CRYPTOSPORIDIUM INFECTION IN CATS; EPIDEMIOLOGY AND CROSS TRANSMISSION STUDIES

by

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DECLARATION

I confirm that I have carried out the work presented in this thesis in the following laboratories: Medicine Department, University of Glasgow Veterinary School, Parasitology Department, Moredun Research Institute, Edinburgh and Department of Bacteriology, Stobhill Hospital, Glasgow.

Some of the work embodied in this thesis has been presented in the following publications:

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ABSTRACT

Cryptosporidium species are important gastrointestinal and respiratory coccidian parasites which affect a wide range of host species including man. The aim of the studies described in this thesis was to determine the prevalence of *Cryptosporidium* infection in cats in the Glasgow area.

Staining and concentration techniques for detection of *Cryptosporidium* oocysts in cat faecal specimens were first evaluated and used in the subsequent prevalence study. Modified Ziehl-Neelsen (MZN) and auramine phenol (A-P) techniques were found to be suitable for the screening of cat faeces for *Cryptosporidium* oocysts, and a fluorescein isothiocyanate (FITC) labelled monoclonal antibody (MAb) was used for confirmation of the diagnosis. Formol-ether (F-E) sedimentation and sucrose flotation techniques were effective for the concentration of *Cryptosporidium* oocysts in cat faecal specimens.

In the prevalence study, 26 out of 294 (8.8%) cats from Glasgow and the surrounding area, were positive for *Cryptosporidium* infection. Infection was more common in kittens than in adult cats, and in farm and feral than in domestic cats. However, 74% of the serum samples from cats in the same area were positive for specific anti-*Cryptosporidium* antibody. The prevalence of IgM and IgA isotypes was higher in sick than in healthy groups of cats.

Experimental transmission studies using *Cryptosporidium* spp. isolated from a farm cat into lambs and mice suggested that farm cats may be infected with the same *Cryptosporidium* isolates infecting other animals and that infection may be transmitted between various animal species on farms leading to a wide spread of the parasite.

This thesis concludes that *Cryptosporidium* infection is common in cats in the Glasgow area and that these animals should be regarded as a potential reservoir of infection for other animals and humans, in particular children and immunodeficient individuals.

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LIST OF ACRONYMS.

µl	=	microlitre(s)
g	=	gram(s)
g	=	force of gravity
mg	=	milligram(s)
cm	=	centimetre(s)
cm^2	=	square centimetre(s)
mm	=	millimetre
µm	=	micrometre(s)
M	=	molar
HCl	=	hydrochloric acid
PBS	=	phosphate buffered saline
$ZnSO_4$	=	zinc sulphate
F-E	=	formol-ether
FITC	=	fluorescein isothiocyanate
SDS	=	sodium dodecyl sulphate
FIV	=	feline immunodeficiency virus
FeLV	=	feline leukaemia virus
$K_2Cr_2O_7$	=	potassium dichromate
SG	=	specific gravity
MZN	=	modified Ziehl-Neelsen
A-P	=	auramine phenol
MAb	=	monoclonal antibody
IgG	=	Immunoglobulin G

IgM	=	Immunoglobulin M
IgA	=	Immunoglobulin A
r	=	coefficient of correlation
PI	=	post-infection
STDEV	=	standard deviation
SEM	=	scanning electron microscope
$\leq$	=	less than or equal to
$>$	=	greater than

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DEDICATION.

I dedicate this thesis to my parents Bibiana Angelo and Michael Mtambo.

GENERAL INTRODUCTION.

Cryptosporidium has emerged as one of the most important coccidian parasites affecting domestic animals and man. Although the parasite was first reported in 1907, its significance was only appreciated at the beginning of the last decade when infections were associated mainly with diarrhoea in calves (Panciera, Thomassen and Garner, 1971; Tzipori, Campbell, Sherwood, Snodgrass and Whitelaw, 1980a). Nevertheless, in medical practice *Cryptosporidium* was regarded as an opportunistic parasite mainly causing diseases in immunocompromised individuals (Weisberger, Hutcheon, Yardly, Roche, Hillis and Charache, 1979; Lasser, Lewin and Ryning, 1979; Stemmermann, Hayashi, Glober, Oishi and Frankel, 1980; Bird and Smith, 1980). However, the parasite was subsequently described in immunocompetent humans associated with diarrhoea. *Cryptosporidium* is now known to be widely spread causing gastrointestinal and respiratory infections in a broad range of host species. Diarrhoea is one of the main features in young and immunocompromised individuals while subclinical infection seems to be much more common in adult immunocompetent hosts (reviewed by Fayer and Ungar, 1986; Current, 1989; Casemore, 1990; Current and Garcia, 1991).

Transmission of *Cryptosporidium* is faecal-oral via any route whereby material contaminated with viable oocysts can reach the mouth. Various sources of oocysts have been incriminated including, infected animals, contaminated environments and water (reviewed by Casemore, 1990; Smith and Rose, 1990; Fayer, Speer and Dubey, 1990a). Zoonotic transmission has been documented

and in several occasions domestic cats have been implicated as the source of infection for humans (Koch, Shankey, Weinstein, Dye, Abt, Current and Eyster, 1983; Egger, Mai Nguyen, Schaad and Krech, 1990). Indeed, infection resulting from *Cryptosporidium* has been observed in diarrhoeic and non-diarrhoeic cats (Iseki, 1979; Bennett, Baxby, Blundell, Gaskell, Hart and Kelly, 1985; Poonacha and Pippin, 1982), and in cats with immunosuppression due to feline leukaemia virus (FeLV) infection (Monticello, Levy, Bunch and Fairley, 1987; Goodwin and Barsanti, 1990). Nevertheless, little is known about the prevalence of *Cryptosporidium* infection in cats, its association with diarrhoea, with FeLV or with the recently reported feline immunodeficiency virus (FIV) in cats.

Studies of the prevalence of *Cryptosporidium* have been conducted in humans and animals based upon the demonstration of oocysts in faecal specimens using Sheather's sucrose flotation (Sheather, 1923), Giemsa and modified Ziehl-Neelsen (MZN) staining (Hart, Baxby and Blundell, 1984; Reynolds, Morgan, Chanter, Jones, Bridger, Debney and Bunch, 1986; Snodgrass, Terzolo, Sherwood, Campbell, Menzies and Synge, 1986; Coleman, Klei, French, Chapman and Corstvet, 1989). Limited seroepidemiological surveys of *Cryptosporidium* have been performed using indirect immunofluorescence antibody techniques (IFAT) (Tzipori and Campbell, 1981; Campbell and Current, 1983; Koch, Phillips and Current, 1985; Casemore, 1987) or enzyme-linked immunosorbent assay (ELISA) (Ungar, Soave, Fayer and Nash, 1986). In cats, the prevalence of *Cryptosporidium* has only recently been determined in Japan, using sucrose flotation (Uga, Matsumura, Ishibashi, Yoda, Yatomi and Kataoka, 1989) and a combination of sucrose flotation and Kinyoun's acid-fast staining (Arai, Fukuda, Hara, Funakoshi, Kaneko, Yoshida,

Asahi, Kumada, Kato and Koyama, 1990). At present, only one serological survey of 23 cats is documented (Tzipori and Campbell, 1981). Techniques used routinely for the demonstration of *Cryptosporidium* oocysts in faecal specimens include, staining with MZN, auramine phenol (A-P), Giemsa, and sucrose and zinc sulphate flotation methods (reviewed by Fayer and Ungar, 1986). However, in several reports staining and concentration techniques failed to detect oocysts in cat faecal specimens whereas numerous endogenous developmental stages of *Cryptosporidium* were seen on histological examination of the same animals (Poonacha and Pippin, 1982; Monticello *et al.,* 1987). These findings probably resulted from the inherent inefficiency of the techniques, and/or low numbers of oocysts in the faecal specimens examined. Several evaluations of techniques for the detection of *Cryptosporidium* have been performed using materials from humans and cattle (Casemore, Armstrong and Sands, 1985b; Garcia, Bruckner, Brewer and Shimizu, 1983; Garza, Hopfer, Eichelberger, Eisenbach and Fainstein, 1984; McNabb, Hensel, Welch, Heijbel, McKee and Istre, 1985). Some workers found sucrose flotation superior to formalin-ether sedimentation (Ma, 1984; McNabb *et al.,* 1985) while others reported formalin-ether or PBS-ether more efficient (Casemore *et al.,* 1985b; Tzipori, 1988). No equivalent investigations have been carried out using faecal samples from household pets. Cryptosporidiosis is rare in dogs (Augustin-Bichl, Boch and Henkel, 1984) but relatively common in cats (Bennett *et al.,* 1985; Uga *et al.,* 1989; Arai *et al.,* 1990). In view of the zoonotic potential of *Cryptosporidium* and the closeness of companion animals to humans, studies of feline cryptosporidiosis would contribute to the knowledge of the epidemiology and facilitate control of the disease.

The objectives of this thesis include: the comparative evaluation of conventional staining and concentration techniques for detection of *Cryptosporidium* oocysts in cat faecal specimens; observations on the prevalence of *Cryptosporidium* infection in cats in the Glasgow area and its association with diarrhoea, feline leukaemia virus and feline immunodeficiency virus infections; and cross-transmission of *Cryptosporidium* sp. from a naturally-infected farm cat to lambs and mice in order to determine host specificity of the isolate.

CHAPTER I.

LITERATURE REVIEW: *CRYPTOSPORIDIUM* INFECTION IN ANIMALS AND MAN.

1.1. HISTORICAL BACKGROUND.

The first documented report of *Cryptosporidium* appeared in 1907, with the description of *C. muris* infection in the gastric glands of the common mouse (Tyzzer, 1907). The same author later reported a similar parasite, designated as *C. parvum*, in the small intestine of the common mouse (Tyzzer, 1912). The parasite has been subsequently reported in a variety of mammalian hosts, birds and reptiles, and such findings have been reviewed extensively by Anderson (1982), Levine (1984), O'Donoghue (1985) and Fayer and Ungar (1986).

The earliest reports suggesting *Cryptosporidium* infection in carnivores were in the Australian dingo (Bearup, 1954), and the Indian jungle cat (Dubey and Pande, 1963). However, it was shown later that the oocysts in both cases resembled sporocysts of the genera *Sarcocystis* or *Isospora* and not *Cryptosporidium* (Upton and Current, 1985; Vetterling, Jervis, Merrill and Sprinz, 1971a). *Cryptosporidium* was associated with diarrhoea for the first time in 1955, in turkey poults and the organism was named *C. meleagridis* (Slavin, 1955). Thereafter, cryptosporidial-associated diarrhoea was diagnosed in calves (Panciera *et al.*, 1971; Meuten, Van Kruiningen and Lein, 1974; Pohlenz, Moon, Cheville and Bemrick, 1978a), and in immunodeficient humans (Meisel, Perera, Meligro and Rubin, 1976; Weisberger *et al.*, 1979; Lasser *et al.*, 1979).

Earlier, *Cryptosporidium* was considered as an extracellular parasite, a feature used to distinguish it from other coccidian parasites (Tyzzer, 1907, 1912; Anderson, 1982; Pohlenz, Bemrick, Moon and Cheville, 1978b; Levine, 1984). However, transmission electron microscopy studies have since shown that *Cryptosporidium* is, in fact, intracellular but extracytoplasmic, developing just under the surface membrane of the host cell plasmalemma (Hampton and Rosario, 1966; Vetterling, Takeuchi and Madden, 1971b; Iseki, 1979; Bird and Smith, 1980; Current, Upton and Haynes, 1986).

