44	13	283

TABLE 2.22 SEASONAL PREVALENCE OF COCCIDIA IN 'PUPPY' (UP TO SIX MONTHS OF AGE) AND 'ADULT' (OVER SIX MONTHS OF AGE) DOGS

	Season	No. examined	No. infected (%)			
			I. canis	*I. ohioensis*	*H. heydorni*	*Sarcocystis* sp.
'Puppies'	Spring + Summer	78	15(19.2)ns	34(43.6)***	4(5.1)ns	48(61.5)ns
	Autumn + Winter	29	3(10.3)	2(6.9)	2(6.9)	15(53.6)
'Adults'	Spring + Summer	215	1(0.5)ns	5(2.3)ns	6(2.8)ns	118(54.9)ns
	Autumn + Winter	118	-	1(0.8)	1(0.8)	74(62.7)
Unknown age		41	-	2	-	28
	Total	481	19	44	13	283

(Asterisks indicate significant association between prevalence and season
ns = no significant association).

that many species of canine and feline coccidia are morphologically indistinguishable has remained largely unchallenged. In the present study, each of the four valid coccidians recorded in cats was found to be matched by a correspondingly similar coccidian in dogs and the opportunity was taken, therefore, to see if these coccidians could be differentiated by statistical means. In all cases statistically significant differences were found between the dimensions of either the oocysts and/or the sporocysts of coccidians infecting the cat and those infecting the dog.

The morphological characteristics of these organisms were similar to those described previously and most were readily identified. The identity of the *Sarcocystis* species recovered, however, is uncertain although at least some from cats were demonstrated to be *S. muris* (see Chapter 3). Apart from this species which infects the mouse, at least six other species of *Sarcocystis* have been shown to be transmitted by cats and seven by dogs (Tables 1.3 and 1.4). The sporocysts of many of these species overlap in size and although the precise species of *Sarcocystis* involved have not been identified, intermediate hosts for ten of them (sheep, cattle, pigs, horses, rats, rabbits) have been found to be infected in New Zealand (Collins, pers. comm.). In addition, goats and red deer have also been found to be infected with *Sarcocystis* in this country (Collins, pers. comm.) and while these sarcocysts have not, as yet, been proved to be transmitted by cats or dogs, it is probable that they are. In New Zealand the feeding of unfrozen or uncooked sheep and goat meat to dogs is forbidden. The feeding of untreated meat of other animals, however is not and no such restrictions apply to cats. Thus although the mean dimensions of sporocysts recovered from cats most nearly approximate in size to those of *S. porcifelis* from pigs and those of dogs to *S. cruzi* from cattle, it is likely that they represent a mixture of several different species.

Surveys of the prevalence of canine and feline coccidia undertaken in different countries have produced differing results. Generally

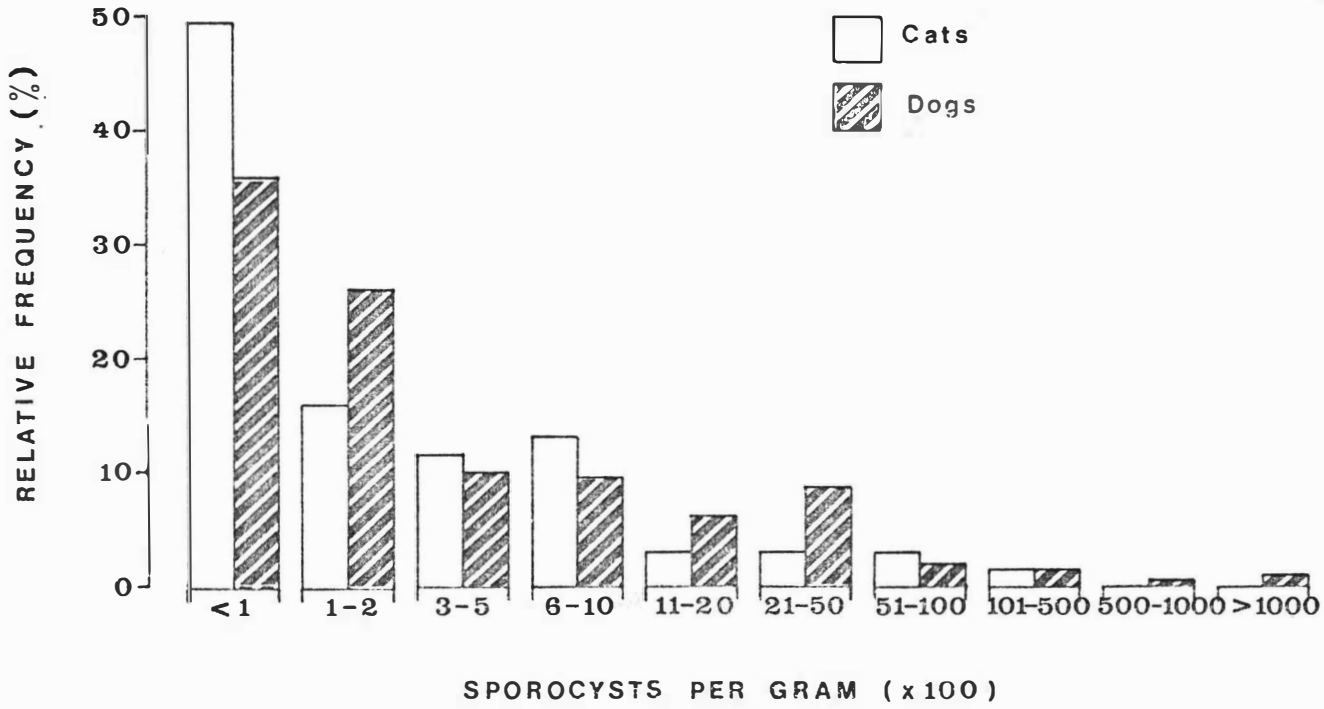


Fig. 2.9 Frequency distributions of *Sarcocystis* sp. counts in cat and dog faeces.

I. felis and *I. rivolta* in cats and *I. canis* and *I. ohioensis* in dogs are commonly encountered. Natural infections of *Sarcocystis*, however, have been reported only infrequently and have usually been found to have a low prevalence (Tables 2.1 and 2.2). In the present study the relatively high prevalence of *Sarcocystis* sp. in cats and dogs indicates that infected intermediate hosts are readily available and may suggest that the feeding of raw meat is more widely practised in this country than elsewhere. Both of the remaining two coccidia recorded, *H. heydorni* from the dog and *T. gondii* from the cat, may also be transmitted by carnivorism. For the former coccidian this route is the sole means of transmission (Heydorn, 1973; Dubey and Fayer, 1976) while for the latter it is the most efficient (Dubey and Frenkel, 1972a; Wallace, 1973b). Despite the fact that these two species have a similar mode of transmission to that of *Sarcocystis* their prevalences were much lower. This may be explained, in the case of *H. heydorni* by the fact that cattle are the only likely source of infection (Heydorn, 1973; Dubey and Fayer, 1976) and in the case of *T. gondii*, by a shorter period of patency and a longer and more substantial immunity in the definitive host (Frenkel *et al.*, 1970; Wallace, 1973b; Dubey and Frenkel, 1974).

Although it is widely held that coccidiosis is more common in puppies and kittens than in adult animals (Levine, 1973) few studies have provided data to support this view; Lodal (1973) found that 82% of coccidia infected cats were juvenile while Knoch *et al.*, (1974) found young cats more commonly excreted oocysts than old cats. A similar tendency for both cats and dogs to show a decreasing prevalence of coccidial infection with increasing age was found in the present investigation although some differences between coccidial species were apparent. Infections of *Isospora* species were more common in 'kittens' and 'puppies' than in 'adults', a result which may reflect the importance of the direct faecal-oral route in the transmission of this genus (Frenkel and Dubey, 1972a). Similarly, as in the present study, most isolations of *T. gondii* have also been made from kittens under 6 months of age (Janitschke

and Kuhn, 1972; Pampiglione *et al*., 1973; Wallace, 1973b; Dubey 1977a) although occasionally they have been recovered from older cats as well (Werner and Walton, 1972; Wallace, 1973b). The source of the present *Toxoplasma* infections is unknown; the young age of infected cats, in one case less than two months of age, may suggest that they were acquired by ingesting oocysts. However, since even some of the youngest cats examined must have had access to raw meat, as shown by the presence of sarcocystan sporocysts in their faeces (Table 2.9), the possibility that they were acquired by carnivorism cannot be excluded.

Sarcocystis infections in definitive hosts are characterised by long periods of sporadic sporocyst shedding and reinfection is easily achieved (Rommel *et al*., 1972; Heydorn and Rommel, 1972a; Fayer, 1974). In view of these findings, which indicate a low level of acquired immunity, the less apparent decline in the prevalence of *Sarcocystis* infection with increasing host age as compared to the previously mentioned coccidia is not surprising. In dogs the prevalence of *Sarcocystis* infection was found to decline less rapidly with increasing host age than in cats and the numbers of sporocysts shed tended to be greater. Both these findings may suggest that immunity to *Sarcocystis* infection, such as it is, is stronger in cats than dogs; the latter result, however, may be more simply explained by differences in the number of *Sarcocystis* species transmitted by the two hosts in New Zealand or, since schizogony does not occur in this genus (Heydorn and Rommel, 1972b; Fayer, 1974; Munday *et al*., 1975), by the greater volumes of meat likely to be consumed by dogs than cats. As with *Sarcocystis* infection, immunity to *H. heydorni* in dogs is also considered to be weak. The absence of any significant difference between the prevalence of this coccidian in 'puppies' and 'adults' recorded here may support this view although the numbers infected were too small to be certain.

Such factors as sex and environment might also be expected to influence the prevalence of coccidial infections in cats and dogs. Females are likely to experience a lowered resistance to infection at times of parturition and lactation and, as a result of closer contact with kittens and puppies, may face greater exposure to oocysts than males. Males, on the other hand, by their more adventurous nature, may have greater opportunity for contact with infected intermediate hosts, as may both sexes in rural environments. Data relating to the effect of such factors, however, are few; Knoch *et al.*, (1974) found no difference in oocyst excretion between male and female cats, while information regarding the association between environment and prevalence appears to be absent. In the present study, the sex of the host was found to have no significant influence on the prevalence of any coccidian in either cats or dogs whereas a comparison between town and country hosts revealed that *I. felis* was significantly more common in country 'kittens' than in town 'kittens' and that *Sarcocystis* species were significantly more common in country 'puppies' than in town 'puppies'. In view of the fact that samples from only a small number of country 'kittens' and 'puppies; were examined, however, these findings must be interpreted with caution.

The assessment of possible seasonal effects on the prevalence of canine and feline coccidia is complicated by the capacity of these species to utilise intermediate hosts as a means of transmission. Obviously seasonal climatic conditions will have some effect on the ability of oocysts to sporulate and survive. Tissue cysts, on the other hand, will be largely unaffected by such changes although it seems probable that small intermediate and transport hosts will be more active and available during Spring and Summer than during Autumn and Winter. A seasonal occurrence of oocyst excretion in cats was observed by Knoch *et al.*, (1974) who found more animals infected in the Summer half-year than the in Winter half-year in Germany. Wallace (1973b) also isolated oocysts of *T. gondii* more frequently from cats during the former period than the latter in

Hawaii, while a similar tendency for coccidia to be more common in dogs in Spring and Summer was observed in the current study. In contrast, all coccidia recorded in cats in the present investigation were found to be more prevalent in the Aumumn-winter season. The reasons for these differences are unclear but may partially relate to disparities in the seasonal occurrence of cat births between countries and to seasonal differences in the breeding of cats and dogs here. Indeed, cats examined during Autumn and Winter contained a greater proportion of 'kittens' than did those examined during Spring and Summer. Tests undertaken to determine the significance of the association between season and prevalence, however, suggest that the seasonal fluctuations in the occurrence of at least some species of coccidia in cats were not entirely attributable to seasonal variations in the age-structure of the host population sampled.

3. THE EXPERIMENTAL INDUCTION OF *SARCOCYSTIS* INFECTIONS IN MICE

3.1 INTRODUCTION

Recently it has been established that the life histories of a number of species of *Sarcocystis* involve an obligatory two-host cycle in which the ingestion of typical cysts present in the musculature of intermediate hosts results in the excretion of coccidian stages in the faeces of cats and dogs (Rommel *et al.*, 1972; Heydorn and Rommel, 1972a, b). For most species, these coccidian stages have been shown to take the form of free, fully-sporulated isosporan-type sporocysts (Ford, 1974; Rommel *et al.*, 1972; Munday and Corbould, 1974). Such sporocysts are not infective for the definitive host but when ingested by the appropriate intermediate host lead to the development of intramuscular cysts once more (Fayer, 1974; Rommel *et al.*, 1974).

Whilst such a cyclic transmission, involving the cat as the definitive host, has been described for *S. muris* of the mouse, the infective stages in the faeces of cats have been variously linked to *Toxoplasma* - like oocysts (Wallace, 1973a), oocysts of *Isospora felis* (Powell and McCarley, 1975) and to free sporulated isosporan sporocysts (Ruiz and Frenkel, 1976), although the first report has since been disproved (Wallace, 1975).

Sarcocystis infection in mice has not been reported in New Zealand. The discovery of such an infection would be of interest since it would provide a useful small animal model enabling the parasite to be studied under carefully controlled laboratory conditions. The present study was undertaken in an attempt to experimentally induce *Sarcocystis* infections in mice using isolates of *I. felis* and free isosporan sporocysts recovered from the faeces of naturally infected cats.

3.2 MATERIALS AND METHODS

3.2.1 Recovery of oocysts and sporocysts:

Faeces of naturally infected cats were broken up in tap water with an electric beater and strained through a sieve (aperture 250 μm). The filtrate was centrifuged at 400g for 5 minutes and the supernatant discarded. The sediment was resuspended in sucrose solution (specific gravity 1.2), centrifuged again and the top layer containing oocysts or sporocysts removed. This procedure was repeated twice. The oocysts and sporocysts were washed clean or sugar solution by repeated suspension and centrifugation in tap water and finally suspended in either 2.5% potassium dichromate or 2% sulphuric acid. Free sporocysts were then stored at $4^{o}C$ until used while oocysts of *I. felis* were placed in petri dishes at 22^{o} - $26^{o}C$ for sporulation. Following sporulation these oocysts were also stored at $4^{o}C$.

3.2.2 Experimental animals :

Laboratory mice and rats were obtained from the Small Animal Production Unit, Massey University. They were fed throughout only on pelleted rations and water. Stock mice were examined for spontaneous *Sarcocystis* infections on numerous occasions before, during and after the experiment but none were found.

Kittens were obtained from a single litter raised in isolation with their mother. The mother cat was fed on tinned fish and milk. After weaning two kittens were removed, placed in separate cages and fed on the same diet. Faeces from both kittens were examined periodically for the presence of coccidia. Small numbers of *I. felis* were recovered from both. However, excretion of these oocysts had ceased entirely before the experiment started.

3.2.3. Infection and examination of rats and mice :

Shortly before they were to be used, oocysts and sporocysts were washed free of storage solution by repeated suspension and centrifugation in tap water. Rats and mice were infected orally using a syringe fitted with narrow bore vinyl tubying. Rats, but not mice, were lightly anaesthetised with chloroform before infection. Following slaughter mice and rats were skinned and fresh spreads of diaphragm and abdominal muscle examined for sarcocysts. Portions of the rest of the carcase musculature were fixed in 10% formalin and sections examined microscopically after staining with haematoxylin and eosin. In addition approximately 5g of muscle from mice and 20g from rats were digested according to the technique described by Seneviratna *et al.*, (1975). For this purpose 2.5 ml of digestion fluid per 1g of finely chopped muscle was used and the samples incubated at 40^{o}C for 2 hours. The digestion fluid contained 1.3g of pepsin, 3.5ml of HCl and 2.5g of NaCl in 500ml of water. After digestion the material was sieved (aperture 250 µm), centrifuged at 10,000g for 10 minutes and the sediment examined microscopically for *Sarcocystis* bradyzoites.

3.3 EXPERIMENTAL PROCEDURES AND RESULTS

3.3.1 Experiments with *I. felis*:

(a) Ten mice were divided into three groups and placed in separate cages. Three mice in groups I and II were each infected with between 2,000 and 4,000 sporulated oocysts of two different pure isolates of *I. felis* . Group III, of four mice, was maintained as an uninfected control group. Mice were killed between 79 and 126 days after infection and examined. No sarcocysts were observed in any mouse.

(b) Twelve mice were divided into four equal groups and placed in separate cages. Pure oocysts of *I. felis* isolated from the faeces of 10 naturally infected cats were pooled.

Group I mice were infected with approximately 20,000, group II with 50,000g and group III with 100,000 of these oocysts. Group IV was maintained as an uninfected control. All mice were killed 173 days later but no sarcocysts were found.

3.3.2 Experiments with free sporocysts:

(a) Free isosporan sporocysts recovered from the faeces of 66 naturally infected cats were pooled into 11 groups of 6. Nine of the 11 pools contained free sporocysts only while of the remaining two, one contained, in addition to these sporocysts, small numbers of unsporulated *I. felis* and the other unsporulated *T. gondii* and *I. rivolta* . Each pool of sporocysts was fed to a group of three mice and all groups were maintained in separate cages. Twenty-seven mice, including at least two from each group, were killed between 108 and 198 days post infection. The remaining six mice were retained for other purposes. Eighteen of these mice from 9 of the 11 groups were found to be infected with sarcocysts. These sarcocysts, which were macroscopically evident as whitish intramuscular streaks, measured up to 12mm long and 125 to 187 µm wide. Cysts were found in all skeletal muscles and in some mice it was estimated that up to 40% of the muscle fibres were infected (Plate 3.1). However, even heavy infections appeared to have little clinical effect on mice. Microscopically the cysts were found to have smooth, thin walls (Plate 3.2A) and were divided into compartments by septa. Each cyst contained thousands of sausage-shaped bradyzoites with bluntly rounded ends (Plate 3.2B). Twenty bradyzoites from freshly macerated cysts measured 13.0 to 14.7 (13.7 ± 0.2) by 3.3 to 4.6 (3.9 ± 0.2) µm. Cysts observed in all infected mice appeared macroscopically and microscopically similar.

Plate 3.1 Ventral aspect of laboratory mouse experimentally infected with *Sarcocystis muris* for 184 days. The bradyzoite-filled sarcocysts are clearly evident as whitish intra-muscular streaks.

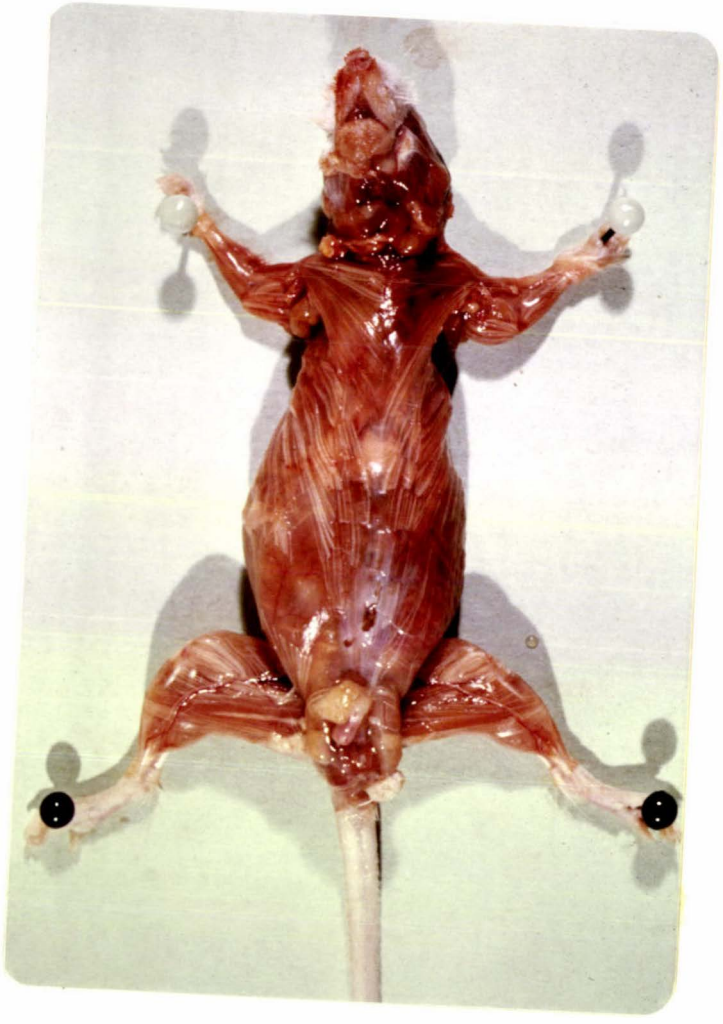
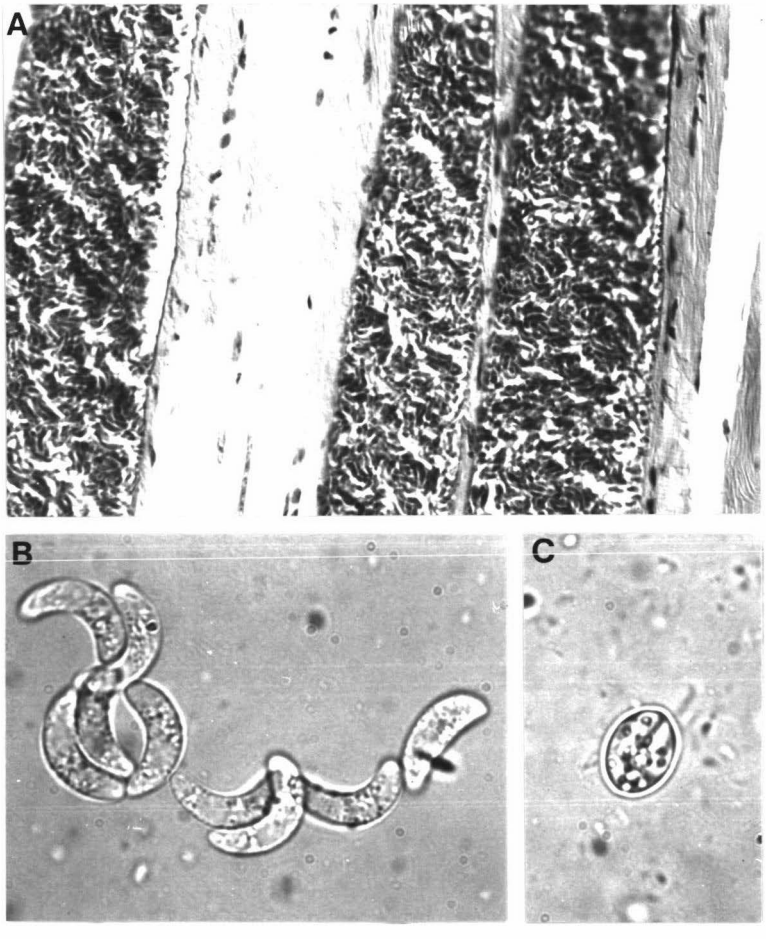


Plate 3.2 Some life cycle stages of *S. muris*.

A.: Section of mouse skeletal muscle showing bradyzoite-filled sarcocysts (haematoxylin and eosin, x 750). B.: Bradyzoites from freshly macerated cysts (unstained, x 3000). C.: Free sporocyst of *S. muris* from the faeces of an experimentally infected kitten (x 3000).


A
B
C

(b) Two heavily *Sarcocystis* infected mice, from two of the groups which had been infected with sporocysts alone 184 days previously, were skinned and heads, tails, feet and internal organs removed. The carcases were cut up into small pieces and fed to a 12 week-old kitten. Another kitten, the littermate of the first, was kept as an uninfected control. Faecal samples were collected daily from both kittens and examined. The infected kitten began passing fully sporulated isosporan sporocysts five days after ingesting the mice. No other coccidian species was excreted by this kitten and no coccidia were found in the faeces of the control. Fifty of these sporocysts (Plate 3.2C), which lacked a stieda body and contained four sporozoites and a scattered residuum, measured 9.8 to 11.4 (10.8 ± 0.1) by 8.2 to 9.8 (8.7 ± 0.1) μm and had a length-width ratio of 1.17 to 1.4 (1.24 ± 0.01). Twelve days after the first kitten was infected three *Sarcocystis* infected mice from two other groups dosed only with free sporocysts were fed to the kitten previously used as a control. Sporocysts identical to those described above were recovered from the faeces of this kitten six days later.

(c) Doses of between 600 and 1,000 sporocysts harvested from the above two kittens were given orally to each of three mice and two rats. A further three mice and two rats were maintained as uninfected controls and all groups were kept in separate cages. All animals were killed 104 days after infection and examined. Light *Sarcocystis* infections, identical to those described previously, were found in all sporocyst infected mice. No sarcocysts were found in any of the control mice and none were found in the rats.

3.4 DISCUSSION

Recent reports indicate that the mouse (*Mus musculus*) may be host to at least three species of *Sarcocystis*. One unnamed species with cysts of up to 1.8mm long and bradyzoites of 6 to 7 by 2 to 3 µm is transmitted by an owl (Cerna, 1976; Munday, 1977). Another species, also unnamed and of unknown origin, forms microscopic cysts surrounded by prominent radial spines (Cosgrove, G.E., cited in Ruiz and Frenkel, 1976). The third species, *S. muris*, which is transmitted by the cat, has large, smooth-walled cysts containing bradyzoites of 10 to 16 by 3 to 6 µm (Powell and McCarley, 1975; Ruiz and Frenkel, 1976).

Sarcocysts found in the muscles of mice in the present study closely resemble those of the latter species, an identification which is supported by the occurrence of infective stages in the faeces of cats. In contrast to the findings of Powell and McCarley (1975) however, isolates of *I. felis* used in this investigation did not produce such cysts in mice. Similar observations have been made by others (Dubey and Frenkel, 1972b; Frenkel and Dubey, 1972a; Melhorn and Markus, 1976) who have found that, in mice, infections of *I. felis* form only monozoic 'cysts' in lung, liver, lymph nodes and spleen. While it is possible that Powell and McCarley's (1975) findings may be related to the existence of a different strain of *I. felis* a more likely explanation has been suggested by Ruiz and Frenkel (1976). They considered that mice used by these workers may have been infected with both *I. felis* and *Sarcocystis* ; in mice only the sarcocysts would have been grossly evident whereas in cat faeces the more obvious *I. felis* oocysts drew attention and the smaller *Sarcocystis* sporocysts may have been overlooked[1]. This suggestion is supported by the present findings since sarcocysts were found only in mice infected with free sporocysts and only such sporocysts were excreted by kittens fed infected mice.

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1. A recent report (Last and Powell, 1978) has revealed that the mice used by Powell and McCarley (1975) were indeed infected with both *I. felis* and *S. muris* and that the source of the *S. muris* infection was a small sarcocystan sporocyst and not *I. felis* as previously supposed.

The sporocysts shed by these kittens were very similar in size to those described by Ruiz and Frenkel (1976) although the observed prepatent periods were somewhat shorter than the 8 to 11 days recorded by these authors.

A comparison between the dimensions of these sporocysts and those previously recovered from the faeces of naturally infected cats (Chapter 2) reveals that the former are shorter than the latter with little overlapping between the two (Fig 3.1). One possible explanation for this apparent anomaly is that the *Sarcocystis* sporocysts recovered from the naturally infected cats consisted of a mixture of species, of which *S. muris* was but one. By feeding such sporocysts to mice all species other than *S. muris*, which may initially have been present in only low numbers, would be eliminated. Subsequent feeding of these mice to kittens would, therefore, result in the excretion of *S. muris* sporocysts only. Alternatively, since sporocysts from only 8 of the cats on which the original measurements were based were used in the present experiment, it is possible that the initial *S. muris* infections may have originated from unmeasured sporocysts isolated from a number of the remaining 58 cats.

The prevalence of *S. muris* sporocysts in the naturally infected cats cannot be determined with any accuracy since sporocysts from six cats were used to infect each group of mice. Assuming that the faeces of one cat in each infective pool contained all such sporocysts then this species must have been present in at least 9 of the 66 cats (13.6%) tested. Although natural infections of *S. muris* have not been reported in mice in New Zealand, morphologically similar sarcocysts have been found to be very common in rats *(Rattus norvegicus)* (Collins, pers. comm.). The definitive host for this rat sarcocyst is unknown. However, it is conceivable that it is the cat[2], since rats from a particularly

2. Although one species of *Sarcocystis (S. cymruensis)* of *Rattus norvegicus* in Wales has been found to be transmitted by the cat (Ashford, 1978) the relationship between this species and the sarcocyst in New Zealand rats has yet to be determined.

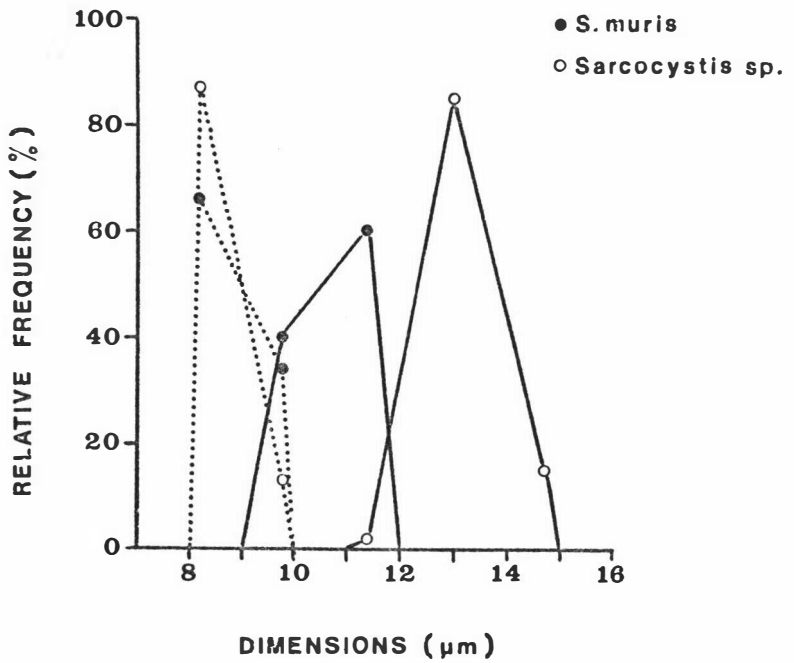


Fig. 3.1 Comparison between the lengths (solid lines) and widths (broken lines) of *Sarcocystis muris* sporocysts recovered from the faeces of experimentally infected kittens and *Sarcocystis* sp. sporocysts recovered from the faeces of naturally infected cats.

heavily infected population have commonly been found to form part of the diet of cats inhabiting the same area (author's personal observation). Despite similarities between sarcocysts found in rats and mice and despite the possibility that the cat may be definitive host for both, the present results indicate that they are different species. This finding, which is similar to that of Ruiz and Frenkel (1976), is consistent with the work of others which suggests that species of *Sarcocystis* are highly host-specific for their intermediate hosts (Gestrich *et al.*, 1974, 1975a; Munday and Rickard, 1974; Munday, 1976; Rickard and Munday, 1976).

4. THE OCCURRENCE OF *BESNOITIA* SPECIES IN NEW ZEALAND CATS

4.1 INTRODUCTION

After completion of the survey of the identity and prevalence of coccidian parasites in New Zealand cats and dogs (Chapter 2) the author was given the opportunity of examining the faeces of five feral cats. In the faeces of one of these cats a small number of unsporulated oocysts were found. Sporulation of these oocysts showed them to be of the isosporan type, intermediate in size between those of *I. rivolta* and *T. gondii* and unlike any of the species previously recorded.

This chapter describes these oocysts and records the experimental procedures undertaken to identify them.

4.2 MATERIALS AND METHODS

4.2.1 Feral cats :

In October 1977, faecal samples obtained from the rectums of five feral cats were given to the author. The cats were of mixed sexes and ages and had all been shot on the Feilding rubbish dump. This dump, which is situated 19 kilometres north-west of Palmerston North, supports a large resident population of rats (*Rattus norvegicus*) and several domestic cats which have reverted to a wild state but, apparently, no mice (Blackmore, pers. comm.).

4.2.2 Recovery of oocysts :

Oocysts present in the faeces of naturally and experimentally infected cats were recovered, sporulated and examined as previously described (Chapter 2).

4.2.3 Experimental animals :

Laboratory mice, rats, rabbits and guinea pigs obtained from

the Small Animal Production Unit, Massey University were fed on pelleted rations and water; experimental kittens were raised in isolation on a diet of tinned fish and milk. Periodic examination of the faeces of these kittens before the experiment started revealed the presence of *I. felis* . However, excretion of these oocysts had ceased by the time the kittens were used.

4.2.4 Infection and examination of intermediate hosts :

Immediately prior to use sporulated oocysts were washed free of storage solution by repeated suspension and centrifugation in tap water. Rats, mice, rabbits and guinea pigs were infected orally using a syringe fitted with narrow bore vinyl tubing. At necropsy, muscle, brain and viscera were examined for cysts under a dissecting microscope and suspicious nodules excised, macerated in physiological saline, and examined for bradyzoites. In addition portions of most organs and tissues were fixed in 10% formalin and examined in sections stained with haematoxylin and eosin.