1.2. CLASSIFICATION AND TAXONOMY OF *CRYPTOSPORIDIUM.*

Cryptosporidium belongs to the phylum Apicomplexa, class Sporozoasida and family Cryptosporidiidae. The main characteristic of the family is the development of endogenous stages of the parasite just below the surface epithelial plasma membrane of the host cell. Meronts have a knob-like attachment organelle, while microgametes are not flagellated. Members of the family Cryptosporidiidae are homoxenous, and the only known genus is *Cryptosporidium*. The main characteristics of the genus *Cryptosporidium* are the presence of the oocyst containing four sporozoites and a residual body, and the lack of a sporocyst (Levine, 1985).

For a long time the taxonomy of *Cryptosporidium* had been in dispute, apparently due to lack of adequate information with regard to intracellular and extracellular location of the parasite (Tzipori, Angus, Campbell and Grey, 1980b; Levine, 1984; Current, 1985). Since Tyzzer (1907) first reported *C. muris*, a further 19 species have been named in different animal hosts,

according to the species of the infected host (Levine, 1984; Current *et al.,* 1986). However, cross-transmission studies have revealed that *Cryptosporidium* is a parasite with little or no host specificity, and such findings have resulted in different opinions on the taxonomy of the organism (Tzipori *et al.,* 1980b; Levine, 1984; Current, 1985). Levine (1984), considered only four species as valid: *C. muris* (Tyzzer, 1907) the type species for mammals, *C. meleagridis* (Slavin, 1955) for birds, *C. crotali* (Triffit, 1925) for reptiles, and *C. nasorum* for fish (Hoover, Hoerr, Carlton, Hinsman and Ferguson, 1981). Later workers have demonstrated that *C. muris* and *C. parvum*, species formerly regarded as synonyms (Levine, 1984), are now known to be distinct but that *C. crotali*, previously described in reptiles, is not a valid species (Vetterling *et al.,* 1971a; Upton and Current, 1985). However, *C. serpentis* (Levine, 1981), once considered as a synonym of *C. crotali* (Levine, 1984) is now confirmed a valid species for reptiles (Upton, McAllister, Freed and Barnard, 1989; Smith, 1990). In addition to *C. serpentis*, several other subspecies of *Cryptosporidium* have been reported in reptiles, based on variations in oocyst size (Upton *et al.,* 1989). *C. parvum* is currently recognised as the type species for all mammalian hosts causing intestinal infections, whereas *C. muris* is responsible for infection in the stomach of the common mouse and abomasum of the bovine and gazelle (Tyzzer, 1907, 1912; Anderson, 1987; Pospischil, Stiglmair-Herb, Von Hegel and Wiesner, 1987). Since *C. parvum* is considered to be the most common species responsible for intestinal cryptosporidiosis in various mammalian hosts, it has been suggested that intestinal cryptosporidial isolates from various mammalian hosts be reported either as *C. parvum*, *Cryptosporidium* sp. or *Cryptosporidium* isolate, pending a review of the taxonomy (Angus, 1987; Current, 1988).

However, several *C. parvum* isolates differ in virulence in various hosts (Mead, Humphreys, Sammons and Sterling, 1990). The species of cryptosporidia currently recognized as valid are shown in Table 1.1.

Species	Host	Oocyst size
C. muris	mice, cattle	7.4 x 5.6 µm
C. parvum	mammals	5.0 x 4.5 "
C. baileyi	poultry	6.2 x 4.6 "
C. meleagridis	poultry	5.2 x 4.6 "
Cryptosporidium sp.	quails	~ 5.0 "
Cryptosporidium sp.	guinea pigs	~ 5.0 "
*C. serpentis**	reptiles	6.2 x 5.3 "

+ = After Current (1989); * = Upton *et al.*, (1989); ~ = approximately

Table 1.1. Valid *Cryptosporidium* species of birds, reptiles and mammals+

1.3. *CRYPTOSPORIDIUM* INFECTION IN VARIOUS HOSTS.

1.3.1. INFECTION IN HUMANS.

The first reports of *Cryptosporidium* infection in humans were published in 1976 (Nime, Burck, Page, Holscher and Yardley, 1976; Meisel *et al.,* 1976). However, the clinical importance of *Cryptosporidium* was only realised when the parasite was associated with diarrhoea in both immunodeficient and immunocompetent humans (Fayer and Ungar, 1986; Current, 1989; Hinnant, Schwartz, Rotterdam and Rudski, 1989). Human cryptosporidiosis has a world-wide distribution, and the disease is common in children and immunocompromised individuals, especially humans with the acquired immunodeficiency syndrome (AIDS)(Fayer and Ungar, 1986; Sinski, Szklarczyk, Oralewska and Socha, 1988; Cruickshank, Ashdown and Croese, 1988; Current, 1989).

Surveys on the prevalence of human cryptosporidiosis have been conducted in the developed and less developed countries, and in urban and rural areas (Fayer and Ungar, 1986; Cruickshank *et al.,* 1988; Sinski *et al.,* 1988; Current, 1989; Roberts, Green, Ma, Carr and Ginsberg, 1989). Prevalence levels of 1 to 2% of screened individuals have been reported in Europe, 0.6 to 4.3% in north America and 3 to 4% and 10 to 20% in developing countries (Fayer and Ungar, 1986). The prevalence of *Cryptosporidium* infection is higher in children than adults (Sinski *et al.,* 1988; Current, 1989). Serological surveys have also indicated a higher prevalence of IgG and/or IgM antibodies specific for *Cryptosporidium* in humans in developing countries (Current, 1989).

Cryptosporidium infection in humans is frequently sub-clinical (Roberts et

al, 1989), or characterised by self-limiting diarrhoea of less than 20 days duration (Tzipori, Smith, Birch, Barnes and Bishop, 1983; Angus, 1987; Sinski *et al.,* 1988). However, in immunocompromised patients the disease is usually severe, prolonged and characterised by profuse life-threatening diarrhoea (Reese, Current, Ernst and Bailey, 1982; Fayer and Ungar, 1986; Angus, 1987; Current, 1989). Other clinical features of human cryptosporidiosis include, anorexia, mild pyrexia, vomiting and abdominal discomfort (Tzipori *et al.,* 1983; Angus, 1987). In addition, respiratory infections have been observed in immunodeficient patients associated with chronic coughing, dyspnoea, bronchitis, and pneumonitis (Forgacs, Tarshis, Ma, Federman, Mele, Silverman and Shea, 1983; Borgaerts, Lepage, Rouvonoy and Vandepitte, 1984; Lewis, Hart and Baxby, 1985). *Cryptosporidium* infection has also been found in gall bladder epithelia of immunocompromised patients, associated with cholecystitis (Pitlik, Fainstein, Rios, Guarda, Mansell and Hersh, 1983; Hinnant *et al.,* 1989) and in one account, oocysts were detected in the sputum of a patient with AIDS (Miller, Wasseheit, Kirihara and Coyle, 1984).

Several authors have suggested the zoonotic transmission of oocysts from livestock and household pets to humans (Anderson, Donndelinger, Wilkins and Smith, 1982; Reese *et al.,* 1982; Blagburn and Current, 1983; Koch *et al.,* 1983; Bennett, *et al.,* 1985; Lewis *et al.,* 1985; Pohjola, Oksanen, Jokipii and Jokipii, 1986a; Current, 1989; Nagy, Janko and Molnar, 1989). Recently, respiratory cryptosporidiosis in an immunodeficient patient has been associated with *C. baileyi* infection, the first report of an avian *Cryptosporidium* species causing human infection (Ditrich, Palkovic, Sterba, Prokopic, Loudova and Giboda, 1991). Although domestic cats are frequently implicated as sources of human

cryptosporidiosis (Koch *et al.,* 1983; Bennett *et al.,* 1985; Hart *et al.,* 1984; Lewis *et al.,* 1985; Current, 1985), human-to-human transmission appears to be more common, especially amongst workers in hospitals and children in day care centres (Baxby, Hart and Taylor, 1983; Koch *et al.,* 1985; Current, 1989). The importance of water as a source of human cryptosporidiosis was realised when *Cryptosporidium* oocysts were detected in treated and untreated water samples (Musial, Arrowood, Sterling and Gerba, 1987; Rush, Chapman and Ineson, 1987; Ongerth and Stibbs, 1987; Smith, Girdwood, Patterson, Hardie, Greene, Benton, Tulloch, Sharp and Forbes, 1988; Smith and Rose, 1990; Hayes, Matee, O'Brien, McKinley, Logsdon, Rose, Ungar, Word, Pinsky, Cummings, Wilson, Long, Hurwitz and Juranek, 1989). Other possible sources of human infection with *Cryptosporidium* include: improperly pasteurized milk, hospital and laboratory equipment, and contaminated farm environment (Angus, 1983; Current, Reese, Ernst, Bailey, Heyman and Weinstein, 1983; Current, 1985; O'Donoghue, 1985; Fayer and Ungar, 1986).

1.3.2. INFECTION IN RUMINANTS.

Enteric cryptosporidiosis is common in cattle, being associated with diarrhoea in newborn calves (Schmitz and Smith, 1975; Pohlenz *et al.*, 1978a; Tzipori *et al.,* 1980a; Fayer and Ungar, 1986; Harp, Woodmansee and Moon, 1990). In sheep and goats, the disease occurs in lambs (Barker and Carbonell, 1974; Berg, Peterson and Freeman, 1978; Tzipori, Angus, Campbell and Clerihew, 1981b; Anderson, 1982; Angus, Sherwood, Hutchison and Campbell, 1982; Hiepe, Ruth, Heike and Schuster, 1985), and kids (Mason, Hartley and Tilt, 1981; Tzipori, Campbell and Angus, 1982; Matovelo, Landsverk and Posada, 1984).

Cryptosporidium also affects young deer (Tzipori, Angus, Campbell and Sherwood, 1981c; Angus, 1987; Angus, 1990; Simpson, 1992). Abomasal cryptosporidiosis has been reported in calves (Upton and Current, 1985; Moon and Woodmansee, 1986; Anderson, 1987), and mountain gazelles (Pospischil *et al.*, 1987).

In ruminants, enteric cryptosporidiosis is characterised by profuse diarrhoea and dehydration in newborn animals, and severe disease can occur when *Cryptosporidium* is combined with rotavirus, coronavirus and *E. coli* infections (Pohlenz *et al.*, 1978a; Snodgrass, Angus, Gray, Keir and Clerihew, 1980; Angus, 1983; Tzipori, 1983; Angus, 1987). *Cryptosporidium*-induced diarrhoea can cause high mortality in lambs, whereas in goats, mortality is low. In young deer, the main clinical signs include, inappetence and profound lethargy, and affected animals usually die after a short period of illness. Infection in calves, lambs and kids normally occurs during the first or second week of life. (Angus, 1987).

The prevalence of *Cryptosporidium* infection in cattle, sheep, goats and deer is not precisely known. However, surveys of infection in the UK have demonstrated that *Cryptosporidium* is second only to rotavirus as a major enteropathogen in calves (Snodgrass *et al.*, 1986; Reynolds *et al.*, 1986). Studies on the incidence of cryptosporidiosis in newborn lambs in the former German Democratic Republic (G.D.R.), showed an incidence of 32% before weaning. Eighty two percent of the overall incidence, involved lambs of 2 to 3 weeks of age (Hiepe *et al.*, 1985). *Cryptosporidium* has also been found to cause high mortalities in farmed red deer, one of the expanding areas in farming in Britain (Angus, 1988). Outbreaks of cryptosporidiosis in calves and lambs are

considered to be seasonal, and more cases have been reported during late winter and spring, followed by a second peak in autumn born calves (Angus, 1988). In the U.S.A., the prevalence of *C. muris* oocysts in the faeces of dairy cattle has been found to be 1.74%, more oocysts were detected in faeces from cows in milk (8.7%) than dry cows (6.7%) (Anderson, 1990).

Infected calves, wild rodents, dogs and cats have been reported as possible sources of cryptosporidiosis for cattle (Fayer and Ungar, 1986). Contamination of the environment and feeding utensils with oocysts are also sources of infection for farm animals, and the disease is more commonly found in artificially reared calves than sucking animals (Tzipori *et al.,* 1982; Angus, 1983; Current *et al.,* 1983; Current, 1985; O'Donoghue, 1985; Angus, 1988). Presently, there is no specific therapy for bovine cryptosporidiosis (Fayer and Ungar, 1986; Angus, 1987).

1.3.3. INFECTION IN HORSES.

Cryptosporidium infection is not uncommon in horses and has been diagnosed in both diarrhoeic and non-diarrhoeic animals (Fayer and Ungar, 1986; Fernandez, Gomez-Villamandos, Carrasco, Perea, Quezada and Gomez, 1988; Coleman *et al.,* 1989). Foals with combined immunodeficiency syndrome have succumbed to severe infections with *Cryptosporidium* and organisms have been found along the epithelia of small intestines, common bile duct, and major pancreatic ducts (Snyder, England and McChesney, 1978; Gibson, Hill and Huber, 1983; Mair, Taylor, Harbour and Pearson, 1990). *Cryptosporidium* infection has also been diagnosed in diarrhoeic and non-diarrhoeic immunocompetent foals (Gajadhar, Caron and Allen, 1985; Coleman *et al.*, 1989). While the prevalence of

Cryptosporidium in horses is not known, there would appear to be a wide distribution of the parasite in this species (Tzipori and Campbell, 1981; Angus, 1988; Coleman *et al.,* 1989).

1.3.4. INFECTION IN PIGS.

Natural and experimental *Cryptosporidium* infections have also been demonstrated in pigs (Kennedy, Kreitner and Strafuss, 1977; Tzipori, McCartney, Lawson and Rowland, 1981a; Fayer and Ungar, 1986; Tacal, Sobieh and El-Ahraf, 1987; Sanford, 1987; Lindsay, 1991). In most cases, infection is asymptomatic (Kennedy *et al.,* 1977), but non-haemorrhagic diarrhoea and unthriftiness have been reported in several cases (Sanford, 1987). Nevertheless, in the UK, *Cryptosporidium* infection in pigs does not appear to be an economic threat to pig farmers (Angus, 1988).

1.3.5. INFECTION IN DOGS.

Cryptosporidium infection would appear to be rare in dogs (Augustin-Bichl *et al.,* 1984; Pohjora, 1984; Simpson, Burnie, Miles, Scott and Lindsay, 1988; El-Ahraf, Tacal, Sobih, Amin, Lawrence and Wilcke, 1991). However, infections have been reported in diarrhoeic and non-diarrhoeic dogs (Wilson, Holscher and Lyle, 1983; Fukushima and Helman, 1984; Siski, Gosser and Styer, 1984; Turnwald, Barta, Taylor, Kreeger, Coleman and Pourciau, 1988; Greene, Jacobs and Prickett, 1990). *Cryptosporidium* has also been associated with pups immunosuppressed following infection with canine distemper (Fukushima and Helman, 1984; Turnwald *et al.,* 1988). Serological surveys revealed the presence of specific antibodies to *Cryptosporidium* in 16 of 20 dogs examined (Tzipori and

Campbell, 1981). In one study conducted in Edinburgh, of 101 faecal samples examined from dogs in a rescue centre, none was found positive for *Cryptosporidium* oocysts (Simpson *et al.,* 1988). These workers suggested that a similar survey be conducted in rural dwelling dogs. The clinical features of infection in dogs are not specific, however histopathological changes and the location of the endogenous stages of *Cryptosporidium* resemble those in other animals with enteric infections (Wilson *et al.,* 1983; Sisk *et al.,* 1984; Fukushima and Helman, 1984; Turnwald *et al.,* 1988).

1.3.6. INFECTION IN RHESUS MONKEYS.

In non-human primates, *Cryptosporidium* infection has been described in macaques (Fayer and Ungar, 1986; Blanchard, Baskin, Murphey-Corb and Martin, 1987). Diarrhoea has been the most common clinical feature in infected juvenile rhesus monkeys (Kovatch and White, 1972; Wilson, Day and Brummer, 1984). Disseminated cryptosporidiosis has also been associated with immunosuppression due to simian immunodeficiency virus infection (Blanchard *et al.,* 1987). Developmental stages of the parasite were found on the epithelia of the small intestine, common bile duct, intrahepatic ducts, pancreatic duct and gall bladder (Kovatch and White, 1972; Cockerell, Valerio and Garner, 1974 ; Wilson *et al.,* 1984; Blanchard *et al.,* 1987).

1.3.7. INFECTION IN SMALL MAMMALS.

Mice and rats are affected by two species of *Cryptosporidium*: *C. muris* which infects the stomach (Tyzzer, 1907, 1910; Iseki, 1986) and *C. parvum* which affects

the intestines (Tyzzer, 1912; Reese *et al.,* 1982; Goebel and Braendler, 1982; Fayer and Ungar, 1986). *Cryptosporidium* infection has also been found in raccoons, rabbits, guinea pigs and squirrels (Jervis, Merill and Sprinz, 1966; Vetterling *et al.,* 1971a; Fayer and Ungar,1986). However, *Cryptosporidium* sp. affecting guinea pigs may be a distinct species as it only affects this species (Vetterling *et al.,* 1971a; Current, 1989). Generally, cryptosporidiosis in small mammals occurs as an asymptomatic infection (Tyzzer, 1907,1910,1912; Vetterling *et al.,* 1971a; Inman and Takeuchi, 1979; Reese *et al.,* 1982; Current and Reese, 1986; Fayer and Ungar, 1986).

1.3.8. INFECTION IN BIRDS.

Cryptosporidium infection has been reported in a variety of avian species causing enteric and respiratory diseases (Fayer and Ungar, 1986; Blagburn, 1988). Two species of *Cryptosporidium* are considered specific for avian hosts, *C. meleagridis* (Slavin, 1955) and *C. baileyi* (Current *et al.,* 1986). *C. meleagridis* has been associated with diarrhoea in turkeys (Slavin, 1955), whereas *C. baileyi* has been responsible for infections of the respiratory tract and the bursa of Fabricius in broiler chickens (Current *et al.,* 1986). The infectivity of *C. baileyi* for other avian species has also been demonstrated, reflecting the lack of host specificity of the parasite (Lindsay, Sundermann and Blagburn, 1988). The species of *Cryptosporidium* responsible for enteric infections in birds other than turkeys is not known, and, in addition, *Cryptosporidium* sp. affecting quails, is distinct from *C. baileyi* and *C. meleagridis* (Current *et al.,* 1986; Lindsay *et al.,* 1988). Recently, *Cryptosporidium* sp. oocysts have been demonstrated in cloacal

scrapings and faecal specimens of gulls (*Larus* spp.) (Smith, personal communication). Experimental transmission studies have also revealed that *C. parvum* can cause respiratory infection in broiler chickens when inoculated by the intratracheal route (Lindsay, Blagburn and Ernest, 1987; Palkovic and Marousek, 1989).

In birds, respiratory cryptosporidiosis is characterised by gurgling respiration, rales, sneezing, coughing, sinusitis, serous ocular discharges and depression (Hoerr, Ranck and Hastings, 1978; Mason and Hartley, 1980; Dhillon, Thacker, Dietzel and Winterfield, 1981; Itakura, Goryo and Umemura, 1984; Glisson, Brown, Brugh, Page, Kleven and Davis, 1984; Whittington and Wilson, 1985; Ranck and Hoerr, 1987). Diarrhoea occurs as a result of intestinal infection (Lindsay *et al.,* 1988). Conjuctivitis and infections of the bursa of Fabricius and the cloaca have also been associated with *Cryptosporidium* infection in birds (Doster, Mahaffey and McClearen, 1979; Mason and Hartley, 1980; Randall, 1982, 1986).

Histologically, endogenous stages of *Cryptosporidium* in birds are similar to those reported in mammalian hosts (Itakura, Nakamura, Umemura and Goryo, 1985; Blagburn, 1988; Sundermann, Lindsay and Blagburn, 1987); however, unlike *C. parvum* three generations of meronts have been reported for *C. baileyi* (Current *et al.,* 1986).

1.3.9. INFECTION IN FISH AND SNAKES.

Cryptosporidium infection in fish was first reported in the naso tang (*Naso lituratus*) and the parasite designated *C. nasorum* (Hoover *et al.,* 1981). Later

Cryptosporidium was found in the carp (Pavlasek, 1983a), and cichlid fish (Landsberg and Paperna, 1986). Recently, *Cryptosporidium* infection has been described in the wild brown trout (*Salmo trutta*) in the Sheffield area (Rush, Chapman and Ineson, 1990). The main clinical signs in infected fish included, intermittent anorexia, regurgitation of food, passage of faeces containing undigested food, and emaciation. Pathological lesions involved atrophy of skeletal muscles, and, on histological examination, organisms were found on the tips of mucosal folds of the microvillous surface of the intestines (Hoover *et al.*, 1981).

In snakes, *Cryptosporidium* infection has been associated with midbody swelling, chronic hypertrophic gastritis and bronchopneumonia (Brownstein, Stranberg, Montali, Bush and Fortner, 1977; Fayer and Ungar, 1986; Sinski and Czarnogrecka, 1989). The main clinical signs were weight loss and regurgitation of undigested food. On histological examination, endogenous stages of *Cryptosporidium* were found on the brush border of the gastric epithelium associated with hyperplasia of the gastric mucosa, fibroplasia and collagenization of the submucosa. Changes in the lamina propria included, oedema and accumulation of inflammatory cells (Brownstein *et al.*, 1977; Fayer and Ungar, 1986). Mature snakes are more commonly affected by cryptosporidiosis than young ones (Brownstein *et al.*, 1977; Sinski and Czarnogrecka, 1989). At present, *C. serpentis* is considered as a valid species of *Cryptosporidium* in reptiles, with the possible existense of several subspecies (Upton *et al.*, 1989; Smith, 1990).

1.4. *CRYPTOSPORIDIUM* INFECTION IN CATS.

1.4.1. INTRODUCTION.

Cryptosporidium infection in domestic cats was first reported in Japan, in three 1 month old kittens, and two adult cats, none of which showed signs of clinical illness (Iseki, 1979). *Cryptosporidium* was later reported in cats with diarrhoea (Poonacha and Pippin, 1982: Bennett *et al.,* 1985), and non-diarrhoeic cats (Bennett *et al.,* 1985; Koch *et al.,* 1983), and in cats with immunosuppression due to feline leukaemia virus infection (Monticello *et al.,* 1987; Goodwin and Barsanti, 1990).

The species of *Cryptosporidium* recovered from domestic cats was first designated as *C. felis* (Iseki, 1979). This author regarded *C. felis* as a distinct species following failure of the parasite to cause infection in mice and guinea pigs. Nevertheless, *C. felis* was later included within the *C. muris* group together with *C. parvum* and other *Cryptosporidium* species found in mammalian hosts (Levine, 1984). However, *C. parvum* is now known as the only valid species for enteric infections of all mammalian hosts (Current, 1988). A dubious species, *Cryptosporidium curyi* was reported in eight cats, with oocysts measuring 32.3 x 31.1 μm and four naked sporozoites, but the validity of this species has not been confirmed (Ogassawara, Benassi, Larsson and Hagiwara, 1986).