4.3 EXPERIMENTAL PROCEDURES AND RESULTS

4.3.1 The initial isolate :

The small numbers of unsporulated oocysts (Plate 4.1D) recovered from the faeces of a young female feral cat were sporulated in 2.5% potassium dichromate at 22^0- 26^0C for 7 days. These oocysts, which lacked micropyles and were spherical to subspherical in shape, were found, after completion of sporulation, to contain two tetrazoic sporocysts without stieda bodies (Plate 4.1E). Five of these sporulated oocysts measured 18.0 to 19.5 (18.6 ± 0.4) by 14.7 to 16.3 (16.0 ± 0.3) µm. The oocysts were divided into five approximately equal lots and fed to three mice and two rats which were killed between 33 and 78 days later. No evidence of infection was found in any of the mice but in both rats a few small, but macroscopically visible, circular cysts measuring up to 260 µm in diameter were apparent in the diaphragm

and abdominal muscles. Microscopically the cysts were found to exhibit characteristics typical of those of the genus *Besnoitia*. Each cyst was surrounded by a thick cyst wall (7 - 11 µm thick) enclosing several hypertrophied and hyperplastic host cell nuclei (Plate 4.1B) and contained thousands of crescentic-shaped bradyzoites with pointed ends (Plate 4.1C). Ten bradyzoites from freshly macerated cysts measured 9.8 to 11.4 (11.1 ± 0.2) by 1.6 to 3.3 (2.8 ± 0.3) µm.

4.3.2 Transmission experiments :

(a) Portions of tissue including muscle, heart and brain from one of the rats which had been dosed with the initial oocyst isolate 78 days previously were fed to two 10 week-old kittens. A further kitten, of similar age, was kept as an uninfected control. Faecal samples were collected daily from all three kittens and examined. Both infected kittens began passing unsporulated oocysts, similar to those originally recovered from the feral cat, 11 days after ingesting rat tissue and continued to do so for 15 and 19 days respectively. No other coccidian species were excreted by these kittens and no coccidia were found in the faeces of the control animal.

The oocysts were sporulated in 2% sulphuric acid and examined. The sporulated oocysts, which had smooth colourless walls about 0.6 µm thick, were sperical to subspherical in shape. Micropyles, polar granules and oocyst residua were absent. One hundred sporulated oocysts from the two kittens measured 14.7 to 18.0 (16.9 ± 0.1) by 13.0 to 16.3 (14.6 ± 0.1) µm (Fig. 4.1) with length-width ratios of 1.00 to 1.38 (1.16 ± 0.01). Within each oocyst were two elliptical sporocysts with smooth colourless walls each containing four sporozoites and a dispersed granular residuum. Sporocyst stieda bodies were absent. Fifty of these sporocysts measured 9.8 to 13.0 (11.8 ± 0.2) by 6.5 to 8.2 (7.4 ± 0.1) µm (Fig 4.1) with length-width ratios of 1.2 to 2.0 (1.62 ± 0.03).

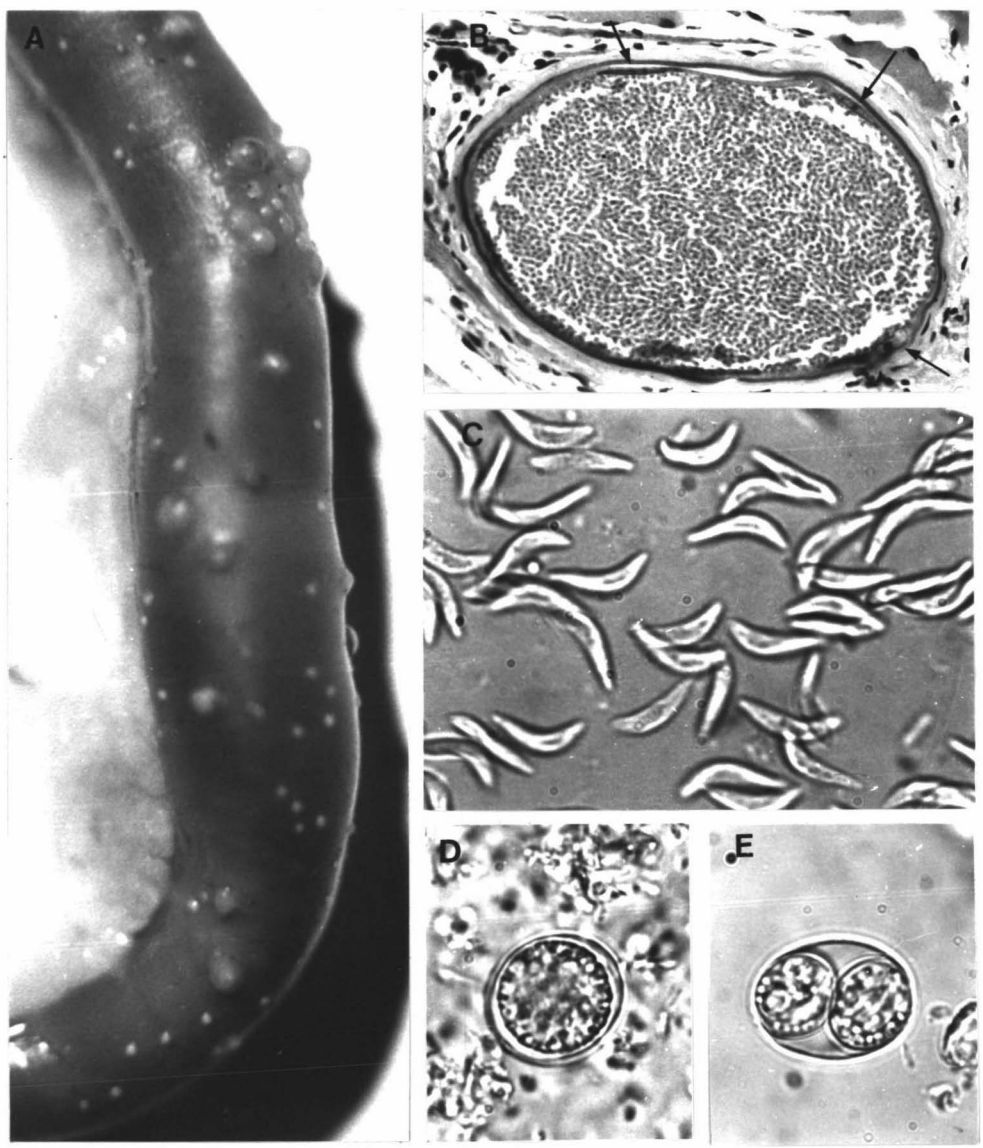
Plate 4.1 Some life cycle stages of *Besnoitia* sp.

A. Cysts on the intestine of a rat 91 days after infection with oocysts (x 4).

B. Section of rat skeletal muscle showing bradyzoite-filled cyst; note cyst wall enclosing enlarged host-cell nuclei (arrows) (haematoxylin and eosin x 750).

C. Bradyzoites from freshly macerated cysts (unstained, x 3000).

D. and E. Unsporulated and sporulated oocysts recovered from the faeces of cats (x 3000).



(b) The above oocysts were used to infect two rabbits, two guinea pigs, three rats and three mice. Rats and Guinea pigs each received approximately 100,000, rabbits 200,000 and mice 40,000 oocysts. The animals were killed between 57 and 91 days post infection and examined. *Besnoitia* infections, identical to those described previously were found in all of the rats, both of the rabbits and two out of the three mice. No cysts were found in either of the two guinea pigs. The heaviest infections were recorded in the rats where the greatest concentration of cysts was found on the walls of the ileum and caecum (Plate 4.1A). Smaller numbers of cysts were also found in skeletal muscle, heart and brain of this host. Much smaller numbers of cysts were found in the rabbits and infected mice and although the distribution of infection in these hosts was similar to that recorded in rats, predilection for the intestine was less obvious.

Confirmation of the absence of *Besnoitia* infection in the guinea pigs was sought by feeding tissues from both animals to the control kitten used in the previous experiment. Daily examination of the faeces of this kitten for 20 days following this feeding failed to detect any oocyst excretion. Elimination of the possibility that this failure may have been due to any host immunity was obtained by the subsequent excretion of *Besnoitia* oocysts by this kitten 12 days after ingesting tissue from one of the infected rats.

4.4 DISCUSSION

The detection of *Besnoitia* in New Zealand, for the first time, is of considerable interest since some species of this genus are known to be pathogenic for domestic livestock (see section 1.7.2). The identification of such species is based primarily on the identity of their natural intermediate hosts, their geographical distribution and on the form, size and location of their tissue cysts. As pointed out by Frenkel (1973), however, the morphology of the cyst

wall is an unreliable characteristic since it is of host origin and it seems likely that the size of the cyst will be dependent on the age of infection. Currently six species of *Besnoitia* are recognised, the geographical distribution and natural intermediate host ranges of which have recently been reviewed by Frenkel (1977). These include *B. besnoiti* of cattle in Europe and Asia and wild ungulates in Africa ; *B. bennetti* of horses in Europe, Africa and North America; *B. tarandi* of Alaskan reindeer and caribou; *B. jellisoni* of deer mice (*peromyscus* sp.) and Kangaroo rats (*Dipodomys* sp.) in North America; *B. darlingi* of lizards (genera *Basiliscus* and *Ameiva)* and opossums *(Didelphis marsupialis)* in Panama and North America and *B. wallacei* in Hawaii; the natural intermediate host of the latter species is unknown although it has been shown experimentally to infect *Mus musculus, R. norvegicus, R. exulans* but not *R. rattus* (Frenkel, 1977).

Until recently the life cycles of these species have remained obscure. It is now known, however, that at least three of them *(B. besnoiti, B. wallacei, B. darlingi)* may be transmitted in the faeces of cats as an isosporan oocyst (Peteshev *et al.*, 1974; Wallace and Frenkel, 1975; Smith and Frenkel, 1977). The definitive hosts of the remaining species are unknown; faecal transmission of neither *B. bennetti* nor *B. tarandi* has been investigated while an oocyst stage of *B. jellisoni* has not been detected despite the feeding of tissue cysts of this species to 20 potential feline host candidates (13 domestic cats, 6 bobcats, 1 cougar) or to several other carnivores (3 foxes, 3 dogs, 1 coyote, 8 procyonids, 4 skunks, 1 owl, 1 hawk) and some snakes (1 boid, 1 colubrid and 3 viperids) (Wallace and Frenkel, 1975).

Like the former three species, the present species of *Besnoitia* is also transmitted as an isosporan oocyst in the faeces of cats. A comparison between the dimensions of these oocysts and those of *B. besnoiti, B. darlingi* and *B. wallacei* (Table 1.3) reveals that they are most nearly approximate to those of *B. wallacei*

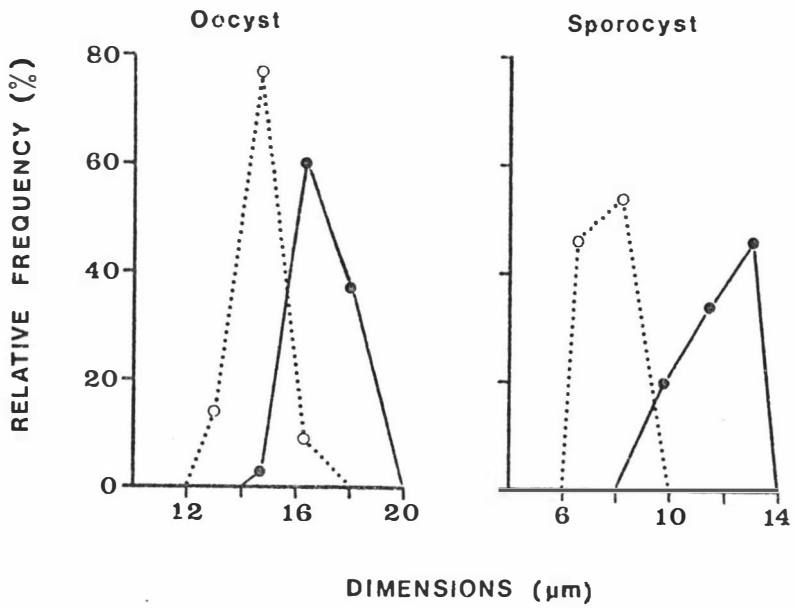


Fig 4.1. Frequency distribution of the lengths (solid lines) and widths (broken lines) of oocysts and sporocysts of *Besnoitia* sp. (oocysts n = 100, sporocysts n = 50).

although they are slightly larger. Similarly, the intermediate host range of this species is also closer to that of *B. wallacei* than to that of *B. besnoiti* or *B. darlingi*. In the present study rats (*R. norvegicus*) appeared to be more suitable experimental intermediate hosts than either mice or rabbits with infection tending to be concentrated on the intestine. Similar observations have been made for *B. wallacei* by Wallace and Frenkel (1975) and Frenkel(1977) who also observed higher infection rates and better persistence of this species in *R. norvegicus* than in other intermediate hosts tested. Whether or not *B. wallacei* is infective for rabbits and guinea pigs or whether the present species will infect *R. exulans* and *R. rattus*, however, has not been determined. Like *B. wallacei* the natural intermediate host of the present species is uncertain although there is strong circumstantial evidence to suggest that it is the rat (*R. norvegicus*). Support for this assumption has been provided by the finding of naturally infected rats on the Feilding dump (Collins, pers comm.). Evidence that the rabbit may

also be a natural intermediate host of this species is provided by the same worker who recovered oocysts of similar form and size to those described in the present study from cats fed wild rabbits. Attempts to reinfect rabbits with these oocysts, however, were not successful (Collins, pers comm.).

In addition to their natural intermediate hosts, some species of *Besnoitia* have been shown experimentally to be capable of infecting a number of other animals: *B. besnoiti* has been transmitted to sheep, goats, rabbits, mice, hamsters, gerbils *(Meriones tristani)* and ground squirrels (*Citellus* sp.) (Pols, 1960; Neuman, 1962; Bigalke, 1967; Peteshev *et al.*, 1974) while *B. jellisoni* has been transmitted to laboratory mice, Norway rats, hamsters and guinea pigs (Frenkel, 1955). Infection of laboratory mice and hamsters with *B. darlingi* has also been achieved (Smith and Frenkel, 1977). Thus although the current evidence indicates that the natural intermediate hosts of the present species are rats and perhaps rabbits, the possibility that domestic livestock may also be infected cannot be excluded and requires investigation.

5. STUDIES ON THE ACTIVATION AND EXCYSTATION OF *I. FELIS* AND *I. RIVOLTA* SPOROZOITES

5.1 INTRODUCTION

For most of the species of coccidia that have been studied, the liberation of motile sporozoites from the sporocyst and oocyst has been found to follow a sequential process involving two stimuli. The first, or pretreatment stimulus affects the permeability of the oocyst wall; the second, or treatment stimulus acts on the sporocysts and possibly the sporozoites. Trypsin and bile are recognised as the most important factors in the second stimulus (Jackson, 1962). The initial stimulus, however, appears to differ for coccidia infecting different hosts. Thus for some species of poultry coccidia mechanical breakage of the oocyst wall, such as may occur in the gizzard, has been found necessary (Doran and Farr, 1962; Farr and Doran, 1962), whereas for coccidia infecting sheep, chemical alteration by factors within the rumen may be required (Jackson, 1962).

In vitro the permeability of the oocyst wall can be enhanced chemically by incubation in a reducing medium under CO_2 (Jackson, 1962; Hibbert and Hammond, 1968; Bunch and Nyberg, 1970). A similar effect may be achieved by removal of the outer wall layers using sodium hypochlorite (Speer *et al.*, 1973; Nyberg and Knapp, 1970) and both techniques have been used successfully to study the excystation process of coccidia. With the former procedure, however, the exposure time necessary to achieve the optimum effect has been found to vary from species to species (Bunch and Nyberg, 1970).

The requirements for the successful *in vitro* excystation of the canine and feline coccidia are largely unknown. Only those of two species, *I. canis* and *S. cruzi* , of the dog have been briefly examined (Bunch and Nyberg, 1970; Speer *et al.*, 1973; Fayer and Leek, 1973) and no information concerning the excystation of

any of the feline species has been reported.

The present study was undertaken to determine the effect of various factors on the activation and excystation of sporozoites of two of the cat coccidia, *I. felis* and *I. rivolta*.

5.2 MATERIALS AND METHODS

5.2.1 Collection and storage of oocysts:

Oocysts of *I. felis* and *I. rivolta* excreted in the faeces of experimentally infected kittens were recovered, cleaned and sporulated as previously described (Chapter 3). Unless otherwise stated all oocysts used in these experiments were sporulated in 2.5% potassium dichromate and stored in the same solution at 4°C for two to three months before use.