1.4.2. MORPHOLOGY OF THE OOCYST AND ENDOGENOUS STAGES OF *CRYPTOSPORIDIUM.*

The morphology of oocysts and endogenous stages of *Cryptosporidium* in cats is similar to that in other mammalian species (Pohlenz *et al.,* 1978b; Iseki, 1979;

Poonacha and Pippin, 1982; Current and Reese, 1986). Cryptosporidial isolates from mammalian and animal hosts can be differentiated on the basis of oocyst size, and the location of the endogenous stages of the parasite in host cells (Current and Reese, 1986). *C. parvum* is confined to the brush borders of intestinal epithelial cells of mammalian species and measures approximately 5.0 x 4.5 µm (4.5-5.4 x 4.2-5.0 µm) (Upton and Current, 1985). *C. muris*, found in the gastric glands of the common mouse (Tyzzer, 1910,1912) and the abomasum of cattle (Anderson, 1987) and gazelles (Pospischil *et al.,* 1987) is larger, measuring 7.4 x 5.6 µm (7.4-7.9 x 5.3-6.5 µm) (Upton and Current, 1985). *C. parvum* oocysts from domestic cat isolates measure approximately 5.0 x 4.5 µm (Iseki, 1979), from humans 5.2 x 5.1 µm (4.6-5.6 x 4.2-5.6 µm) (Current and Reese, 1986), whereas *C. baileyi* and *C. meleagridis* from avian species measure approximately 6.2 x 4.6 µm (6.3-5.6 x 4.8-4.5 µm), and 4.5 x 4.0 µm (4.5-6.0 x 4.2-5.3 µm), respectively (Current *et al.,* 1986; Lindsay and Blagburn, 1990).

Endogenous stages of the parasite appear as round to ovoid bodies, 2 to 6 µm in diameter, located on the microvillous region of the host intestinal epithelial cells (Meuten *et al.,* 1974; Kennedy *et al.,* 1977; Brownstein *et al.,* 1977; Snyder *et al.,* 1978; Inman and Takeuchi, 1979; Bird and Smith, 1980; Hoover *et al.,* 1981; O'Donoghue, 1985). Of the reported cases in cats, cryptosporidial forms ranged from 1 to 5 µm in size (Iseki, 1979; Poonacha and Pippin, 1982).

1.4.3. ULTRASTRUCTURE OF *CRYPTOSPORIDIUM* SP.

Much of the ultrastructural studies of *Cryptosporidium* sp. in cats were performed by Iseki (1979), who found that the morphological characteristics were similar to

those reported in other mammalian hosts (Iseki, 1979; Current and Reese, 1986). When examined under transmission electron microscopy the trophozoite appeared spherical to ovoid in shape, containing a large nucleus, nucleolus, golgi complex, rough endoplasmic reticulum, micronemes and ribosomes (Iseki, 1979). The attachment area between the trophozoite and the host cell is composed of membraneous folds continuous with the parasite pellicle, below which is a thick electron-dense band (Iseki, 1979). The membrane covering the parasite originates from the host cell microvillus, and the parasite assumes an intracellular but extra-cytoplasmic location (Vetterling *et al.,* 1971b; Iseki, 1979). A mature trophozoite is oval or round, 2.0 to 2.5 μm in diameter, with a relatively large nucleus 1.0 to 1.3 μm in diameter, containing a prominent nucleolus. A mature meront is round, 4 to 5 μm in diameter and possesses eight banana-shaped merozoites. In other mammalian species, two types of meronts are present; one type contains eight merozoites and is known as the first generation meront, while the other type contains four merozoites, and is known as the second generation meront (Vetterling *et al.,* 1971a; Pohlenz *et al.,* 1978b; O'Donoghue, 1985; Current and Reese, 1986). In birds, infection with *C. baileyi* results in three generations of the meronts known as Types I, II and III, with eight, four and eight merozoites, respectively (Current *et al.,* 1986). In cats, only one mature meront stage containing eight merozoites is known (Iseki, 1979).

The anterior region of the merozoite contains several dense bodies (rhoptries), micronemes and membrane-bound electron-pale vacuoles. The mature merozoite has a centrally-located nucleus without a nucleolus, and measures 5.0 μm long by 1 μm wide. The rough surfaced endoplasmic reticula are located at the posterior region of the merozoite while the conoid is at the

anterior end. Each merozoite is surrounded by a pellicle composed of three (outer, middle and inner) unit membranes, the outer membrane is separated from the other two by an electron-pale space. On the outer surface of the middle unit membrane there are several electron-dense fibril-like structures which are distributed at regular intervals around the merozoite. At the end of merogony the parasitophorous vacuole contains free merozoites, a small 0.5 to 0.8 μm residual mass of cytoplasm and an attachment organ. (Iseki, 1979).

The microgametocyte (microgamont) is spherical to subspherical in shape, measuring 4.0 μm to 5.0 μm in diameter, and contains 14 to 16 bullet-shaped non-flagellated microgametes (Iseki, 1979; O'Donoghue, 1985; Current and Reese, 1986). The macrogamont measures approximately 4 μm to 5.5 μm in diameter and possesses numerous polysaccharide granules, electron-dense bodies, wall-forming bodies, membrane-bound vesicles and rough-surfaced endoplasmic reticula (Vetterling *et al.,* 1971a,b; Iseki, 1979; Current and Reese, 1986).

At the area of attachment between the parasite and the host cell, there is a thick electron-dense adhesion zone, formed by the host cell (Vetterling *et al.,* 1971b; Iseki, 1979; Bird and Smith, 1980; Lumb, Lanser and O'Donoghue, 1988). The parasite pellicle at the adhesion zone is convoluted repeatedly, forming a comb-like metabolic lamella designated "feeder organelle" by Tyzzer (1912), presumably increasing the surface area for nutrient absorption (Hampton and Rosario, 1966; Vetterling *et al.,* 1971b; Iseki, 1979; Matovelo *et al.,* 1984). The "feeder organelle" develops when the trophozoite is formed and persists throughout endogenous development of the parasite (Iseki, 1979; Current and Reese, 1986).

The exogenous stage which is the oocyst, consists of a wall enclosing four sporozoites, and a crystalline residual body (Pohlenz *et al.,* 1978b; Iseki, 1979; O'Donoghue, 1985; Current *et al.,* 1986). Studies using a bovine isolate of *C. parvum* have shown that the oocyst wall is made up of two layers, the inner and outer layers (Reduker, Speer and Blixt, 1985a; Current and Reese, 1986). Within the inner layer, opposite the region of amylopectin accumulation, there is a suture of approximately 3 μm in length (Reduker, Speer and Blixt, 1985b; Current and Reese, 1986). Sporozoites are C-shaped and each is surrounded by a pellicle similar to that occurring in the merozoites, and contains a prominent nucleus, micronemes, electron-dense bodies, electron-pale vacuoles and highly condensed ribosomes in its cytoplasm (Iseki, 1979).

1.4.4. LIFE CYCLE.

The life cycle of *Cryptosporidium* in domestic cats is similar to that in other mammalian hosts (Pohlenz *et al.,* 1978b; Iseki, 1979; Current *et al.,* 1986; Current, 1988). The detailed life cycle of *Cryptosporidium* in cats was described by Iseki (1979), following experimental infections using *Cryptosporidium* oocysts from naturally-infected cats (Iseki, 1979) (Figure 1.1). Following ingestion of the sporulated oocysts, excystation occurs in the gastrointestinal tract (Iseki, 1979; Fayer and Leek, 1984; Reduker *et al.,* 1985b; Current *et al.,* 1986). The sporozoites then become motile and invade intestinal epithelial cells to undergo asexual proliferation, known as merogony or schizogony (Iseki, 1979; Fayer and Leek, 1984; Reduker *et al.,* 1985b; Current *et al.,* 1986; Current and Reese, 1986; Sundermann *et al.,* 1987). During this penetration process the microvillus invaginates, and its membrane extends along the surface of the sporozoite to

cover the parasite completely (Iseki, 1979).

The nucleus of the trophozoite (uninucleated meront) divides several times to produce eight merozoites (Pohlenz *et al.,* 1978b; Iseki, 1979; Current and Reese, 1986). Mature merozoites break through the parasitophorous envelope to invade new microvilli, and develop into second generation meronts (Iseki, 1979; Angus, 1983; Current and Reese, 1986). In cats, the number of merozoites in the second generation meront has never been verified (Iseki, 1979). It is not known how often the schizogonic stage is repeated but after several divisions the merozoites invade new cells and differentiate into the micro- and macrogamonts. The macrogamont develops into a macrogamete, while the microgamont produces up to 16 bullet shaped microgametes. (Iseki, 1979; Goebel and Braendler, 1982; Current and Reese, 1986). Mature microgametes are released by rupture of the parasitophorous envelope, and they exhibit gliding or jerky forward movements which facilitate penetration into the macrogamete. It has been postulated that the host cell membrane covering the macrogametes possesses specific receptors for the attachment and penetration of the microgametes. (Current and Reese, 1986).

Following fertilization, the zygote is produced which develops into an oocyst. In several studies, two types of oocysts have been reported, the first type has a thick wall, while the other type has a thin wall. Thick walled oocysts are already sporulated, and are passed intact in faeces, whereas, thin walled oocysts may rupture within the intestinal lumen, to cause autogenous reinfection of the host cells (Current and Reese, 1986). In the cat, the prepatent period ranges between 5 to 6 days post-infection whereas, the patent period is 7 to 10 days. *Cryptosporidium* oocysts were seen in faeces of one cat up to 5 months

post-infection. The number of oocysts discharged in the faeces of infected cats decreases with time, and intermittent discharge of oocysts has also been observed (Iseki, 1979). Unlike the situation in other species, where the second generation meronts have four merozoites, Iseki (1979) could not verify the number of merozoites in the second generation meronts of cat origin. In other mammalian species, the prepatent period ranges from 2 to 9 days, while the patent period is variable (O'Donoghue, 1985). However, recently intrauterine infection has been documented in the deer (Simpson, 1992). In young susceptible hosts, the life cycle may be completed within 3 to 4 days (Angus, 1987).

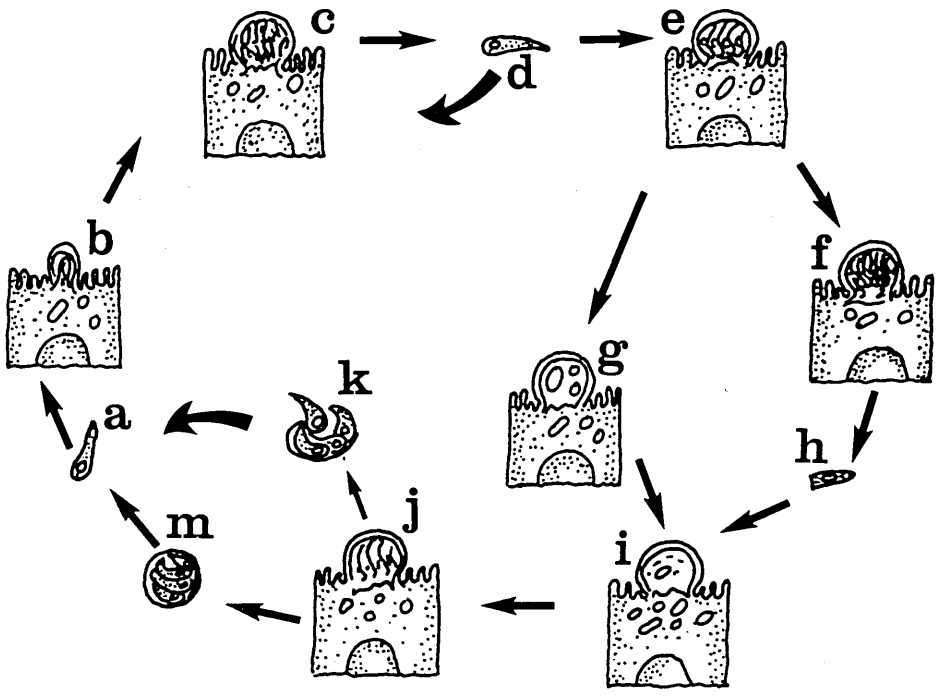

FIGURE 1.1. Diagrammatic representation of the life cycle of *C. parvum* in mammalian host. **a.** A sporozoite; **b.** A trophozoite; **c.** First generation meront with eight merozoites; **d.** A merozoite released from a mature meront; **e.** Second generation meront with four merozoites; **f.** microgamont; **g.** Macrogamete; **h.** Microgamete released from a microgamont; **i.** A zygote; **j.** A mature oocyst; **k.** Thin walled oocyst; **m.** Thick walled oocyst.

1.4.5. EPIDEMIOLOGY.

Prevalence and geographical distribution.

Little is known about the prevalence of *Cryptosporidium* infection in cats (Monticello *et al.,* 1987). Infection has been reported in cats in England (Bennett *et al.,* 1985), U.S.A. (Poonacha and Pippin, 1982; Monticello *et al.,* 1987; Koch *et al.,* 1983), Japan (Iseki, 1979; Arai *et al.,* 1990) and Czechoslovakia (Pavlasek, 1985). In a recent survey in Tokyo, Japan, 23 out 608 domestic cats were infected with *Cryptosporidium*, a prevalence of 3.8% (Arai *et al.,* 1990). The prevalence of infection was higher in kittens than adult cats but none of the infected cats had diarrhoea (Arai *et al.,* 1990).

Transmission and sources of infection.

Cryptosporidiosis is currently recognized as a zoonosis and the disease has been described in human patients who have been in contact with infected cats or other animals (Anderson *et al.,* 1982; Reese *et al.,* 1982; Blagburn and Current, 1983; Koch *et al.,* 1983; Bennett *et al.,* 1985; Lewis *et al.,* 1985; Pohjola *et al.,* 1986a). Transmission of *Cryptosporidium* infection in cats is faecal-oral by ingestion of materials contaminated with *Cryptosporidium* oocysts (Iseki, 1979). The source from which companion animals acquire *Cryptosporidium* infection is not clearly understood (Sisk *et al.,* 1984). Although rodents have been suggested as a possible source of *Toxoplasma gondii* infection in stray and farm cats, no report has associated rodents as possible sources of *Cryptosporidium* infection in cats. Infection has been described in both a cat and its owner, but it was difficult

to incriminate either of them as a source of infection for the other (Koch *et al.,* 1983; Hart *et al.,* 1984; Lewis *et al.,* 1985; Bennett *et al.,* 1985). On the other hand, pet cats and occasionally dogs, have been implicated as sources of human cryptosporidiosis, especially to children and immunocompromised individuals (Angus, 1983; Current *et al.,* 1983; Koch *et al.,* 1983; Sisk *et al.,* 1984; Bennett *et al.,* 1985; Current, 1985; Fayer and Ungar, 1986; Monticello *et al.,* 1987; Sinski *et al.,* 1988).

Viability of *Cryptosporidium* oocyst.

The viability of *Cryptosporidium* oocysts from the cat is not known. However, *Cryptosporidium* oocysts from some other mammalian hosts have been found to be extremely resistant to a variety of commonly used disinfectants (Blewett, 1988; Campbell, Tzipori, Hutchison and Angus, 1982; Angus *et al.,* 1982), including iodophores, cresilic acid, sodium hydroxide, sodium hypochlorite, benzylkonium chloride (Campbell *et al.,* 1982), and the aldehyde-based disinfectants Tegodor (Th.Goldschmidt) and formula-H (Hoechst) (Angus *et al.,* 1982).

At one time, ammonia and formal saline were the only known effective disinfectants against *Cryptosporidium* oocysts (Campbell *et al.,* 1982). However, Blewett (1988), reported some other disinfectants effective against *Cryptosporidium* oocysts, including Oocide (Antec International Ltd.), hydrogen peroxide, and Exospor (Alcide Corporation). Oocide is an oocysticide compound containing a biocide together with ammonia and sodium hydroxide. The latter two are partially effective but in combination with the former constitute a very strong disinfectant against *Cryptosporidium* oocysts (Blewett,

1988). Exspor (Alcide Corporation), a chloride dioxide based disinfectant is ineffective in materials containing proteins, hence its use against *Cryptosporidium* oocysts is limited (Blewett, 1988).

Cryptosporidium oocysts can survive a wide range of climatic changes (O'Donoghue, 1985). However, freeze drying and exposure to temperatures of -70°C or above 65°C for 30 minutes destroy the viability of the oocysts (Campbell *et al.,* 1982; Tzipori, 1983). Irrespective of the method of storage, the infectivity of *Cryptosporidium* oocysts for mice is lost when exposed to a temperature of 4°C for 2 to 6 month period (Moon and Bemrick, 1981; Sherwood, Angus, Snodgrass and Tzipori, 1982).

1.4.6. PATHOGENICITY AND PATHOGENESIS.

Pathogenicity.

The pathogenicity of *Cryptosporidium* sp. in domestic cats is not clearly understood. While Iseki (1979) found no clinical signs in any cats with *Cryptosporidium* infection, other workers have associated diarrhoea with the parasite (Poonacha and Pippin, 1982; Bennett *et al.,* 1985). Furthermore, *Cryptosporidium* has been associated with chronic diarrhoea in cats with immunosuppression due to feline leukaemia virus infection (Monticello *et al.,* 1987; Goodwin and Barsanti, 1990). Nevertheless, on several occasions the parasite has also been detected in faecal specimens from asymptomatic cats (Iseki, 1979; Koch *et al.,* 1983; Bennett *et al.,* 1985; Arai *et al.,* 1990).

Pathogenesis.

As in other mammalian species, the pathogenesis of *Cryptosporidium* diarrhoea in the cat is not clear (Tzipori *et al.,* 1983). The occurrence of diarrhoea in animals with *Cryptosporidium* infection is considered to be associated with reduced digestive enzyme activity and malabsorption due to stunting and fusion of intestinal villi (Angus, 1987; Tzipori *et al.,* 1983). Decreased amounts of lactase in the intestinal lumen impairs digestion of lactose and an osmotic effect is created in the gut lumen. The combined effect of malabsorption and the osmotic effect of lactose, presumably plays a role in the pathogenesis of diarrhoea in patients with cryptosporidiosis. (Angus, 1987; Tzipori *et al.,* 1983). Malabsorption of dietary nutrients has also been assessed using pulmonary hydrogen gas (H_2) concentrations, in calves experimentally infected with *Cryptosporidium* isolate (Holland, Herdt and Refsal, 1989). However, further studies are necessary for a better understanding of the pathogenesis of cryptosporidiosis in both animals and humans (Sinski *et al.,* 1988).

1.4.7. EXPERIMENTAL TRANSMISSION STUDIES.

Experimental transmission studies of *Cryptosporidium* infection in domestic cats have been successfully carried out using *Cryptosporidium* isolates from cats (Iseki, 1979; Asahi, Koyama, Funakoshi, Yamaura, Shirasaka and Okutomi, 1991), cattle (Augustin-Bichl *et al.,* 1984; Pavlasek, 1983b), and immunodeficient humans (Current *et al.,* 1983). Cats have also been experimentally-infected with a *C. muris* (strain RN 66) from a house rat (Iseki, Maekawa, Moriya, Uni and Takada, 1989). Attemps to infect mice, guinea pigs (Iseki, 1979; Asahi *et al.,*

1991), rabbits (Iseki, 1979), rats and dogs (Asahi *et al.*, 1991) with *Cryptosporidium* isolates from cats were unsuccessful. No studies have yet involved the use of *Cryptosporidium* oocysts from the cat into calves, lambs or avian species, or the use of oocysts from birds into cats.

Mild diarrhoea was observed in cats which were experimentally infected with *Cryptosporidium* isolate from a calf (Pavlasek, 1983b), but no significant clinical sign was observed with oocysts from immunodeficient patients (Current *et al.*, 1983). Recent studies have revealed severe gastric infections in cats, following oral inoculation with *C. muris* (strain RN 66) isolated from the house rat (Iseki *et al.*, 1989). The author considered *C. muris* (strain RN 66), to be better adapted in cats than mice. Hitherto, natural infection with *C. muris* has never been reported in cats.

1.4.8. CULTIVATION OF CRYPTOSPORIDIUM IN CELL CULTURES AND CHICKEN EMBRYOS.

Complete development of *C. parvum* has been demonstrated in cell cultures of human foetal lungs, primary chicken kidney, porcine kidney, baby hamster kidney, and human rectal tumours (Woodmansee and Pohlenz, 1983; Current and Haynes, 1984; Naciri, Yvore, Boissieu and Esnault, 1986). Recently, asexual developmental stages of *C. parvum* have been demonstrated in a differentiated human enterocyte cell line (Flanigan, Aji, Marshall, Soave, Aikawa and Kaetzel, 1991). *C. baileyi* from broiler chickens has been grown in avian embryos and the oocysts retained infectivity after 10 to 20 passages in chicken embryos (Lindsay *et al.*, 1988).

1.4.9. CLINICAL SIGNS AND PATHOLOGY.

Clinical Signs.

Cryptosporidiosis in cats is an enteric infection with or without signs of diarrhoea (Iseki, 1979; Poonacha and Pippin, 1982; Bennett *et al.,* 1985; Monticello *et al.,* 1987). However, clinical cases of cryptosporidiosis in cats are very rare, infection being predominantly asymptomatic (Iseki, 1979; Bennett *et al.,* 1985; Current *et al.,* 1983; Angus, 1987; Arai *et al.,* 1990). Nevertheless, persistent diarrhoea, anorexia, depression, weight loss, and dehydration have been associated with *Cryptosporidium* infection in cats (Poonacha and Pippin, 1982; Bennett *et al.,* 1985; Monticello *et al.,* 1987). Kittens (Bennett *et al.,* 1985; Arai *et al.,* 1990) and adult cats with immunosuppression due to FeLV infection (Monticello *et al.,* 1987; Goodwin and Barsanti, 1990) are considered to be more susceptible to cryptosporidiosis. However, the association between *Cryptosporidium* infection and immunosuppressive viral conditions like FeLV and the recently reported feline immunodeficiency virus (FIV) (Pedersen, Ho, Brown and Yamamoto, 1987), is not clearly known.

Pathological features.

Gross and microscopic pathological lesions in cats with *Cryptosporidium* infection are similar to those reported in other animal species (Monticello *et al.,* 1987). Histopathological changes have been reported in the small and large intestines of infected cats (Poonacha and Pippin, 1982; Iseki, 1979). Lesions are found in the small intestines, caecum and colon (Monticello *et al.,* 1987; Poonacha and

Pippin, 1982; Iseki, 1979). Histopathological lesions are characterised by atrophy and fusion of intestinal villi, cryptitis, distended crypts accompanied by accumulation of necrotic cell debris, and infiltration of the lamina propria by mononuclear cells and neutrophils (Poonacha and Pippin, 1982; Sisk *et al.,* 1984; Monticello *et al.,* 1987; Wilson *et al.,* 1983; Current and Reese, 1986; Tzipori, 1983). Other changes include the alteration of glandular structure with mild hyperplasia of the crypt epithelium (Panciera *et al.,* 1971; Poonacha and Pippin, 1982; Monticello *et al.,* 1987). In the caecum and colon, lesions include mild hyperaemia and dilatation of the crypts, usually filled with mucus and necrotic cell debris (Poonacha and Pippin, 1982; Moon and Bemrick,1981; Moon, Schwartz, Welch, McCann and Runnels, 1982a).