5.2.2 CO_2 pretreatment:

Oocysts were washed free of storage solution by suspension and centrifugation in four changes of tap and one of distilled water. Equal volumes of oocyst suspension in distilled water and freshly prepared 0.04M cysteine hydrochloride were placed in 100 ml Erlenmeyer flasks giving a final concentration of 0.02M cysteine hydrochloride. The flasks, which were fitted with inlet and outlet valves, were flushed for two minutes with compressed CO_2. They were then sealed and incubated at 39°C in a water bath for intervals of 1, 4, 8 or 24 hours. Following incubation the oocysts were washed twice with distilled water and twice with Ringer's solution, in which they were finally suspended.

5.2.3 Sodium hypochlorite pretreatment:

One ml aliquots of cleaned oocyst suspensions in distilled water were made up to 10 ml with 0.5% sodium hypochlorite in graduated centrifuge tubes and placed in an ice-bath for intervals of 15,

30 or 60 minutes and stirred frequently. After the required interval in the ice-bath each suspension was washed free of sodium hypochlorite with five changes of tap water, one of distilled water and two of Ringer's solution.

5.2.4 Treatment with excysting medium:

Pretreated and non-pretreated oocysts suspended in Ringer's solution were added to equal volumes of freshly prepared excysting medium consisting of bile, trypsin (1:300) and buffer and incubated under air in a water bath. The interval and temperature of incubation varied according to the experiment, as did the concentration of trypsin and bile, the type of bile used and the buffer. Natural biles used in these experiments were pooled from at least five animals and were frozen until use, while all buffers were prepared following the methods of Gomori (1955).

5.2.5 Determination of activation:

Wet mount slides were prepared after the appropriate incubation period in excysting medium and examined microscopically for activated oocysts. For this purpose and at each observation interval, 100 normally sporulated oocysts were randomly counted to determine the percentage activated. Unsporulated or abnormally sporulated oocysts were not counted and oocysts were considered to be activated when at least one sporozoite from one sporocyst was free in the oocyst.

5.3 EXPERIMENTAL PROCEDURES AND RESULTS

All results stated herein represent the means of at least four experiments ± the standard error.

5.3.1 Effect of type and duration of pretreatment:

Cleaned *I. felis* and *I. rivolta* oocysts were divided into eight lots; four were pretreated with CO_2 and cysteine hydrochloride

for periods of 1, 4, 8 and 24 hours and three were pretreated with sodium hypochlorite for 15, 30 or 60 minutes. The final lot was not subjected to any pretreatment. All pretreated and non-pretreated oocysts suspended in Ringer's solution were then added to equal volumes of excysting medium consisting of 10% (V/V) ovine bile, 0.5% (W/V) trypsin and tris buffer (pH 7.5). They were then incubated at 39°C for six hours after which the percentage activation was determined.

The results, which are presented in Table 5.1 revealed that *I. felis* and *I. rivolta* sporozoites were capable of activation in non-pretreated oocysts. For both species, however, the levels of activation were greater in pretreated than in non-pretreated oocysts and these differences tended to increase with increasing duration of pretreatment. Sodium hypochlorite pretreatment appeared more effective for the activation of *I. felis* than CO_2 pretreatment whereas for *I. rivolta* the converse appeared to be the case and the highest level of activation of this species was attained in oocysts pretreated with CO_2 for 24 hours.

5.3.2 Effects of storage solution and storage duration:

Freshly harvested oocysts of *I. rivolta* were divided into two lots and sporulated in either 2.5% potassium dichromate or 2% sulphuric acid for six days at a temperature of 22 to 26°C. Following sporulation oocysts from each solution were further subdivided into two portions. One portion was used to determine the percentage activation immediately following sporulation and the other to determine the percentage activation after 100 days storage at 4°C in the same solution in which they were sporulated. In both cases and for each sporulation and storage solution, aliquots of oocysts were divided into three lots; one was pre-treated with CO_2 and cysteine hydrochloride for eight hours, the second was pretreated with sodium hypochlorite for 30 minutes while the third was not pretreated. Pretreated and non-pretreated oocysts were then incubated for six hours at 39°C in the same excysting

TABLE 5.1 EFFECT OF VARIOUS PRETREATMENTS ON THE ACTIVATION OF *I. FELIS* AND *I RIVOLTA* SPOROZOITES AS DETERMINED AFTER 6 HOURS TREATMENT IN TRYPSIN AND BILE

Pretreatment	Concentration	Duration (hrs)	Percent Activation* I.felis	I. rivolta
None	-	-	60.8 ± 2.8	36.0 ± 2.4
Cysteine hydrochloride and CO_2	0.02M	1	74.5 ± 2.2	57.8 ± 1.3
		4	70.5 ± 3.0	61.5 ± 2.7
		8	74.8 ± 1.5	66.8 ± 2.5
		24	81.0 ± 1.5	70.3 ± 2.2
Sodium hypochlorite	0.5%	0.25	78.3 ± 2.1	56.5 ± 2.1
		0.5	90.8 ± 2.0	56.5 ± 1.3
		1	90.3 ± 2.5	63.0 ± 0.8

* Mean of 4 replications ± SE

medium as used in the previous experiment and the percentage activation determined.

The results of this experiment (Figure 5.1) revealed two main differences in the effect of sporulation and storage solution on the activation of *I. rivolta* sporozoites. Firstly, whereas greater levels of activation were always attained in pretreated than in non-pretreated oocysts sporulated and stored in potassium dichromate, this was not the case for oocysts sporulated and stored in sulphuric acid. For the latter oocysts the levels of activation attained following either no pretreatment or CO_2 pretreatment were similar while pretreatment with sodium hypochlorite appeared mildly inhibitory. The second main difference related to the effect of duration of storage on activation. For oocysts sporulated and stored in potassium dichromate the level of activation recorded after 100 days exposure had declined to almost half that recorded after six days exposure. For oocysts sporulated and stored in sulphuric acid, however, the level of activation was found to decline less and at 100 days the level of activation recorded in all such oocysts averaged only 10.3% below that recorded at six days.

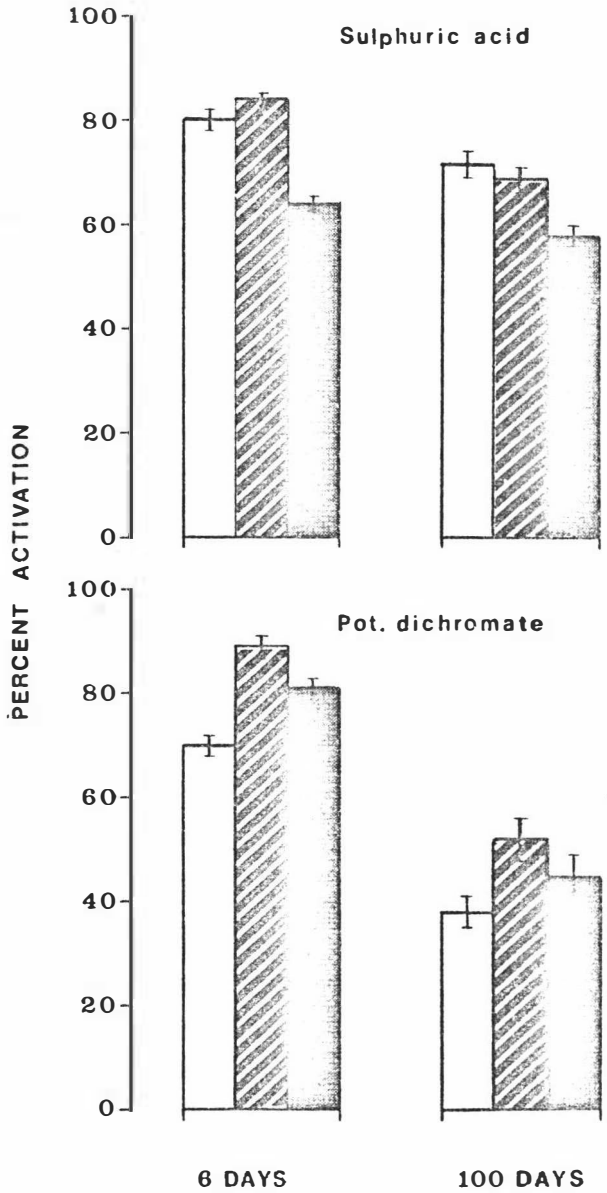


Fig 5.1. Effect of storage solution and storage duration on the activation of *I. rivolta* sporozoites. (Open bars = non-pretreated oocysts, cross-hatched bars = CO_2 pretreated and stippled bars = sodium hypochlorite pretreated oocysts).

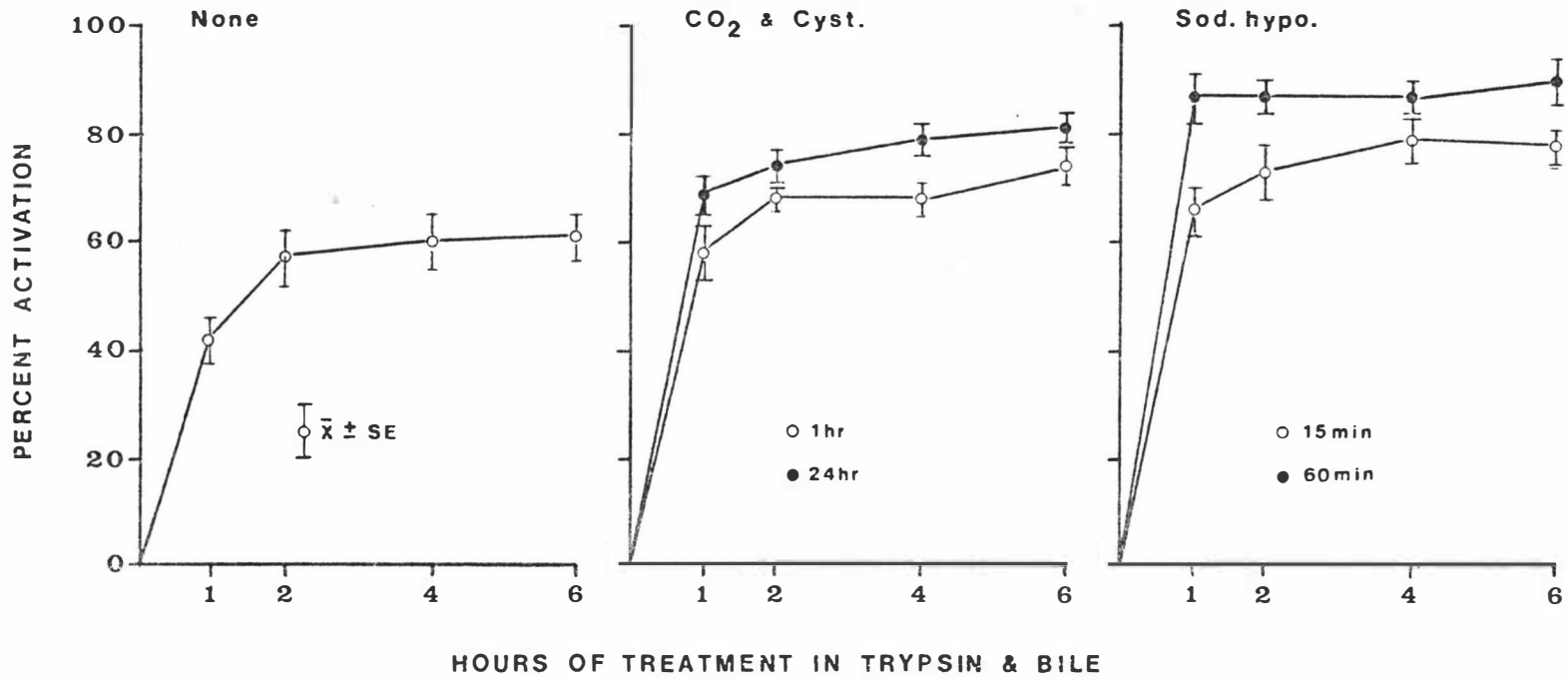


Fig. 5.2 Effect of duration of treatment in excysting medium on the activation of *I. felis* sporozoites following : no pretreatment; CO_2 pretreatment for 1 or 24 hours, or sodium hypochlorite pretreatment for 15 or 60 minutes.

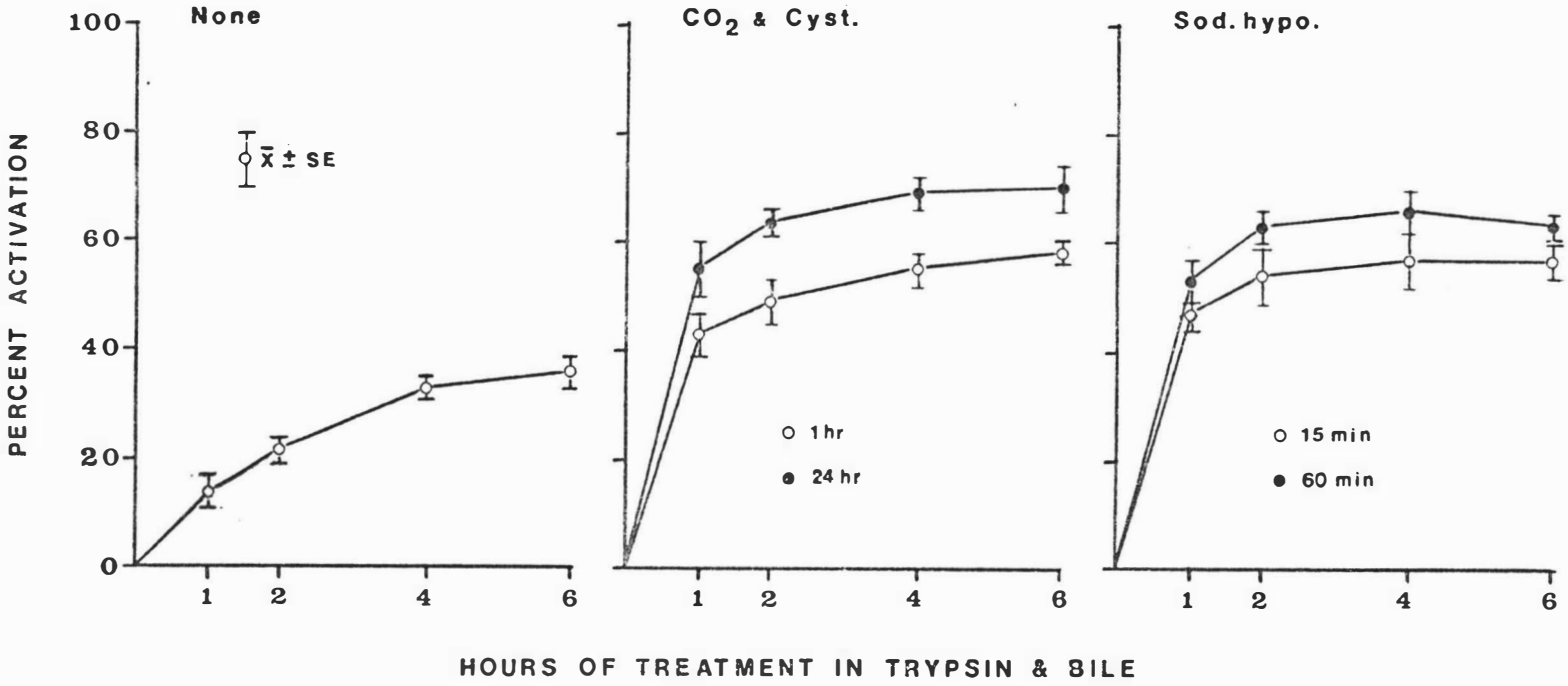


Fig. 5.3 Effect of duration of treatment in excysting medium on the activation of *I. rivolta* sporozoites following: no pretreatment; CO_2 pretreatment for 1 or 24 hours, or sodium hypochlorite pretreatment for 15 or 60 minutes.

5.3.3 Effect of duration of treatment in excysting medium:

Oocysts of *I. felis* and *I. rivolta* subjected to : (a) no pretreatment, (b) CO_2 and cysteine hydrochloride pretreatment for one or 24 hours and (c) sodium hypochlorite pretreatment for 15 or 60 minutes, were incubated at 39°C in the same excysting medium as used in the previous two experiments. Samples were removed after one, two, four and six hours incubation in excysting medium and examined for activated sporozoites.

The results, which are presented in Figures 5.2 and 5.3, revealed that although the levels of activation recorded varied according to the nature and duration of pretreatment, as previously observed (Exp. 5.3.1), the patterns of activation following treatment in excysting medium were basically similar. For both species, the percentage of oocysts containing activated sporozoites tended to increase with increasing intervals of exposure to excysting medium. With the exception of non-pretreated oocysts of *I. rivolta* , however, the levels of activation attained after two hours exposure were only slightly less than those recorded after six hours.