Histologically, cryptosporidial forms appear as round, or coccoid basophilic bodies, measuring 1 to 6 μm in size, attached to the microvillous region of villus epithelium and luminal epithelium of the crypts (Poonacha and Pippin, 1982; Monticello *et al.,* 1987; Sisk *et al.,* 1984; Fukushima and Helman, 1984).

1.4.10. DIAGNOSIS.

Diagnosis of feline cryptosporidiosis based on clinical signs is difficult due to the non-specific clinical presentation of infected cats (Iseki, 1979; Koch *et al.,* 1983; Bennett *et al.,* 1985; Monticello *et al.,* 1987). However, cats with persistent diarrhoea in the presence of FeLV infection should be suspected of having cryptosporidiosis (Monticello *et al.,* 1987). Laboratory diagnosis of *Cryptosporidium* infection in cats has been achieved using direct smear staining

of faecal specimens with Giemsa and safranin methylene blue and concentration methods with zinc sulphate and sucrose flotation methods (Iseki, 1979; Koch *et al.,* 1983; Bennett *et al.,* 1985; Lewis *et al.,* 1985).

A number of techniques have been employed for the diagnosis of *Cryptosporidium* infection in man and animals, including faecal examination for *Cryptosporidium* oocysts (Garcia *et al.,* 1983; Casemore, Sands and Curry, 1985a; Pohjola, Neuvonen, Niskanen and Rantama, 1986b; McLauchlin, Casemore, Harrison, Gerson, Samuel and Taylor, 1987; Angus, 1987), serology (Tzipori and Campbell, 1981; Campbell and Current, 1983; Van Opdenbosch and Wellemans, 1985; Casemore, 1987), animal inoculation tests (Reese *et al.,* 1982), and histopathology (Sisk *et al.,* 1984; O'Donoghue, 1985; Monticello *et al.,* 1987; Angus, 1987).

Various techniques, ranging from simple staining to monoclonal antibody tests, are used for detection of *Cryptosporidium* oocysts in faecal samples from man and animals suspected of being infected (Garcia *et al.,* 1983; O'Donoghue, 1985; Casemore *et al.,* 1985b; Sterling and Arrowood, 1986; McLauchlin *et al.,* 1987; Baron, Schenone and Tanenbaum, 1989; Rusnak, Hadfield, Rhodes and Gaines, 1989; Arrowood and Sterling, 1989). Simple staining procedures include modified Ziehl-Neelsen (MZN) (Henriksen and Pohlenz, 1981; Garcia *et al.,* 1983; Angus, 1987), auramine phenol (A-P) staining (Casemore *et al.,* 1985b; Giemsa staining (Pohlenz *et al.,* 1978a), and safranin with malachite green or methylene blue (Baxby, Blundell and Hart, 1984). Other methods occasionally used are negative staining with nigrosin (Pohjola, 1984) and iodine wet mounts (Ma and Soave, 1983).

MZN and A-P staining techniques are preferred and used routinely for

diagnosis of *Cryptosporidium* infection in humans and animals (Angus, 1987; McLauchlin *et al.,* 1987). In both methods faecal smears are fixed with methanol (Henriksen and Pohlenz, 1981; Casemore *et al.,* 1985b; Angus, 1987) or by heat (Garcia *et al.,* 1983). With MZN staining, fixed smears are stained with strong carbol fuchsin for 5 to 30 minutes, then differentiated in acid alcohol solution (1% to 3% concentrated HCl in 96% ethanol) until no more colour leaches out, then rinsed with tap water and counter stained in 0.4% to 5% malachite green, for 1 to 5 minutes (Henriksen and Pohlenz, 1981; Casemore *et al.,* 1985b; Angus, 1987). The smear is examined under bright field light microscopy, at x400 or x1000 magnification. *Cryptosporidium* oocysts appear as deep red or pink stained round bodies against a green background (O'Donoghue, 1985; Henriksen and Pohlenz, 1981).

In A-P staining the alcohol-fixed faecal smears are dipped in auramine phenol stain for 5 minutes, rinsed with tap water, then counterstained for 5 to 10 seconds in strong carbol fuchsin stain (Casemore *et al.,* 1985b). The smear is examined using fluorescence microscopy and the oocysts appear as bright apple green, fluorescing round bodies, against a dark background (Casemore *et al.,* 1985b). Positive faecal smears stained with A-P should always be confirmed by the MZN staining technique (McLauchlin *et al.,* 1987).

Direct and indirect immunological methods are extensively used for the demonstration of *Cryptosporidium* antigens in faecal specimen of humans and animals (Casemore *et al.,* 1985a,b; McLauchlin *et al.,* 1987; Sterling and Arrowood, 1986; Pohjola *et al,* 1986a; Snyder, 1988; Ungar, 1990). Immunological techniques, including monoclonal antibody (MAb), indirect immunofluorescence antibody and latex agglutination tests have been used for

detection of *Cryptosporidium* oocysts in faecal and water samples (Casemore *et al.,* 1985a,b; McLauchlin *et al.,* 1987; Sterling and Arrowood, 1986; Pohjora *et al.,* 1986b; Casemore, 1987; Arrowood, Mead, Maht and Sterling, 1989; Smith, McDiarmid, Smith, Hinson and Gilmour, 1989). Immunofluorescence antibody techniques have been found to be more sensitive than conventional tinctorial methods such as the MZN and A-P (McLauchlin *et al.,* 1987; Casemore, 1989). Immunophoretic and immunoblot analysis have also been performed for detection of *Cryptosporidium* oocyst antigens using immune sera from infected humans and animals (Lumb *et al.,* 1988; Mead, Arrowood and Sterling, 1988).

The examination of *Cryptosporidium* oocysts is made easier following concentration of faecal specimens (Pohjora *et al.,* 1986b). Oocyst concentration can be achieved either by flotation or sedimentation methods (Willson and Acres, 1982; Garcia *et al.,* 1983; McNabb *et al.,* 1985; Pohjola *et al.,* 1986b). Solutions used for oocyst flotation, include concentrated sucrose (Reese *et al.,* 1982), zinc sulphate ($ZnSO_4$) (Iseki, 1979), and a solution of sucrose or $ZnSO_4$ of specific gravity of 1.18 g/l is best suited for these techniques (Iseki, 1979; Snyder, 1988). Examination of the concentrated oocysts should be performed by phase-contrast microscopy (Willson and Acres, 1982; Angus, 1990). Oocyst concentration is also carried out by sedimentation, using the formol-ether (F-E) or PBS-ether technique (Casemore *et al.,* 1985b; Tzipori, 1988).

Specific antibodies against *Cryptosporidium* have been demonstrated in various animal species and humans (Tzipori and Campbell, 1981; Campbell and Current, 1983; Van Opdenbosch and Wellemans, 1985; Casemore, 1987). Sero-epidemiological studies have been conducted in human hospitals (Koch *et al.,* 1985) and in humans and animals resident in semi-rural areas (Tzipori and

Campbell, 1981; Van Opdenbosch and Wellemans, 1985; Casemore, Jessop, Douce and Jackson, 1986; Casemore, 1987; Ungar, Gilman, Lanata and Perez-Schael, 1988). The presence of antibody to *Cryptosporidium* may only indicate previous exposure to the parasite (Angus, 1987). Recently, the presence of smooth muscle auto-antibodies has been associated with *Cryptosporidium* infection in human patients (Boopucknavig and Bunyaratvej, 1988).

Animal inoculation tests are performed by orally inoculating samples of concentrated fresh faeces from a suspected subject into suckling, specific pathogen free (SPF), laboratory animals (Reese *et al.,* 1982). Diagnosis is confirmed following the demonstration of *Cryptosporidium* endogenous stages in histologic sections of the intestine of the test animals (Reese *et al.,* 1982). Prior to inoculation, 2.5% (w/v) of sodium dichromate solution should be added to the sample, in order to inactivate other possible enteropathogens (Iseki, 1979; Reese *et al.,* 1982; Sherwood *et al.,* 1982). However, it has been reported that some *Cryptosporidium* isolates failed to cause infection in SPF mice (Tzipori *et al.,* 1982; O'Donoghue, 1985).

Diagnosis of cryptosporidiosis is confirmed histologically following the demonstration of endogenous stages of *Cryptosporidium* in intestinal sections of suspected animals (Tzipori, 1983; Angus, 1983; O'Donoghue, 1985). Biopsy materials from the colon, ileum or rectum of human patients have been used for the diagnosis of cryptosporidiosis (Nime *et al.,* 1976; Lasser *et al.,* 1979; Bird and Smith, 1980; Weinstein, Edenlstein and Madara, 1981; Sloper, Dourmashkin, Bird, Slavin and Webster, 1982; Ma and Soave, 1983; Payne, Lancaster and Heinzman, 1983). Due to rapid autolytic post-mortem changes in the gut, sections for histological processing should be obtained as soon as

possible after death (Pohlenz *et al.,* 1978a; Angus, 1987). Nevertheless, tissue biopsy has been discouraged by some workers because of the invasiveness of the procedure (Ma and Soave, 1983; Garcia *et al.,* 1983; Casemore *et al.,* 1985a).

Diagnosis of *Cryptosporidium* infection in cats can be difficult due to the small size of the oocysts and the inefficiency of concentration methods (Monticello *et al.,* 1987). In several instances, both direct staining of faecal smears with Giemsa and sucrose flotation methods have failed to detect *Cryptosporidium* oocysts in faecal specimens from infected cats, whereas, numerous endogenous stages of the parasite were seen on histological examinations (Poonacha and Pippin, 1982; Monticello *et al.,* 1987). Antibodies to *Cryptosporidium* endogenous stages have been demonstrated in the sera of cats and other mammalian hosts (Tzipori and Campbell, 1981).

1.4.11. IMMUNITY TO *CRYPTOSPORIDIUM* INFECTION.

Several studies have been conducted into the immune mechanisms associated with cryptosporidiosis in humans and animal hosts other than cats. Both humoral and cell-mediated immunity have been reported to be necessary for recovery from *Cryptosporidium* infection in humans and animals (Current *et al.,* 1983; Heine, Pohlenz, Moon and Woode, 1984; Arrowood *et al.,* 1989; Whitmire and Harp, 1990). Specific antibodies of IgM, IgG, and IgA isotypes against *Cryptosporidium* have been demonstrated in sera from humans (Campbell and Current, 1983; Ungar *et al.,* 1986; Casemore, 1987; Ungar *et al.,* 1988) and sheep (Hill, Blewett, Dawson and Wright, 1990). Faecal IgA has also been reported to play an important role in recovery from cryptosporidiosis in lambs (Hill *et al.,* 1990). Age-related resistance and specific acquired immunity have

been described in calves exposed to a *C. parvum* isolate (Harp *et al.,* 1990). However, these studies indicated that exposure to the parasite is not necessary for the development of resistance to *Cryptosporidium* infection in mice of over 3 weeks of age. It is suggested that resistance to infection in adult mice is due to the development of mature intestinal flora which render the intestinal environment hostile to establishment of *Cryptosporidium* infection (Harp *et al.,* 1990).

The role of lactogenic antibodies in relation to immunity against *Cryptosporidium* infection is not clearly understood (Current, 1989). While studies in humans reported significant resistance to infection in breast-fed infants (Mata, Bolanos, Pizarro and Vives, 1984; Wolfson, Ritcher, Waldron, Weber, McCarthey and Hopkins, 1985; Fayer and Ungar, 1986; Fayer, Andrews, Ungar and Blagburn, 1989a), suckling mice were not protected by immune colostrum from their dams (Moon, Woodmansee, Harp, Abel and Ungar, 1988; Arrowood *et al.,* 1989). The association between immune mechanisms and the occurrence of *Cryptosporidium* infection in cats is not known.