5.3.4 Effect of bile and trypsin concentration:

To determine the effect of bile and trypsin concentration on activation, oocysts of *I. felis* and *I. rivolta* were pretreated for 30 minutes with sodium hypochlorite. Oocysts so pretreated were then added to equal volumes of excysting media consisting of tris buffer (pH 7.5) and either 0.5% trypsin and ovine bile at concentrations of 0, 1, 5, 10, 20 and 50%, or 10% ovine bile and trypsin at concentrations of 0, 1 and 2%. All samples were then incubated at 39°C for two hours and the percentage activation determined.

The results are presented in Figure 5.4. Activation of both species was found to occur only when bile was present. At concentrations of 1% bile, only low levels of activation of *I. felis* (52.7 ± 2.2%)

and *I. rivolta* (5.5 ± 1.0%) were recorded. These levels increased to 81.3 ± 2.6% and 37.5 ± 4.7% respectively, when 5% bile was used. Above this concentration the level of activation of *I. rivolta* was found to further increase with increasing concentrations of bile with the highest level of activation (56.5 ± 0.9%) being recorded in excysting medium containing 50% bile. For *I. felis*, however, concentrations of bile above 5% had little effect on the level of activation.

The presence of trypsin appeared to be less important for the activation of *I. felis* and *I. rivolta* than the presence of bile since the activation of both species was found to occur in its absence. For *I. rivolta* , however, higher levels of activation were recorded whenever trypsin was used than when it was not, although above 0.5%, concentration appeared unimportant. In contrast the activation of *I. felis* was found to occur at similar levels regardless of whether or not trypsin was present.

5.3.5 Effect of bile type or substitute

Oocysts of *I. felis* and *I. rivolta* , pretreated for 30 minutes with sodium hypochlorite, were incubated at 39°C for two hours in various excysting media. These excysting media, which contained 0.5% trypsin and tris buffer (pH 7.5), were made up of one of eight different bile types or substitutes. Frozen sheep, cattle, dog, cat. chicken and pig bile were used at concentrations of 10%. Sodium taurocholate was used at a concentration of 1.5% (w/v) and in one series of experiments 'Tween 80', at a concentration of 1×10^{-4}% (v/v) was substituted for bile.

The results, which are presented in Figure 5.5 revealed that activation of both species occurred in the presence of all bile types but not when 'Tween 80' was substituted for bile. Although all biles appeared equally effective for the activation of *I. felis* this was not the case for *I. rivolta* . For this latter species

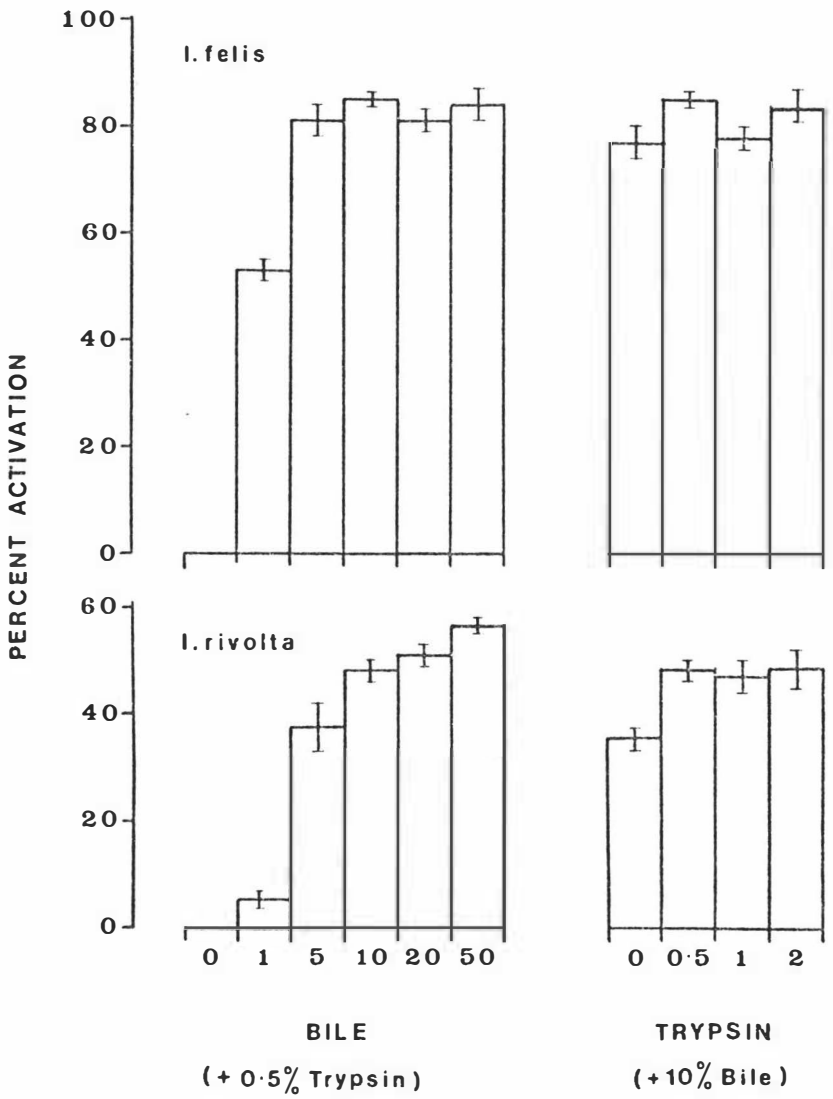


Fig. 5.4 Effect of bile and trypsin concentration on the activation of *I. felis* and *I. rivolta* sporozoites.

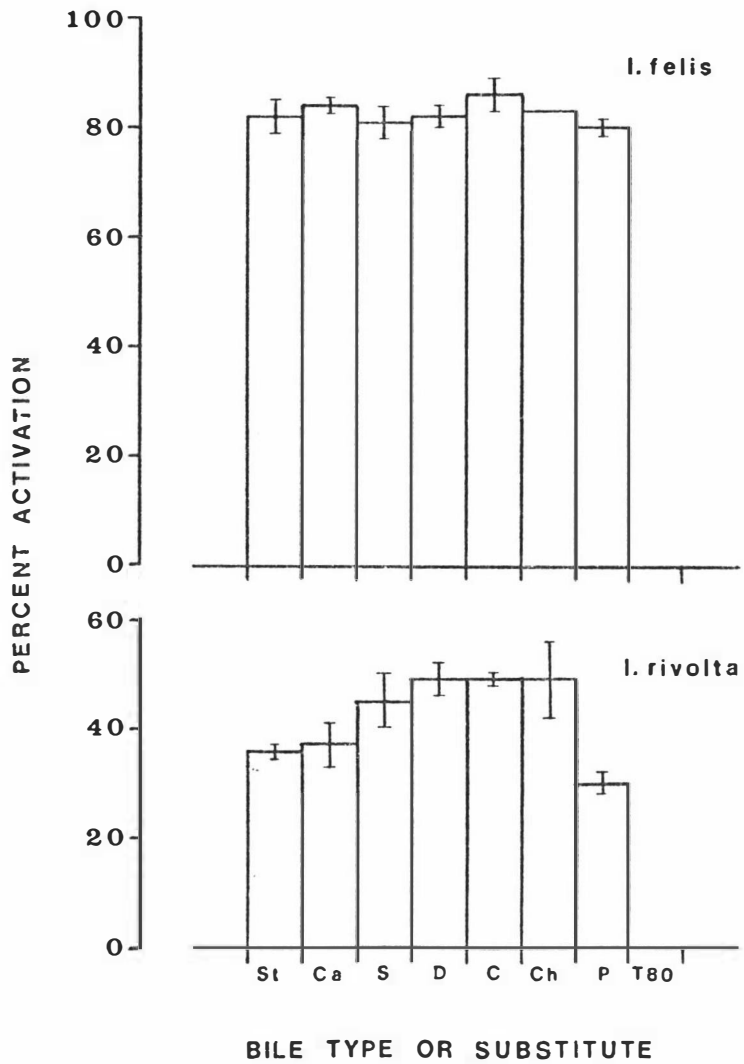


Fig. 5.5 Effect of bile type or substitute on the activation of *I. felis* and *I. rivolta* sporozoites. (St = sodium taurocholate, Ca = cattle bile, S = sheep bile, D = dog bile, C = cat bile, Ch = chicken bile, P = pig bile).

species the highest levels of activation were recorded whenever cat (48.5 ± 1.1%) or dog (49.3 ± 2.9%) bile were used and the lowest (30.0 ± 1.7%) when pig bile was employed. Relatively high levels of activation of *I. rivolta* (48.5 ± 6.5%) were also recorded in the presence of chicken bile; however, this bile appeared more erratic in its effect than did that of cats and dogs .

5.3.6 Effect of treatment temperature:

Following 30 minutes pretreatment with sodium hypochlorite, oocysts of *I. felis* and *I. rivolta* were suspended in excysting medium [10% ovine bile, 0.5% trypsin, tris buffer (pH 7.5)] and incubated at temperatures of 21, 25, 30, 35, 39 or 43°C for either one or six hours.

Activation of both species was found to occur at all temperatures tested following six hours incubation but not at the lowest temperature (21°C) after one hour's incubation (Figure 5.6). For *I. felis* the percentage activation was found to increase with increasing temperature up to 39°C and level off thereafter. A similar pattern was observed for *I. rivolta* although a levelling off at higher temperatures was not so apparent for this species. For both species higher levels of activation were recorded after six hours incubation than after one hour's incubation at all temperatures tested. However, for *I. felis* the differences in activation level between the two incubation intervals was found to decrease with increasing temperature.

5.3.7 Effect of hydrogen ion concentration:

In these experiments oocysts of *I. felis* and *I. rivolta* were pretreated for thirty minutes with sodium hypochlorite. They were then incubated at 39°C for two hours in excysting media containing 10% ovine bile, o.5% trypsin buffered to one of eight pH levels.

The buffers used in these experiments and their pH ranges were : tris mealeate [tris (hydroxymethyl) aminomethanemaleate], pH 5.0 to 6.5; tris [tris (hydroxymethyl) aminomethane], pH 7.0 to 8.5 and a sodium carbonate-bicarbonate buffer, pH 9.0 to 10.0

Hydrogen ion concentration appeared to have little influence on the activation of either *I. felis* or *I. rivolta* and percentage activation was found to vary little between the pH levels tested (Figure 5.7). For *I. felis* 70.1 ± 3.0% contained activated sporozoites at pH 5.0. At pH 6.0 and 7.0 the percentage activation increased to 73.6 ± 2.4 and 81.8 ± 1.5% respectively, and the maximum level of activation (87.0 ± 2.0%) was recorded at pH 7.5. The percentage activations at pH's 8.0, 8.5 and 9.0, which were 84.8 ± 1.2, 84.3 ± 1.6 and 84.1 ± 3.2 respectively, had declined to 78.8 ± 2.1% at pH 10. For *I. rivolta* 46.4 ± 2.2% of oocysts contained activated sporozoites at pH 5.0. This increased slightly to 49.8 ± 2.5, 49.0 ± 0.7 and 48.9 ± 3.1% at pH's 6.0, 7.0 and 7.5 respectively. The percentage activation dropped to the lowest level recorded (43.9 ± 1.7%) at pH 8.0 and then rose through 46.8 ± 2.4 and 56.1 ± 4.6% at pH's 8.5 and 9.0 to 60.5 ± 3.2% at pH 10.

5.3.8 The excystation process:

To study the excystation process of *I. felis* and *I. rivolta* , oocysts were pretreated for 30 minutes with sodium hypochlorite and suspended in Ringer's solution as previously described. One drop of this suspension was placed on a slide and to this drop was added a drop of excysting medium [10% ovine bile, 0.5% trypsin, tris buffer (pH 7.5)]. A coverslip was placed over the preparation and sealed with Vaseline. The slide was then examined under a microscope which was fitted with a warm stage set at 39°C.

The process of excystation was found to be essentially similar for both species (plates 5.1 and 5.2). Some oocysts appeared unaltered by the pretreatment regime but in others the oocysts wall was

folded into ridges and depressions and occasionally breaks in the wall were apparent. In oocysts so affected, this folding and wrinkling appeared to increase in prominence with increasing exposure to excysting medium and such changes were frequently accompanied by a flattening and indentation of one or both ends of the oocyst. In both altered and apparently unaltered oocysts, sporozoites were observed to begin movement within the sporocyst after approximately 10 to 15 minutes exposure to excysting medium. Initially, movement was slow and intermittent but progressively increased in vigour until it was fast and almost continuous. Suddenly, without warning, the sporocyst wall ruptured and the sporocyst residuum, closely followed by the sporozoites were expelled into the oocyst. The site of rupture appeared to be randomly located and on some occasions was followed by a second rupture shortly afterwards. Following the escape of the residuum and sporozoites, the walls of the sporocyst appeared to curl inwards to form four longitudinal plates which frequently separated.

The expelled sporocyst residuum, particularly that of *I. felis*, appeared very active and the separated granules were observed to continuously jig around within the oocyst, possibly as a result of Brownian movement. The liberated sporozoites were also very active at this stage and movement was achieved by a series of gliding and flexing motions. In some oocysts the release of the sporozoites from both sporocysts occurred almost simultaneously but more usually one sporocyst was observed to rupture several minutes before the other.

The time taken for the sporozoites to escape from the oocyst was found to vary greatly. In those oocysts in which breaks in the oocyst wall were present, complete excystation, frequently through one or other of the indented ends of the oocyst, was usually accomplished within five to 10 minutes of their liberation from the sporocyst. On some occasions sporozoites from one sporocyst had completed escape from the oocyst before rupture of the second sporocyst had taken place. Sporozoites in apparently

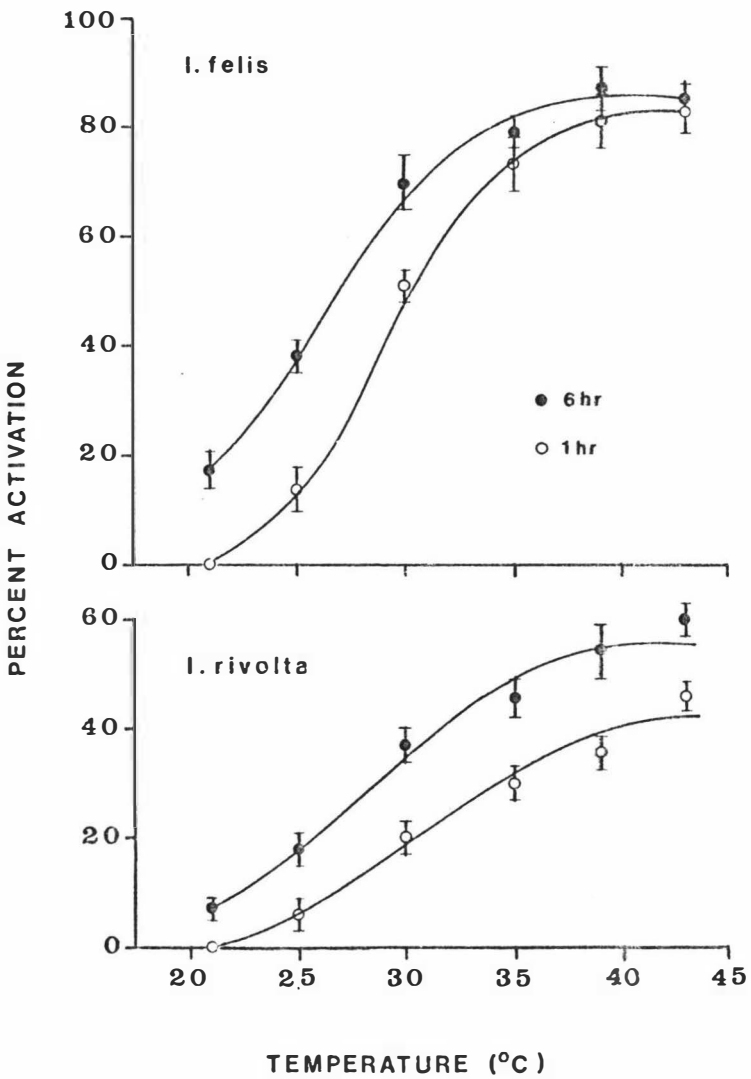


Fig 5.6 Effect of treatment temperature on the activation of *I. felis* and *I. rivolta* sporozoites.

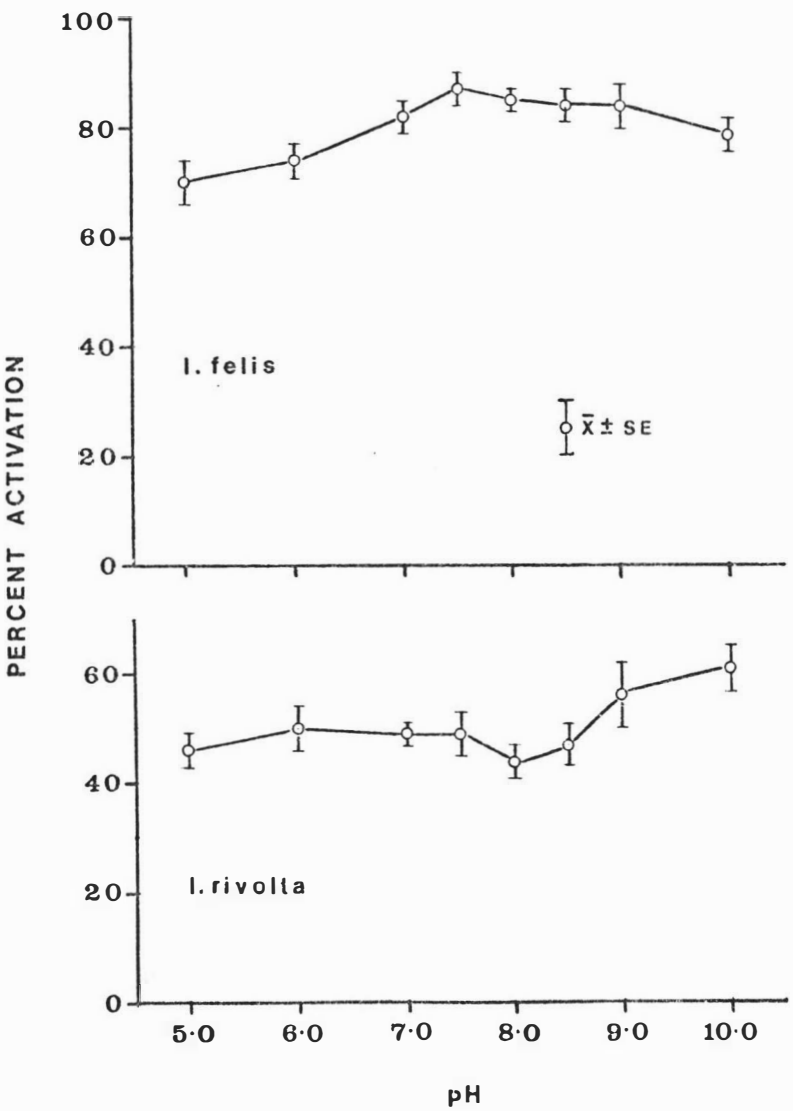


Fig. 5.7 Effect of hydrogen ion concentration on the activation of *I. felis* and *I. rivolta* sporozoites.

Plate 5.1 Photomicrographs of the excystation process of *I. felis* (x 3000). A. Sporulated oocyst before pretreatment. B. Similar oocyst following pretreatment with sodium hypochlorite; note rents and folds in the oocyst wall (arrows). C. Activation of one sporocyst and escape of sporozoites following treatment with trypsin and bile. D. Complete activation of both sporocysts. E. Completely excysted oocyst; note indented end of the oocyst (arrow) through which the sporozoites have escaped and the partially separated plates of the sporocyst wall. F, G and H. Liberated sporozoites. (OW = oocyst wall, SW = sporocyst wall, SR = sporocyst residuum, SP = sporozoite).

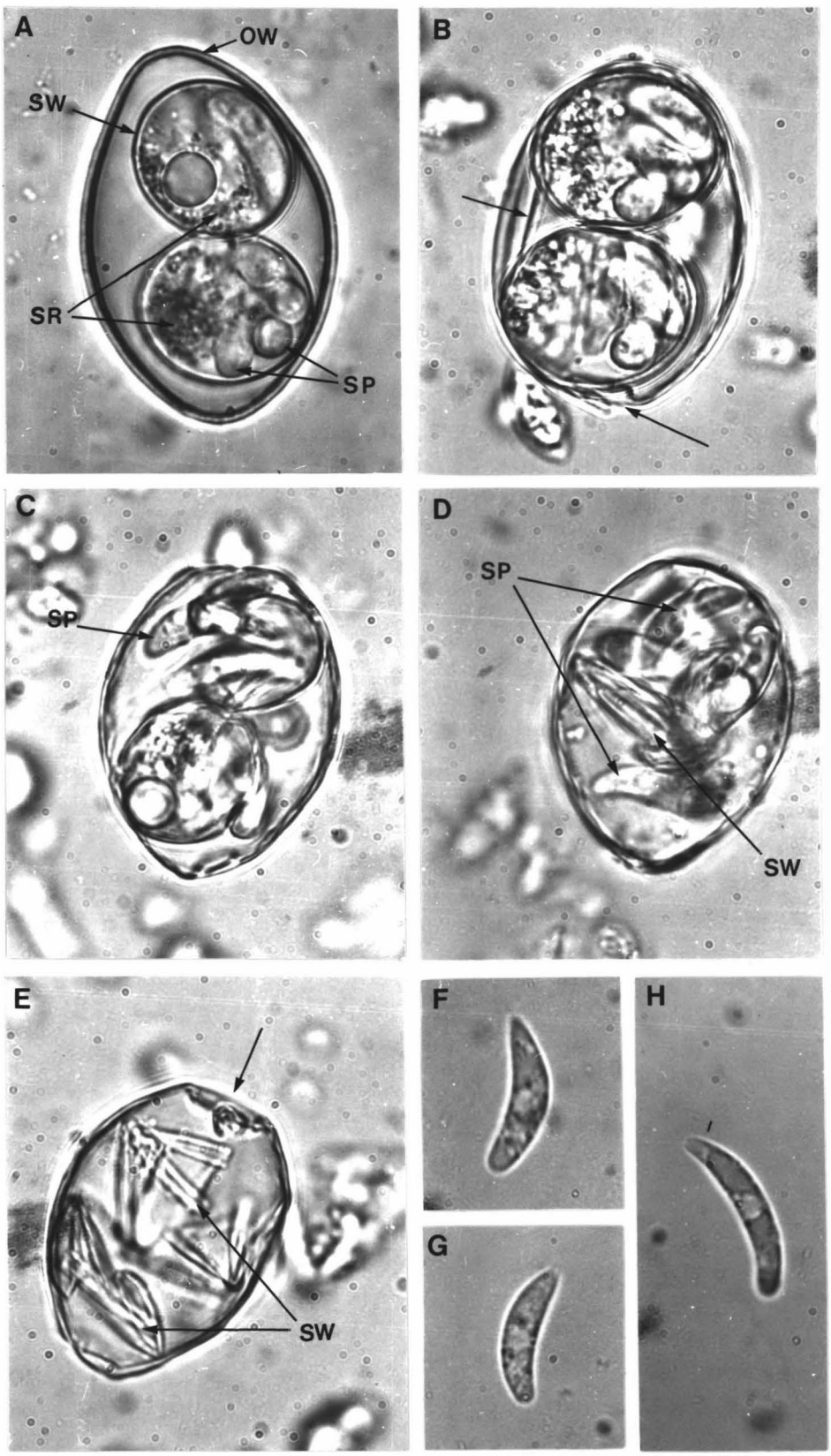
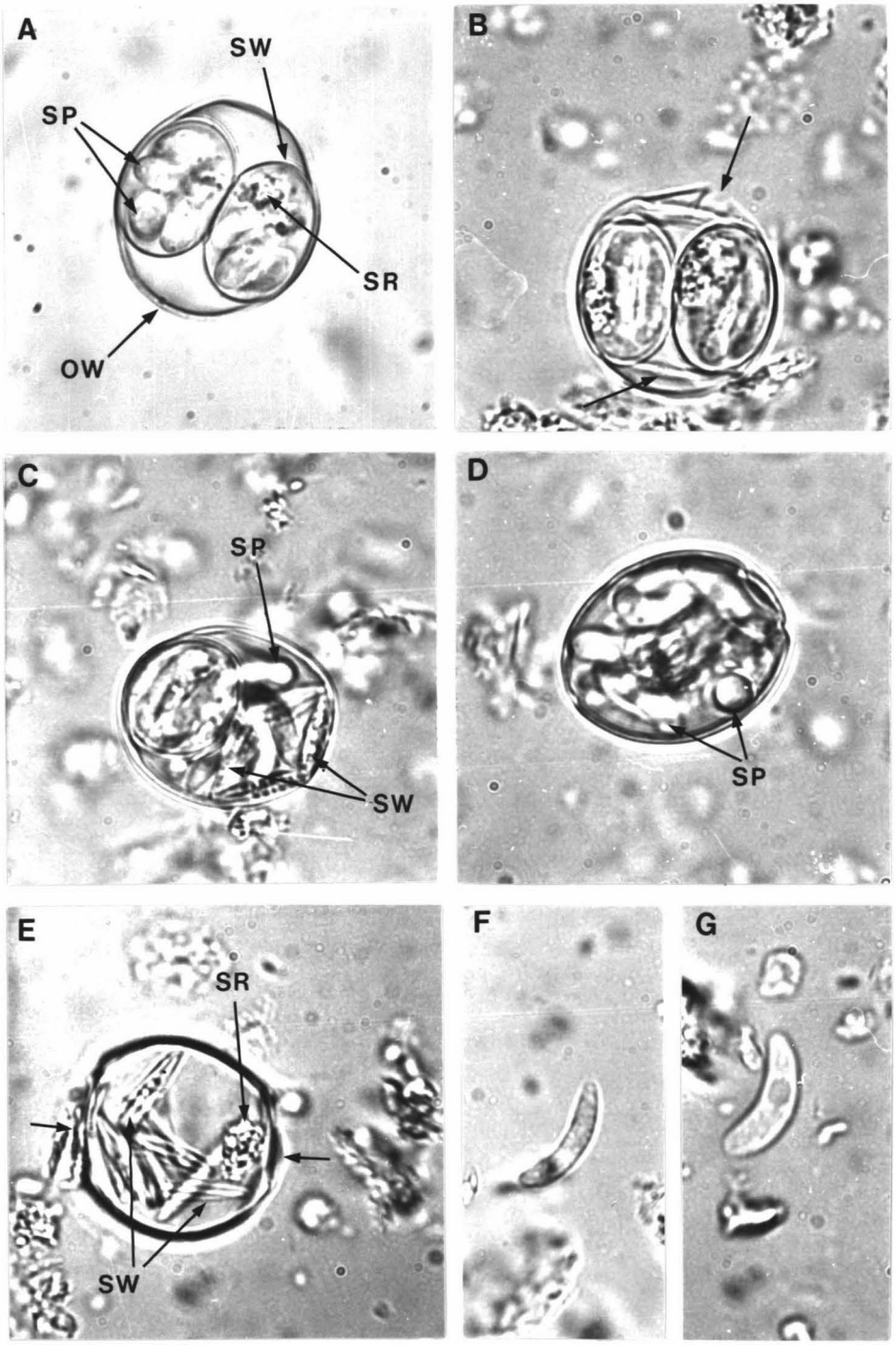

A
OW
SW
SR
SP
B
C
SP
D
SP
SW
E
SW
F
G
H

Plate 5.2 Photomicrographs of the excystation process of *I. rivolta* (x 3000). A. Sporulated oocyst before pretreatment. B. Similar oocyst following pretreatment with sodium hypochlorite; note rents and folds in the oocyst wall (arrows). C. Activation of one sporocyst following treatment with trypsin and bile; collapse of the sporocyst into four plates clearly evident. D. Complete activiation of both sporocysts. E. Completely excysted oocyst showing indented ends through which sporozoites have escaped and the separated plates of the sporocyst wall. F and G. Liberated sporozoites. (OW = oocyst wall, SW = sporocyst wall, SR = sporocyst residuum SP = sporozoite).


A
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undamaged oocysts however frequently appeared unable to escape and were observed to move around within the oocyst in a futile search for an exit for at least an hour until all movement ceased.

5.4 DISCUSSION

A direct comparison of the present results with those of previous workers is complicated by the fact that different investigators have used different techniques, terms and criteria to study the liberation of motile sporozoites from sporocysts and oocysts. Thus in some studies the term "excystation" has been used to describe the escape of sporozoites from both mechanically-freed sporocysts and from intact oocysts while estimates of "percentage excystation" have been variously based on: (a) counts of partially and completely empty sporocysts (Doran and Vetterling, 1969), (b) counts of completely empty oocysts (Hibbert and Hammond, 1968; Hibbert *et al.*, 1969), (c) counts of sporozoites free from sporocysts (Farr and Doran, 1962; Wang and Stotish, 1975) and (d) on counts of sporozoites free from oocysts (Doran and Farr, 1962). To further complicate an already confused situation, other workers (Nyberg and Hammond, 1964; Nyberg *et al.*, 1968; Bunch and Nyberg, 1970; Jensen *et al.*, 1976) have used another term, "percentage activation", to indicate the proportion of oocysts which are either partially or completely empty of sporozoites.

It is obvious, therefore, that there is a need for a uniform system of terminology. In studies on intact oocysts an appropriate system would be to restrict the term "activation" to the escape of the sporozoites from sporocysts and "excystation" to their subsequent escape from the oocyst. "Activation" and "excystation" may be classified as either partial (escape of at least one sporozoite) or complete (escape of all sporozoites). Under this system the liberation of sporozoites from mechanically-freed sporocysts should only be referred to as "activation" whereas for those coccidia in which the sporocysts are naturally shed free (e.g. *Sarcocystis* sp.) the terms "activation" and "excystation" may be considered synonymous.

This terminology was adopted in the present study which revealed that although activation was usually greater in pretreated than in non-pretreated oocysts of *I. felis* and *I. rivolta* , activation of both species could occur in its absence. Similar findings have been reported for *I. canis* of the dog (Bunch and Nyberg, 1970; Speer *et al.*, 1973) but not for the only other canine coccidian studied, *S. cruzi* (Fayer and Leek, 1973). Sporocysts of *S. cruzi* are only infective for cattle (Fayer, 1974; Fayer *et al.*, 1976a) and like other coccidia which are infective for this host the activation of this species has been found to be dependent on pretreatment (Fayer and Leek, 1973). Indeed, apart from the previously mentioned canine and feline species of *Isospora*, coccidial oocysts from almost all other hosts have also been found to require at least some form of pretreatment for activation to occur. Although there are exceptions to this generalisation much of the information concerning these exceptions is contradictory. Thus while some investigators (Ikeda, 1960; Smetana, 1933; Bunch and Nyberg, 1970) have been able to obtain activation and excystation in non-pretreated oocysts of *E. tenella*, *E. stiedae* and *E. Bilamellata*, other workers (Nyberg *et al.*, 1968; Goodrich, 1944; Hibbert and Hammond, 1968), have not. The reasons for these contradictions are unclear but may relate, at least in part, to differences in both the method and duration of oocyst storage. In the present investigation the level of activation of *I.rivolta* was found to be less dependent on pretreatment for oocysts stored in sulphuric acid than for those stored in potassium dichromate and it is possible, therefore, that similar differences in the effect of such solutions may be more exaggerated for other coccidian species. Unfortunately, however, information concerning the preservation of oocysts has not always been provided by earlier workers.

The inhibitory effect of potassium dichromate on the subsequent activation of coccidial oocysts has been previously noted by Jackson (1962) who suggested that this solution may reduce the permeability of the oocyst wall to excysting medium. This suggestion is

supported by the present findings which indicate that sulphuric acid may be a more suitable long-term oocyst preservative than potassium dichromate. Why pretreatment with sodium hypochlorite should apparently be mildly inhibitory for the activation of *I. rivolta* oocysts stored in sulphuric acid, however, is unclear. It is not likely that such an effect was merely due to a toxic effect of sulphuric acid on the sporozoites since non-pretreated oocysts stored in the same solution were apparently not affected. Nor does it seem likely that this effect was due to the toxicity of the sodium hypochlorite since; (a) a similar phenomenon was not observed in oocysts stored in potassium dichromate and (b) sodium hypochlorite has been used previously at much higher concentrations than employed in the present investigation without hindering the ability of other coccidia to excyst (Nyberg and Knapp, 1970; Speer *et al.*, 1973). It would appear, therefore, that a more likely explanation may be that the combined effects of sulphuric acid and sodium hypochlorite altered the oocyst or sporocyst walls in some way so that they were less permeable. The precise nature of such changes, if any, are unknown but are worthy of further investigation.

Sporozoites of different coccidian species are known to activate and excyst at different rates; in certain instances the rate of excystation may be directly correlated with the distance which must be travelled in the digestive tract to reach the usual site of endogenous development (Duszynski and Brunson, 1973). Like *I. canis* , which has been found to activate faster than coccidia from other hosts (Bunch and Nyberg, 1970), activation of both *I. felis* and *I. rivolta* was observed to occur rapidly after contact with excysting medium. The relative rapidity of this response for all these species of *Isospora* is not altogether surprising in view of the comparatively short length of the canine and feline small intestine.