1.4.12. TREATMENT AND CONTROL.

There is no specific treatment for cryptosporidiosis in animals or humans (Fayer and Ungar, 1986; Angus, 1987; Current, 1988; Soave, 1988). A variety of broad spectrum antibiotics, anticoccidials, anthelmintics, and other antiprotozoal agents, have been tested in animals and humans (Morin, Lariviere and Lallier, 1976; Stemmermann *et al.,* 1980; Weinstein *et al.,* 1981; Tzipori *et al.,* 1982; Sloper *et al.,* 1982; Angus, Hutchison, Campbell and Snodgrass, 1984). The only drug which was found effective, was lasalocid, but at a toxic dose to calves

(Moon, Woode and Ahrens, 1982b). There are no reports of attempts to treat cryptosporidiosis in cats. In humans, treatment with bovine transfer factor and hyperimmune bovine colostrum appeared to be effective in the eradication of diarrhoea and oocyst shedding in immunocompromised patients with cryptosporidiosis (Tzipori, Roberton and Chapman, 1986; Louie, Borkowsky, Klesius, Haynes, Gordon, Bonk and Lawrence, 1987). In experimentally-infected mice, immunotherapy with hyperimmune bovine colostrum greatly reduced parasite load in histologic sections of intestine examined at 72 hours postinfection (Fayer, Guidry and Blagburn, 1990b). Bovine transfer factor and hyperimmune bovine colostrum were also effective in the prevention of *Cryptosporidium* infection in orally-inoculated calves (Fayer, Klesius and Andrews, 1987; Fayer *et al.*, 1989a) and mice (Fayer, Perryman and Riggs, 1989b; Perryman, Riggs, Mason and Fayer, 1990). Nevertheless, some workers failed to alter the course of cryptosporidiosis in humans (Saxon and Weinstein, 1987; Chng, Shaw, Klesius and Saxon, 1989) and mice (Moon *et al.*, 1988; Arrowood *et al.*, 1989) using oral bovine transfer factor and hyperimmune bovine colostrum. Spiramycin (Rovamycin(R)) is the only drug currently being used to treat cryptosporidiosis in human patients (Saez-Llorens, Odio and Umana, 1989), though some workers have found it to be ineffective in some studies (Wittenberg, Miller and Van den Ende, 1989). Treatment of *Cryptosporidium*-induced diarrhoea is mainly symptomatic, with fluid replacement required in severely dehydrated patients (Current *et al.*, 1983; O'Donoghue, 1985; Angus, 1987; Current, 1988). Supportive or symptomatic treatment may be necessary in immunocompetent cats with cryptosporidiosis; however, in animals undergoing immunotherapy, as in neoplastic diseases,

immunosuppressive treatment should be suspended temporarily to permit self-cure of *Cryptosporidium* infection (Kirkpatrick and Dubey, 1987).

Control of *Cryptosporidium* infection can be achieved by strict hygiene, the isolation of infected animals from healthy ones and chemical sterilization of the immediate surroundings and equipment (O'Donoghue, 1985; Angus, 1987; Blewett, 1988). Disinfection by steam heat, 10% formol saline, 2% to 5% household ammonia (Angus, 1987), hydrogen-peroxide, or Oocide (Antec International Ltd.) has been recommended (Blewett, 1988). Vaccination against rotavirus and *Escherichia coli* is highly recommended, especially young ruminants, which are very susceptible to cryptosporidiosis (Angus, 1987). Animal attendants and research workers should be careful while working with diarrhoeic animals and all samples suspected of containing *Cryptosporidium* oocysts should be preserved in 10% formol-saline (Current *et al.,* 1983; Angus, 1987; Current, 1989). Because of the potential for zoonotic transmission of the parasite, pet owners should be made aware of the possibility of acquiring infection from their animals.

CHAPTER II.

GENERAL MATERIALS AND METHODS.

2.1. INTRODUCTION.

In this study, conventional techniques for the staining and concentration of *Cryptosporidium* oocysts in cat faecal specimens were evaluated and employed in a survey of the prevalence of *Cryptosporidium* infection in the Glasgow area. A serological study was also conducted in cats from the same area using an indirect immunofluorescence antibody test (IFAT). Experimental transmission of a *Cryptosporidium* sp. isolate from the cat to lambs and cat to mice was performed using oocysts recovered from a naturally-infected farm cat.

2.2. MATERIALS AND METHODS.

2.2.1. SOURCE OF ANIMALS.

Domestic, feral (wild) and farm cats from the Glasgow area were screened for *Cryptosporidium* infection. Domestic cats were referred to the Glasgow University Veterinary School (GUVS) by veterinary surgeons in general practice either as clinical cases, or for post-mortem (PM) examination. Feral cats were obtained mainly from the Dog and Cat Home at Cardonald, Glasgow, to which they had been taken for euthanasia. Farm cats were accessed via veterinary surgeons and were sampled either at the farms or at GUVS. Faecal samples were obtained from all farm cats and blood samples from some of them. Occasionally, farm cats were available for PM examination.

For experimental transmission studies, undertaken at the Animal Diseases Research Association, Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH, newborn lambs and mice were used. Two Scottish Blackface cross Suffolk and one Finnish-Landrace male lambs were used in the first experiment. The lambs, obtained from breeding stock at the Moredun Research Institute, were allowed colostrum and then separated from their dams at one day of age. In the second experiment, 2 day old minimally inbred Porton mice obtained from a breeding colony at the Moredun Research Institute were used.

2.2.2. PHYSICAL EXAMINATION AND AGE DETERMINATION.

Animals were examined for physical abnormalities and the ages of the feral and farm cats were estimated following dental examination. In cats, deciduous teeth erupt between 2 and 7 weeks of birth, whereas permanent teeth appear between 4 and 6 months of age (Table 2.1). Cats having all their permanent teeth were regarded as being of more than 6 months of age, or adults, whereas those with deciduous teeth as being less than 6 months of age, or kittens (Sams and Harvey, 1989). Faecal samples were collected from the litter trays in the catteries of the admitted domestic cats, from the sand boxes of some farm cats or from the lumina of the colon and rectum of some of the domestic, farm and all feral cats during post-mortem examination. In 18 farm cats, only colonic and rectal swabs were available.

Type of teeth	Deciduous Teeth (weeks of age)	Permanent Teeth (months of age)
incisors	2-3	3-4
canines	3-4	4-5
premolars	3-6	4-6
molars	-	4-5

(From Sams and Harvey, 1989)

Table 2.1. Tooth eruption schedule for the cat.

2.2.3. BLOOD SAMPLING.

Blood samples were normally taken via jugular puncture with the cat conscious, while restrained using a laboratory coat. In the case of feral and some of the farm cats which were to be euthanased, blood samples were obtained via jugular or cardiac puncture following anaesthesia by intramuscular injection of 0.5 ml ketamine ("Ketaset" C-Vet Limited). Blood was sampled for serum preparation, haematology, biochemistry and FeLV and FIV tests. Following blood collection, all feral and some farm cats were euthanased by intracardiac injection of pentobarbitone sodium ('Euthatal', RMB Animal Health Ltd.), and post-mortem examination was performed.

2.3.4. PROCEDURES FOR DEMONSTRATION OF *CRYPTOSPORIDIUM* OOCYSTS IN FAECAL SPECIMENS.

Faecal smear preparation.

A small amount of the faecal sample, was emulsified in a drop of normal saline to make a uniform faecal smear, on a marked, 1 cm^2 area on a glass slide. The smear was air-dried and fixed for 5 minutes with methanol, then stained with modified Ziehl-Neelsen (MZN), or auramine phenol (A-P) or FITC-labelled monoclonal antibody technique (MAb) as described below.

MZN staining (Casemore *et al.*, 1985b).

Methanol fixed smears were placed on a staining rack, capable of holding 20 slides, and immersed for 15 minutes in a staining jar containing strong carbol-fuchsin (BDH Chemicals Limited Poole, England). The smears were then rinsed in tap water and differentiated in acid alcohol (1% hydrochloric acid [HCl] in alcohol) for 10 to 15 seconds, rinsed again and counterstained with 0.4% malachite green, for 30 seconds. The stained smear was examined under bright field microscopy at x400 magnification.

A-P staining (Casemore *et al.*, 1985b).

Methanol fixed smears were stained for 5 minutes in auramine-phenol ("Lempert" BDH Chemicals Ltd.), then rinsed with tap water, and counterstained briefly for 5 to 10 seconds in strong carbol-fuchsin (BDH Chemicals Ltd.), then rinsed and air-dried. Examination was by fluorescence microscopy using Leitz Orthoplan

isothiocyanate (FITC) filters, at x100 and x400 magnifications.

Monoclonal antibody (MAb) technique (Northumbria Biologicals Limited, Cramlington, England).

A commercially available FITC-labelled MAb (Northumbria Biologicals Ltd.) directed against the oocyst wall of *Cryptosporidium* spp. was used in this study. This MAb has been shown to react specifically with *Cryptosporidium* sp. from a variety of animal hosts (Rose, Landeen, Riley and Gerba, 1989). Faecal smears were fixed in methanol for 2 minutes, air-dried, then overlaid with 10 µl of the reagent. The slides were incubated horizontally in a moist chamber at 37°C for 30 minutes, then rinsed three times in phosphate buffered saline (PBS) pH 7.4 (BDH Chemicals Ltd.) for 10 minutes each and air-dried prior to examination. The slides were mounted with coverslips using the mountant provided with the kit, and examined under fluorescence microscopy with FITC filters at x400 magnification.

2.2.5. OOCYST CONCENTRATION METHODS.

Preparation of Sheather's sucrose (Sheather, 1923) and zinc-sulphate ($ZnSO_4$) solutions.

Using an electronic weighing balance (Precisa Balance Limited McQuilkin and Co. Zurich, Switzerland), 500 g of sucrose (BDH Chemicals Ltd.) were weighed. Sucrose was added slowly to 320 ml of distilled water in a 500 ml glass beaker, and the mixture was continuously mixed and mildly heated (56°C) using a Corning hot plate stirrer (Corning Limited, Halstead, England) until all the sucrose was completely dissolved. The sucrose solution was transferred into a 500 ml measuring cylinder and the specific gravity (SG) of adjusted to 1.18 at room temperature (Snyder, 1988). Sucrose solutions of SG 1.09 and 1.02 were

cylinder and the specific gravity (SG) of adjusted to 1.18 at room temperature (Snyder, 1988). Sucrose solutions of SG 1.09 and 1.02 were also prepared from the Sheather's sucrose solution. The solutions were used the same day of preparation or stored in corked 1000 ml bottles at 4°C until needed. A similar procedure was followed for preparation of 33% $ZnSO_4$ solution, by mixing 331 g of $ZnSO_4$ (BDH Chemicals Ltd.) in 1000 ml distilled water. The specific gravity was similarly adjusted to 1.18.

Sucrose flotation method.

Sheather's sucrose flotation was performed as modified by Snyder (1988) as follows:

Five grammes of cat faecal material were added to 20 ml of distilled water in a 50 ml centrifuge tube, mixed thoroughly and centrifuged for 6 minutes at 400xg. The supernatant was poured off and the sediment mixed with sucrose solution (SG 1.18), and centrifuged for 6 minutes at 400xg. The contents of the top half of the tube were poured off into another 50 ml centrifuge tube, then washed three times by centrifugation with PBS (pH 7.4) to remove excess sucrose. After the final washing, a small amount of sediment was applied to a glass slide, emulsified with a drop of normal saline, then covered with a cover-slip and examined using phase-contrast microscopy. Smears were also prepared for staining with MZN, A-P and/or MAb techniques. Examination was made under bright field microscopy at x400 magnification (Snyder, 1988; Current *et al.*, 1983).