Although both trypsin and bile are generally considered important ingredients of excysting media (Jackson, 1962) the present results indicate that the latter is the most essential for the activation of *I. felis* and *I. rivolta*. For both species activation was found to occur in the presence of bile alone but not when trypsin was used without bile. These findings are similar to those recorded for coccidia of dogs (Fayer and Leek, 1973; Speer *et al.*, 1973), chickens, squirrels and sheep (Hibbert and Hammond, 1968) but not for those of cattle which appear to be more dependent on the presence of trypsin than bile (Hibbert and Hammond, 1968; Hibbert *et al.*, 1969). Above a threshold level of 5%, the concentration of bile appeared to have little effect on the activation of sporozoites. For *I. rivolta* , however, the level of activation was found to further increase with increasing concentrations of bile above 5%. Differences between the two species in this respect may relate to the type of bile used since bile type appeared to be more important for the activation of *I. rivolta* than *I. felis*. Despite these differences both species were found to be capable of activation in a wide range of bile types but not when 'Tween 80' was substituted for bile. This latter result is in contrast to the findings of others (Jackson, 1962; Doran and Farr, 1962) who have reported that this and other surface active agents could be substituted for bile in activating oocysts of *E. arloingi* and free sporocysts of *E. acervulina*.

Like trypsin and bile, temperature and hydrogen ion concentration are also considered to be important factors for the activation and excystation of sporozoites *in vitro*. The effect of temperature has been studied by Hibbert and Hammond (1968) who found that excystation of bovine coccidia occurred between temperatures of 29 to 43°C and Jackson (1962) who reported that *E. arloingi* from sheep would not excyst at temperatures below 31°C. For all these species however, maximum excystation was found to occur between 39 and 41°C (i.e. close to body temperature). In the present investigation optimum activation of *I. felis* and *I. rivolta* was

found to occur at similarly high temperatures. Unlike coccidia of cattle and sheep, however, activation of these two species was also found to take place at temperatures as low as 21°C. Similar findings have been reported for *I. canis* (Speer *et al.*, 1973) suggesting that the ability of sporozoites to activate at relatively low temperatures may be a common feature of coccidia of cats and dogs.

Doran and Farr (1962) reported that activation of free sporocysts of *E. acervulina* occurred between pH's of 5.5 to 8.5 (the range tested) with optimum activation occurring at pH 7.6. In the study of Hibbert *et al.*, (1969) excystation of *E. bovis* was found not to occur at pH levels below 6.0. Above pH 6.0 excystation was found to increase markedly; maximum levels of excystation were recorded at pH's 7.5, 8.0 and 8.5 and declined thereafter. In the present study hydrogen ion concentration (pH 5.0 to 10.0) appeared to have little influence on the activation of either *I. felis* or *I. rivolta* and percentage activation was found to be broadly similar to all pH levels tested. The reasons for these differences in results as compared to those of previous workers are unknown. Perhaps it could be associated with the differing morphology and modes of activation and excystation of the coccidia species used.

Several investigators have described the details of the *in vitro* activation of various species of *Eimeria* and *Isospora* . These studies show two distinct patterns of activation which appear related to the structure of the sporocysts. Oocysts of many species of *Eimeria* , such as *E. bovis* have a micropyle and contain sporocysts with stieda bodies. Although sporocysts of *E. acervulina* also have stieda bodies, those of *I. felis* and *I. rivolta* do not and an oocyst micropyle is similarly absent. In those species which have sporocysts with stieda bodies the sporozoites always escape through a gap at one end of the sporocyst created by the dissolution of this structure (Doran and Farr, 1962; Nyberg and Hammond, 1964; Hammond *et al.*, 1970; Roberts *et al.*, 1970;

Duszynski and Brunson, 1972, 1973; Speer and Duszynski; 1975). In those species which have sporocysts which lack stieda bodies, however, such as *I. canis* and *I. endocallimici*, the sporocyst walls collapse and the sporozoites escape randomly (Speer *et al.*, 1973, 1976; Duszynski and File 1974). Ultrastructural studies of the sporocyst walls of the latter two species (Speer *et al.*, 1973, 1976) have revealed that they consist of two layers - a continuous thin outer layer and a relatively thick discontinuous inner layer of four separate curved plates. Excysting medium apparently acts upon the sites of apposition of these plates resulting in the rupture of the sporocyst wall and their eventual collapse into four pieces (Speer *et al.*, 1973, 1976). The process of activation of *I. felis* and *I. rivolta* observed in the present study was found to be comparable to that described for *I. canis* and *I. endocallimici* suggesting that their sporocyst walls are structually similar.

The subsequent means of escape of the sporozoites from the oocyst has also been found to vary. Thus in species such as *E. bovis* exit of the sporozoites takes place through the micropyle (Nyberg and Hammond, 1964) whereas in those species in which such a structure is lacking, as in *I. canis*, the sporozoites escape through ruptures created at one or both ends of the oocyst (Speer *et al.*, 1973). A similar escape of the sporozoites of *I. felis* and *I. rivolta* was observed in the present investigation. However, since complete excystation was not always accomplished *in vitro* it would appear likely that *in vivo* rupture of the oocyst wall may be further facilitated by enviromental agents prior to ingestion or by other physical and chemical factors following this event, or both.

6. GENERAL DISCUSSION

It is now over 100 years since coccidia were first recorded in the cat and dog. Since that time progress towards an understanding of the identity and biology of these species has advanced tremendously, particularly over the last seven or eight years. The major impetus for this progress was provided by the discovery that *Toxoplasma gondii* , until recently an enigmatic cyst-forming organism of uncertain taxonomic status, could be transmitted in the faeces of cats in the form of a coccidial oocyst. Subsequently, similar heteroxenous coccidian life cycles, involving the cat and dog as definitive hosts, were described for two other economically important parasites, *Besnoitia* and *Sarcocystis* , and a new genus of cyst-forming coccidia, *Hammondia*, was also discovered. These findings and a re-examination of the life cycles of other isosporan parasites of cats and dogs have forced parasitologists to review many of the basic assumptions concerning the coccidia in general and have emphasised the superficiality of our knowledge regarding the canine and feline coccidia in particular.

In New Zealand both toxoplasmosis and sarcosporidiosis are of considerable concern to the sheep industry. Attempts at limiting these infections, however, have been prevented by a lack of information concerning their origin and epidemiology. With the recent realisation of the coccidian nature of these organisms and the recognition of the central importance of the cat and dog in their transmission, the feasibility of instituting control measures has now increased. As a very preliminary first step towards the realisation of this objective, the study described in this thesis was carried out to provide, for the first time, some basic information concerning the coccidian parasites of cats and dogs in New Zealand. The initial part of the study, which was undertaken to determine the identity and prevalence of these protozoa, revealed four genera : *Isospora*, *Toxoplasma*, *Sarcocystis* and *Hammondia*. Although some species (e.g. *H. hammondi*) were not found,

it would, because of the limited geographic nature of the survey, be naive to assume that they are absent from the country as a whole. Indeed, a subsequent investigation led to the recovery of a species of *Besnoitia* from the faeces of a feral cat and it is likely, therefore, that further searches may reveal the existence of other species as well.

The effect of various factors such as host age, host origin, and season on the prevalence of infection was found to vary from species to species. Such variations are readily explicable in terms of differences in routes of transmission, host immunity and intermediate host specificity. Of particular interest in this regard was the observed prevalence of *Toxoplasma* and *Sarcocystis* infections. As recorded in similar surveys which have been carried out elsewhere (Table 2.1) the proportion of cats excreting oocysts of the former coccidian was found to be less than 1%. While these findings may not be totally unexpected inasmuch as cats usually shed *Toxoplasma* oocysts for only a short period of 1 to 3 weeks after primary infection (Table 1.8) and usually not at all following reinfection (Dubey *et al.*, 1970b) they do, however, present something of an anomaly. A recent summary of world wide reports (Vanderwagen *et al.*, 1974) has indicated that up to 66% of cattle and 96% of sheep may exhibit antibodies to *Toxoplasma* . As oocysts appear to be the main source of infection for herbivorous hosts (Hartley and Munday, 1974) these findings appear difficult to reconcile with the small numbers of cats which have been found to be excreting *Toxoplasma* in their faeces. In assessing the infective potential of this source, however, cognisance must be taken of the fact that a single cat may shed several million oocysts during patency (Dubey and Frenkel, 1972a) and that such oocysts may remain infective for at least a year in warm moist climates and even longer under cooler conditions (Yilmaz and Hopkins, 1972; Frenkel *et al.*, 1975). In addition, serelogical surveys in several countries have shown that about 40% of adult cats in nature have antibody to *T. gondii* (Dubey, 1968). Since it

may be assumed that the majority of such cats must have passed oocysts in their faeces at some time, the potential for infection by oocyst ingestion is likely to be much greater than faecal surveys may otherwise suggest.

In contrast to the findings for *Toxoplasma* , sporocysts of *Sarcocystis* were commonly encountered in cat and dog faeces. The patent periods of these coccidia are usually long (Tables 1.8 and 1.9) and although data relating to the number of sporocysts shed during patency are limited, levels of excretion attained are likely to be dependent on the amount of infected meat ingested. In the present investigation sporocyst excretion in naturally infected cats and dogs tended to be low with the majority shedding 200 sporocysts per gram of faeces or less. However, in dogs a mean count of 2,270 sporocysts per gram and in cats 597 per gram of faeces, was recorded. If the faecal output of a medium sized dog is assumed to be 250 grams per day (Anvik *et al.*, 1974) and that of a cat approximately one third of this amount, then these figures can be crudely translanted as representing an average excretion of about half a million sporocysts per day from infected dogs and 50,000 per day from infected cats. These estimates and the relatively high frequency of sporocyst shedding encountered in cats and dogs, may indicate a considerable source of *Sarcocystis* infection for susceptible intermediate hosts. However, an assessment of the epidemiological significance of such data is complicated by the fact that (a) information relating to the resistance and survival of these sporocysts is totally lacking and (b) that sporocysts recovered from the faeces of naturally infected cats and dogs are likely to represent a variable and indeterminate number of species. The specific identity of the sarcocystan sporocysts recovered in the present study are unknown although at least some from cats were demonstrated to be those of *S. muris*. The discovery of the latter species is of some interest since it may provide a useful laboratory animal model enabling *Sarcocystis* infections to be studied under carefully

controlled conditions. Such circumstances are difficult to achieve with cattle and sheep due to the ubiquity of naturally acquired *Sarcocystis* infections (Levine, 1973) and the expense of providing sterile environments for animals.

The detection of *Besnoitia* in New Zealand for the first time is also of more than passing interest since some species of this genus are known to be pathogenic for domestic livestock (Levine, 1973). The specific identity of the present species, however, is unknown although it shows similarities to *B. wallacei*. Despite these similarities and in view of the possible economic importance of this protozoan further investigation is obviously required.

Of the remaining two coccidian genera recorded in this study, *Hammondia* and *Isospora* , the former genus has an obligatory and the latter a faculatively, heteroxenous life cycle. The ability of *Isospora* species to ultilise intermediate hosts as an optional means of transmission has not been fully investigated but species from both cats and dogs have been shown to infect rats, mice and hamsters (Frenkel and Dubey, 1972a). Whether this constitutes the entire intermediate host range of these species, or whether other hosts, such as domesticated livestock, may serve equally well, is unknown. In the present investigation sporozoites of *I. felis* and *I. rivolta* from the cat were found to activate *in vitro* under a wide range of conditions; pretreatment did not appear to be an essential prerequisite for this process and both species exhibited a considerable tolerance to variations in temperature, pH, bile type and bile concentration. The precise relationship between these findings and the situation *in vivo* is unknown. Generally, however, it is considered that the stimuli necessary to achieve activation and excystation *in vitro* are essentially similar to those required *in vivo* (Jackson, 1962). Thus although it is readily conceded that the ability of coccidia to infect any one host is likely to be dependent on more constraints than those governing

activation and excystation alone, it is tempting, nevertheless, to speculate that the present results may indicate a potentially much greater intermediate host range for these species than has hitherto been demonstrated.

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Appendix 1 Synonyms of Canine and Feline Coccidian Species

(After Levine 1977a with modifications)

FELINE COCCIDIA

Isospora felis Wenyon 1923

Synonyms : Diplospora bigemina von Wasielewski 1904 pro parte; Isospora bigemina Swellengrebel 1914; I. rivolta Dobell and O'Conner 1921; I. cati Marotel 1921; Lucetina felis (Wenyon 1923) Henry and Leblois 1926; Levineia felis Dubey 1977; Cystoisospora felis Frenkel 1977.

Isospora rivolta (Grassi 1879) Wenyon 1923

Synonyms : Coccidium rivolta Grassi 1879; Diplospora bigemina von Wasielewski 1904 pro parte; Isospora rivoltae Dobell 1919; Lucetina rivolta (Grassi 1879) Henry and Leblois 1926; Isospora novocati Pellerdy 1974; Levineia rivolta Dubey 1977; Cytoisospora rivolta Frenkel, 1977

Besnoitia besnoiti (Marotel 1912) Henry 1913

Synonyms : Sarcocystis besnoiti Marotel 1912; Gastrocystis besnoiti (Marotel 1912) Brumpt 1913; Globidium besnoiti (Marotel 1912) Wenyon 1926.

Besnoitia wallacei (Tadros and Laarman 1976) Dubey 1977

Synonyms : Besnoitia sp. Wallace and Frenkel 1975

Besnoitia darlingi (Brumpt, 1913) Schneider 1967

Synonyms : Besnoitia panamensis Schneider 1965

Toxoplasma gondii (Nicolle and Manceaux 1908) Nicolle and Manceaux 1909

Synonyms : Leishmania gondii Nicole and Manceaux 1908; Toxoplasma cuniculi Spendore 1908; Toxoplasma canis Mello 1910; Toxoplasma talpae von Prowazek 1910; Toxoplasma columbae Yakimoff and Kohl-Yakimoff 1912; Toxoplasma pyrogenes Castellani 1913; Toxoplasma musculi Sangiorgi 1913; Toxoplasma sciuri Coles 1914; Toxoplasma ratti Sangiorgi 1915; Toxoplasma francae (de Mello 1915) Wenyon 1926; Toxoplasma caviae Carini and Migliano 1916; Toxoplasma

Nikanorovi Zasukhin and Gaisky 1930; Toxoplasma laidlawi Coutelen 1932; Toxoplasma wenyoni Coutelen 1932; Toxoplasma crocidurae Galli-Valerio 1933; Toxoplasma fulicae de Mello 1935; Toxoplasma hominis Wolf, Cowen, and Paige 1939; Toxoplasma gallinarum Hepding 1939.

Hammondia hammondi Frenkel and Dubey 1975

Synonmys : Toxoplasma hammondi Levine 1977.

Sarcocystis hirsuta Moule 1888

Synonmys : Miescheria cruzi Hasselmann 1926 pro parte; Sarcocystis fusiformis Railliet 1897 of Babudieri 1932 pro parte; S. fusiformis (Railliet 1897) Bernard and Bauche 1912; Sarcocystis bovifelis Heydorn, Gestrich, Melhorn and Rommel 1975.

Sarcocystis tenella Railliet 1886

Synonmys : Balbania gigantea Railliet 1886; Sarcocystis ovifelis Heydorn, Gestrich, Melhorn and Rommel 1975.

Sarcocystis porcifelis Dubey 1976

Synonyms : None

Sarcocystis muris (Blanchard 1885) Labbe 1899

Synonyms : Miescheria muris Blanchard 1885; Coccidium bigeminum var. cati Railliet and Lucet 1891; Sarcocystis musculi Blanchard 1885 of Kalyakhin and Zasukhin 1975.

Sarcocystis leporum Crawley 1914

Synonyms : Sarcocystis cuniculi Brumpt 1913

Sarcocystis cymruensis Ashford 1978

Synonyms: None

CANINE COCCIDIA

Isospora canis Nemeseri 1959

Synonyms : *Isospora felis* Wenyon 1923; *Levineia canis* Dubey 1977; *Cytoisospora canis* Frenkel 1977.

Isospora ohioensis Dubey 1975

Synonyms : *Isospora rivolta* (Grassi 1879) Wenyon 1923; *Levineia ohioensis* Dubey 1977; *Cytoisospora ohioensis* Frenkel 1977.

Hammondia heydorni (Tadros and Laarman 1976) Dubey 1977

Synonyms : *Isospora bigemina* (Stiles 1891) Luhe 1906; *Isospora wallacei* Dubey 1976; *Isospora heydorni* Tadros and Laarman 1976; *Hammondia bigemina* Frenkel 1977.

Sarcocystis cruzi Hasselmann 1926

Synonyms : *Miescheria cruzi* Hasselman 1926 *pro parte*; *Sarcocystis fusiformis* Railliet 1897; probably *Sarcocystis iturbei* Vogelsang 1938; *Sarcocystis marcovi* Vershinin 1975 *pro parte*; *Sarcocystis bovicanis* Heydorn, Gestrich, Melhorn and Rommel 1975.

Sarcocystis ovicanis Heydorn, Gestrich, Melhorn and Rommel 1975

Synonyms : *Sarcocystis tenella* Railliet 1886 *pro parte*.

Sarcocystis miescheriana (Kuhn 1865) Labbe 1899

Synonyms : *Synchytrium miescherianum* Kuhn 1865; *Sarcocystis miescheri* Lankester 1882; *Coccidium bigemina* var. canis Railliet and Lucet 1891 *pro parte*.

Sarcocystis bertrami Doflein 1901

Synonyms : *Sarcocystis equicanis* Rommel and Geisel 1975.

Sarcocystis Fayeri Dubey, Streitel, Stromberg and Toussant 1977

Synonyms : None.

Sarcocystis hemionilatrantis Hudkins and Kistner 1977

Synonyms : None.

Appendix 2

Results of χ^2 tests to determine the significance of the differences between the prevalence of coccidia in 'kitten' (up to 6 months of age) and 'adult' (over 6 months of age) cats.

I. felis	'Kittens'	'Adults'	Totals	
+ve	69	20	89	
-ve	102	304	406	$\chi^2_1 = 86.36^{***}$
Totals	171	324	495	$(\chi^2_1 (.001) = 10.83)$

I. rivolta	'Kittens'	'Adults'	Totals	
+ve	7	4	11	
-ve	164	320	484	$\chi^2_1 = 3.00$ (n.s.)
Totals	171	324	495	$(\chi^2_1 (.05) = 3.84)$

T. gondii	'Kittens'	'Adults'	Totals	
+ve	5	0	5	
-ve	166	324	490	$\chi^2_1 = 6.87^{**}$
Totals	171	324	495	$(\chi^2_1 (.01) = 6.64)$

Sarcocystis sp.	'Kittens'	'Adults'	Totals	
+ve	41	40	81	
-ve	130	284	414	$\chi^2_1 = 10.23^{**}$
Totals	171	324	495	

Appendix 3:

Results of χ^2 tests to determine the significances of the differences between the prevalence of coccidia in 'puppy' (up to 6 months of age) and 'adult' (over 6 months of age) dogs.

I. canis	'Puppies'	'Adults'	Totals	
+ve	18	1	19	
-ve	89	332	421	$\chi^2_1 = 49.58^{***}$
Totals	107	333	440	$(\chi^2_1 (.001) = 10.83)$

I. ohioensis	'Puppies'	'Adults'	Totals	
+ve	36	6	42	
-ve	71	327	398	$\chi^2_1 = 91.45^{***}$
Totals	107	333	440	

H. heydorni	'Puppies'	'Adults'	Totals	
+ve	6	7	13	
-ve	101	326	427	$\chi^2_1 = 2.36$ (n.s.)
Totals	107	333	440	

Sarcocystis sp.	'Puppies'	'Adults'	Totals	
+ve	63	192	255	
-ve	44	141	185	$\chi^2_1 = 0.01$ (n.s.)
Totals	107	333	440	

Appendix 4

Results of χ^2 tests to determine the significance of the differences between the prevalence of coccidia in male and female cats

I. felis		Male	Female	Totals	
	+ve	31	30	61	
	-ve	121	152	273	$\chi^2_1 = 0.61$ (n.s.)
	Totals	152	182	334	
I. rivolta		Male	Female	Totals	
	+ve	5	6	11	
	-ve	147	176	323	$\chi^2_1 = 0.09$ (n.s.)
	Totals	152	182	334	
T. gondii		Male	Female	Totals	
	+ve	1	4	5	
	-ve	151	178	329	$\chi^2_1 = 0.49$ (n.s)
	Totals	152	182	334	
Sarcocystis sp.		Male	Female	Totals	
	+ve	35	38	73	
	-ve	117	144	261	$\chi^2_1 = 0.12$ (n.s.)
	Totals	152	182	334	

Appendix 5

Results of χ^2 tests to determine the significance of the differences between the prevalence of coccidia in male and female dogs

I. canis		Male	Female	Totals	
	+ve	2	2	4	
	-ve	231	176	407	$\chi^2_1 = 0.00$ (n.s.)
	Totals	233	178	411	
I. ohioensis		Male	Female	Totals	
	+ve	7	10	17	
	-ve	226	168	394	$\chi^2_1 = 1.14$ (n.s.)
	Totals	233	178	411	
H. heydorni		Male	Female	Totals	
	+ve	7	5	12	
	-ve	226	173	399	$\chi^2_1 = 0.03$ (n.s.)
	Totals	233	178	411	
Sarcocystis sp.		Male	Female	Totals	
	+ve	135	104	239	
	-ve	98	74	172	$\chi^2_1 = 0.00$ (n.s.)
	Totals	233	178	411	

Appendix 6

Results of χ^2 tests to determine the significance of differences between the prevalence of coccidia in town and country cats

I. felis		Town	Country	Totals	
	+ve	75	14	89	
	-ve	408	11	419	$\chi^2_1 = 24.22$ ***
	Totals	483	25	508	$(\chi^2_1 (.001) = 10.83)$

I. rivolta		Town	Country	Totals	
	+ve	10	1	11	
	-ve	473	24	497	†
	Totals	483	25	508	

T. gondii		Town	Country	Totals	
	+ve	5	0	5	
	-ve	478	25	503	†
	Totals	483	25	508	

Sarcocystis sp.		Town	Country	Totals	
	+ve	82	4	86	
	-ve	401	21	422	$\chi^2_1 = 0.02$ (n.s)
	Totals	483	25	508	

(† = insufficient numbers for statistical analysis).

Appendix 7

Results of χ^2 tests to determine the significance of the differences between the prevalence of coccidia in town and country "kittens" (up to 6 months of age).

I. felis		Town	Country	Totals	
	+ve	55	14	69	
	-ve	99	3	102	$\chi^2_1 = 11.97$ ***
	Totals	154	17	171	$(\chi^2_1(.001) = 10.83)$

I. rivolta		Town	Country	Totals	
	+ve	6	1	7	
	-ve	148	16	164	†
	Totals	154	17	171	

T. gondii		Town	Country	Totals	
	+ve	5	0	5	
	-ve	149	17	166	†
	Totals	154	17	171	

Sarcocystis sp.		Town	Country	Totals	
	+ve	39	2	41	
	-ve	115	15	130	$\chi^2_1 = 0.89$ (n.s)
	Totals	154	17	171	

(† = insufficient numbers for statistical analysis).

Appendix 8

Results of χ^2 tests to determine the significance of the differences between the prevalence of coccidia in town and country "adult" (over 6 months of age) cats

I. felis		Town	Country	Totals	
	+ve	20	0	20	
	-ve	296	8	304	†
	Totals	316	8	324	

I. rivolta		Town	Country	Totals	
	+ve	4	0	4	
	-ve	312	8	320	†
	Totals	316	8	324	

T. gondii		Town	Country	Totals	
	+ve	0	0	0	
	-ve	316	8	324	†
	Totals	316	8	324	

Sarcocystis sp.		Town	Country	Totals	
	+ve	38	2	40	
	-ve	278	6	284	$\chi_1^2 = 0.31$ (n.s.)
	Totals	316	8	324	

(† = insufficient numbers for statistical analysis.)

Appendix 9

Results of χ^2 tests to determine the significances of the differences between the prevalence of coccidia in town and country dogs.

I. canis		Town	Country	Totals	
	+ve	18	1	19	
	-ve	324	138	462	$\chi^2_1 = 4.25^*$
	Totals	342	139	481	$(\chi^2_1 (.05) = 3.84)$
I. ohioensis		Town	Country	Totals	
	+ve	36	8	44	
	-ve	306	131	437	$\chi^2_1 = 2.16$ (n.s.)
	Totals	342	139	481	
H. heydorni		Town	Country	Totals	
	+ve	8	5	13	
	-ve	334	134	468	$\chi^2_1 = 0.21$ (n.s.)
	Totals	342	139	481	
Sarcocystis sp.		Town	Country	Totals	
	+ve	196	87	283	
	-ve	146	52	198	$\chi^2_1 = 0.93$ (n.s.)
	Totals	342	139	481	

Appendix 10

Results of χ^2 tests to determine the significance of the differences between the prevalence of coccidia in town and country "puppies" (up to 6 months of age).

		Town	Country	Totals	
I. canis					
	+ve	18	0	18	
	-ve	71	18	89	$\chi^2_1 = 3.05$ (n.s.)
	Totals	89	18	107	$(\chi^2_1(.05) = 3.84)$
I. ohicensis		Town	Country	Totals	
	+ve	31	5	36	
	-ve	58	13	71	$\chi^2_1 = 0.09$ (n.s.)
	Totals	89	18	107	
H. heydorni		Town	Country	Totals	
	+ve	5	1	6	
	-ve	84	17	101	$\chi^2_1 = 0.30$ (n.s.)
	Totals	89	18	107	
Sarcocystis sp.		Town	Country	Totals	
	-ve	48	15	63	
	-ve	41	3	44	$\chi^2_1 = 4.20$*
	Totals	89	18	107	

Appendix 11

Results of χ^2 tests to determine the significance of the differences between the prevalence of coccidia in town and country "adult" (over six months of age) dogs.

I. canis		Town	Country	Totals	
	+ve	0	1	1	
	-ve	224	108	332	†
	Totals	224	109	333	

I. ohioensis		Town	Country	Totals	
	+ve	4	2	6	
	-ve	220	107	327	$\chi^2_1 = 0.17$ (n.s.)
	Totals	224	109	333	

H. heydorni		Town	Country	Totals	
	+ve	3	4	7	
	-ve	221	105	326	$\chi^2_1 = 0.97$ (n.s.)
	Totals	224	109	333	

Sarcocystis sp.		Town	Country	Totals	
	+ve	130	62	192	
	-ve	94	47	141	$\chi^2_1 = 0.01$ (n.s.)
	Totals	224	109	333	

(† = insufficient numbers for statistical analysis).

Appendix 12

Results of χ^2 tests to determine the significance of the association between season and the prevalence of coccidia in cats.

I. felis	Spring	Summer	Autumn	Winter	Totals	
+ve	3	24	49	13	89	$\chi^2_3 = 98.3^{***}$
-ve	84	217	46	72	419	$(\chi^2_3(.001) = 16.27)$
Totals	87	241	95	85	508	

I. rivolta	Spring	Summer	Autumn	Winter	Totals	
+ve	1	2	4	4	11	$\chi^2_3 = 6.92$ (n.s.)
-ve	86	239	91	81	497	$(\chi^2_3(.05) = 7.81)$
Totals	87	241	95	85	508	

T. gondii	Spring	Summer	Autumn	Winter	Totals	
+ve	0	2	3	0	5	
-ve	87	239	92	85	503	$\chi^2_1 = 0.47$ (n.s.)
Totals	87	241	95	85	508	

Sarcocystis sp.	Spring	Summer	Autumn	Winter	Totals	
+ve	19	25	20	22	86	$\chi^2_3 = 14.85^{**}$
-ve	68	216	75	63	422	$(\chi^2_3(.01) = 11.34)$
Totals	87	241	95	85	508	

Appendix 13

Results of χ^2 tests to determine the significance of the association between season and the prevalence of coccidia in "kittens" (up to 6 months of age).

I. felis	Spring	Summer	Autumn	Winter	Totals	
+ve	1	16	40	12	69	
-ve	9	31	28	34	102	$\chi^2_3 = 18.17^{***}$
Totals	10	47	68	46	171	

I. rivolta	Spring	Summer	Autumn	Winter	Totals	
+ve	1	1	2	3	7	
-ve	9	46	66	43	164	$\chi^2_2 = 0.95$ (n.s.)
Totals	10	47	68	46	171	

T. gondii	Spring	Summer	Autumn	Winter	Totals	
+ve	0	2	3	0	5	
-ve	10	45	65	46	166	$\chi^2_2 = 1.91$ (n.s.)
Totals	10	47	68	46	171	

Sarcocystis sp.	Spring	Summer	Autumn	Winter	Totals	
+ve	2	12	10	17	41	
-ve	8	35	58	29	130	$\chi^2_3 = 7.64$ (n.s.)
Totals	10	47	68	46	171	

Appendix 14

Results of χ^2 tests to determine the significance of the association between season and the prevalence of coccidia in "adult" (over 6 months of age) cats.

I. felis	Spring	Summer	Autumn	Winter	Totals	
+ve	2	8	9	1	20	
-ve	68	186	17	33	304	$\chi^2_3 = 39.82^{***}$
Totals	70	194	26	34	324	

I. rivolta	Spring	Summer	Autumn	Winter	Totals	
+ve	0	1	2	1	4	
-ve	70	193	24	33	320	$\chi^2_1 = 5.19^{*}$
Totals	70	194	26	34	324	

T. gondii	Spring	Summer	Autumn	Winter	Totals	
+ve	0	0	0	0	0	
-ve	70	194	26	34	324	†
Totals	70	194	26	34	324	

Sarcocystis sp.	Spring	Summer	Autumn	Winter	Totals	
+ve	15	13	10	2	40	
-ve	55	181	16	32	284	$\chi^2_3 = 28.89^{***}$
Totals	70	194	26	34	324	

(† = insufficient numbers for statistical analysis).

Appendix 15

Results of χ^2 tests to determine the significance of the association between season and the prevalence of coccidia in dogs.

I. canis	Spring	Summer	Autumn	Winter	Totals	
+ve	12	4	1	2	19	$\chi^2_3 = 6.33$ (n.s.)
-ve	167	117	90	88	462	$(\chi^2_3(.05) = 7.81)$
Totals	179	121	91	90	481	

I. ohioensis	Spring	Summer	Autumn	Winter	Totals	
+ve	25	14	3	2	44	
-ve	154	107	88	88	437	$\chi^2_3 = 14.67$**
Totals	179	121	91	90	481	

H. heydorni	Spring	Summer	Autumn	Winter	Totals	
+ve	5	5	0	3	13	
-ve	174	116	91	87	468	$\chi^2_3 = 3.62$ (n.s.)
Totals	179	121	91	90	481	

Sarcocystis sp.	Spring	Summer	Autumn	Winter	Totals	
+ve	112	59	62	50	283	$\chi^2_3 = 9.80$*
-ve	67	62	29	40	198	
Totals	179	121	91	90	481	

Appendix 16

Results of χ^2 tests to determine the significance of the association between season and the prevalence of coccidia in "puppies" (up to 6 months of age).

I. canis	Spring	Summer	Autumn	Winter	Totals	
+ve	11	4	1	2	18	
-ve	29	34	10	16	89	$\chi^2_3 = 5.32$ (n.s.)
Totals	40	38	11	18	107	

I. ohioensis	Spring	Summer	Autumn	Winter	Totals	
+ve	22	12	1	1	36	
-ve	18	26	10	17	71	$\chi^2_3 = 17.62$***
Totals	40	38	11	18	107	

H. heydorni	Spring	Summer	Autumn	Winter	Totals	
+ve	2	2	0	2	6	
-ve	38	36	11	16	101	$\chi^2_2 = 0.13$ (n.s.)
Totals	40	38	11	18	107	

Sarcocystis sp.	Spring	Summer	Autumn	Winter	Totals	
+ve	27	21	6	9	63	
-ve	13	17	5	9	44	
Totals	40	38	11	18	107	$\chi^2_3 = 2.1$ (n.s.)

Appendix 17

Results of χ^2 tests to determine the significance of the association between season and the prevalence of coccidia in "adult" (over 6 months of age) dogs.

I. canis	Spring	Summer	Autumn	Winter	Totals	
+ve	1	0	0	0	1	
-ve	131	83	60	58	332	$\chi^2_1 = 0.09$ (n.s)
Totals	132	83	60	58	333	

I. ohioensis	Spring	Summer	Autumn	Winter	Totals	
+ve	3	2	1	0	6	
-ve	129	81	59	58	327	$\chi^2_3 = 3.08$ (n.s.)
Totals	132	83	60	58	333	

H. heydorni	Spring	Summer	Autumn	Winter	Totals	
+ve	3	3	0	1	7	$\chi^2_3 = 1.42$ (n.s.)
-ve	129	80	60	57	326	
Totals	312	83	60	58	333	

Sarcocystis sp.	Spring	Summer	Autumn	Winter	Totals	
+ve	80	38	38	36	192	
-ve	52	45	22	22	141	$\chi^2_3 = 6.57$ (n.s.)
Totals	132	83	60	58	333	