$ZnSO_4$ flotation method.

The $ZnSO_4$ flotation method was performed as modified by Iseki (1979):

Distilled water was added to 5 g of cat faeces to produce a uniform, thick paste and

a stainless steel tea strainer into a 50 ml centrifuge tube, then centrifuged at 400xg for 5 minutes. The supernatant was discarded and $ZnSO_4$ solution of SG 1.18 was added to fill the tube, followed by centrifugation for 2 minutes at 400xg. After centrifugation, a coverslip was applied gently on to the the positive meniscus of the $ZnSO_4$ solution and examined under bright field and phase-contrast microscopy at x400 magnification. The upper 2 ml of floating material was aspirated with a Pasteur pipette fitted with a rubber bulb, and resuspended in 40 ml of distilled water, followed by centrifugation at 400xg for 5 minutes. The supernatant was discarded and the procedure repeated three times. After the final washing smears were prepared from the sediment, fixed for 5 minutes with methanol and stained with MZN and A-P stains.

Formol-ether (F-E) sedimentation method (Allen and Ridley, 1970).

The formol-ether sedimentation procedure of Allen and Ridley (1970) was performed with slight modifications, as follows:

One gramme of cat faecal sample was mixed with 7 ml of 10% formalin (1 volume of 40% formaldehyde diluted with 9 volumes of distilled water) then thoroughly mixed to make a homogeneous suspension using a whirlmixer (Fisons Scientific Apparatus, Loughborough, England). The suspension was well mixed and sieved using a stainless steel tea strainer, then added to 3 ml of diethyl-ether "GPR" (BDH Chemicals Ltd.) in a glass universal container. The formalin-ether suspension was mixed thoroughly by vigorous shaking for 30 seconds and centrifuged for 3 minutes at 400xg. After centrifugation there were four distinct layers of fluid: the upper one comprised of mainly ether, followed by a fatty plug between the ether layer and a column of clear fluid and at the bottom, sedimented

between the ether layer and a column of clear fluid and at the bottom, sedimented materials. The upper layers of ether and fatty plug were aspirated using a vacuum pump (Charles Austen Pump Ltd.) and the middle column of fluid transferred into another glass universal container leaving only the sediment in the original container. Smears were prepared from the sediment and these were air-dried, fixed with methanol for 5 minutes, and stained with MZN, A-P or MAb and examined for *Cryptosporidium* sp. oocysts. Wet smears were also prepared and examined under phase-contrast and bright field microscopy at x400 magnification. The column fluid was further centrifuged for 5 minutes and the sediment also examined for *Cryptosporidium* oocysts.

Acid flocculation method (Blewett personal communication).

One gramme of faecal sample was added to 9 ml of distilled water in a universal container and the sample well mixed until a homogenous suspension was obtained. One hundred microliters of 2% hydrochloric acid (HCl) was added to the suspension using a micropipette and the sample left to stand for 40 minutes. The supernatant was aspirated into another tube using a long needle and a 20 ml syringe and centrifuged at 400x**g** for 5 minutes. The supernatant was discarded and the sediment was further purified using sucrose density gradient (Woodmansee, 1987). This technique was used for concentration of *Cryptosporidium* oocysts from artificially infected lambs.

Sucrose gradient flotation.

The sucrose density gradient method described by Woodmansee (1987) was used, with minor modifications. Three layers of sucrose solution of SG 1.18, 1.09, and

plastic centrifuge tube. About 15 ml of previously concentrated faeces sample was introduced at the bottom of the tube using a 4 inch needle and 20 ml syringe, then centrifuged at 4°C at 400xg for 5 minutes. The middle (SG 1.09) layer was aspirated carefully and mixed with PBS (pH 7.4) (1 to 10), then centrifuged at 400xg for 5 minutes. The supernatant was discarded and the sediment washed three times with PBS (pH 7.4) by centrifugation at 400xg for 5 minutes. The purified oocysts were stored in 2.5% potassium dichromate ($K_2Cr_2O_7$) at 4°C until used.

2.2.6. OOCYST COUNTING.

Concentrated or non-concentrated *Cryptosporidium* oocysts were counted using an improved-Neubauer haemocytometer. Oocyst sample was diluted 1 to 10 in 0.16% malachite green in 1% sodium dodecyl sulphate (SDS). The sample was applied in the counting chambers using a Pasteur pipette and the oocysts were counted in both chambers at x400 magnification under bright field microscopy. In samples with very low numbers of oocysts, 5 µl of the concentrated material were smeared onto glass slides, air-dried, fixed with methanol and stained with A-P stain. The number of oocysts in the whole smear were counted and the total oocyst count in the sample calculated.

2.2.7. HISTOLOGICAL TECHNIQUES.

Histological techniques were performed for examination of endogenous stages of *Cryptosporidium* in naturally and experimentally infected animals. Sections from the stomach, duodenum, ileum, caecum, colon, and rectum, fixed in 10% BNF (buffered neutral formalin) were trimmed and cut into sections 3 mm thick and post-fixed in mercuric chloride (BDH Chemicals Ltd.) for 24 hours. The tissues were then placed

onto Lab-Tek plastic embedding cassettes marked with a case number of the animal. The cassettes were then placed into an automatic tissue processing machine (Tissue-Tek VIP 1000, Ames Miles Scientific, Slough, England), for dehydration, clearing, and paraffin wax (Difco Polywax, East Molsey, England) impregnation, for 16 hours. Paraffin wax impregnated tissues were then flooded with molten paraffin wax (56°C) using a paraffin dispenser (Tissue-Tek III embedding centre, Ames Miles Ltd.) and cooled by placing on a cold plate (-2°C) for 30 minutes.

The tissues embedded with paraffin wax were then cut into 3 μm thin sections using a Leitz 1512 microtome and mounted on marked glass slides. The sections were dried in an oven at 60°C for 30 minutes, then stained with Mayer's haematoxylin and eosin (H&E).

Haematoxylin and Eosin (H&E) staining technique.

Following drying, the sections were dewaxed in xylene (BDH Chemicals Ltd.) for 5 minutes, then dipped 10 times successively in absolute alcohol, 90% alcohol and 70% alcohol and washed in running water for one minute. The sections were stained with Lugol's iodine for one minute, then bleached in sodium thiosulphate (BDH Chemicals Ltd.) for 1 minute, stained with Mayer's haematoxylin for 5 minutes, then rinsed in running water for one minute. The sections were differentiated in 1% acid alcohol for 15 seconds, rinsed in running water, treated with alkaline water (Scott Water Substitute) for 3 minutes until the colour changed to blue, and then further rinsed in running water for one minute. Staining with Putt's eosin (BDH Chemicals Ltd.) was done for 3 minutes, followed by rinsing in running water. Finally the sections were treated with 70% alcohol (1 minute), 90% alcohol (1 minute), absolute alcohol (1 minute), again

water. Finally the sections were treated with 70% alcohol (1 minute), 90% alcohol (1 minute), absolute alcohol (1 minute), again absolute alcohol (1 minute), and xylene (5 minutes).

Stained sections were mounted using D.P.X. (BDH Chemicals Ltd.), and dried on a hot plate (60°C) for 60 minutes. Examination was performed at x400 and x1000 (using oil immersion) magnifications, under bright field microscopy.

2.2.8. SCANNING ELECTRON MICROSCOPY (SEM).

Preparation of fixative and Buffer.

Karnovsky's fixative.

Two hundred and fifty millilitres of 0.2 M cacodylate buffer was added to 100 ml of 10% paraformaldehyde, 50 ml of 25% glutaraldehyde and topped up with distilled water to 500 ml.

Cacodylate buffer.

Fifty millilitres of 0.4 M sodium cacodylate (BDH Chemicals Ltd.) were added to 8 ml of 0.2 M HCl then made up to 100 ml with distilled water.

Sample preparation and processing.

At necropsy, sections of approximately 2 cm^2 from the jejunum, ileum and caecum were washed gently in 0.1 M cacodylate buffer (pH 7.4) to remove surface blood and mucus, then fixed in 3% buffered glutaraldehyde (BDH Chemicals Ltd.) or

Karnovsky's fixative for a minimum of 24 hours. The tissues were trimmed and washed for 4 hours in cacodylate buffer, then exposed to 70% acetone for 4 hours, followed by 90% acetone (2 hours), 100% acetone (2 hours) and 100% acetone, overnight. Subsequently, the tissues were dried then attached to aluminium stubs using silver paint. The tissues were allowed to dry at 37%, then coated with palladium for 4 minutes, and preserved at 37% until examined. Examination was performed under Philips 501B SEM at an operating kilovoltage of 15kv and using spot sizes ranging from 200 to 1000. An automatic Rolliflex camera with Ilford FPA 120 (125 ASA) film was employed, and black and white prints were prepared.

2.2.9. STATISTICAL METHODS.

Two way analysis of variance (Siegel and Castellan, 1988) was performed followed by Newman-Keuls Range test for multigroup comparisons (Snedecor and Cochran, 1967) for oocyst detection methods (Chapter 3, section 1). The Chi-square test (Siegel and Castellan, 1988) was used for analysis of the results of a survey of the prevalence of *Cryptosporidium* infection in cats (Chapter 3, section 2 and 3). Analyses were conducted at levels of significance of 5% and 1%.

CHAPTER III.

EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN CATS.

SECTION I. COMPARATIVE EVALUATION OF EFFICIENCY OF TECHNIQUES FOR DETECTION OF *CRYPTOSPORIDIUM* OOCYSTS IN CAT FAECAL SPECIMENS.

3.1.1. INTRODUCTION.

Cryptosporidium is a coccidian parasite causing respiratory and gastrointestinal infections in animals and humans (Pohlenz *et al.,* 1978a; Tzipori *et al.,* 1980a; Casemore, 1988; Fayer and Ungar, 1986; Current, 1989). Diagnosis of cryptosporidiosis is based mainly on demonstration of oocysts in faecal specimens from the affected host (Garcia *et al.,* 1983; Casemore *et al.,* 1985a,b; Pohjola *et al.,* 1986b; McLauchlin *et al.,* 1987; Angus, 1987). As in other animal hosts, direct microscopical examination and faecal concentration techniques have been used for diagnosis of *Cryptosporidium* infection in cats (Iseki, 1979; Bennett *et al.,* 1985; Koch *et al.,* 1983; Lewis *et al.,* 1985). However, in several cases direct smear staining and faecal flotation techniques failed to detect any oocyst in infected animals and diagnosis was based on histological findings (Poonacha and Pippin, 1982; Monticello *et al.,* 1987). The relatively small size of *Cryptosporidium* oocysts, coupled with the presence of lipid and cat hair in the faeces and the relative inefficiency of current detection methods, makes it

possible that positive cases might be missed by routine diagnostic procedures (Monticello *et al.,* 1987).

Several techniques for the staining and concentration of *Cryptosporidium* oocysts in faecal specimens have been described (Fayer and Ungar, 1986). Of the staining techniques, MZN and A-P staining have been used frequently in screening faecal specimens for oocysts (McLauchlin *et al.,* 1987), whereas Sheather's sucrose flotation (Sheather, 1923), and F-E sedimentation are commonly employed for oocyst concentration (Casemore *et al.,* 1985b; Smith *et al.,* 1989; Fayer and Ungar, 1986). However, Iseki (1979) used $ZnSO_4$ flotation for the concentration of *Cryptosporidium* oocysts from cat faecal specimens. Currently, monoclonal antibody (MAb) techniques are in common use for confirmation of the diagnosis (McLauchlin *et al.,* 1987; Smith *et al.,* 1989). On several occasions, there have been conflicting findings on the efficiency of Sheather's sucrose flotation and F-E sedimentation techniques (Fayer and Ungar, 1986). Some workers reported Sheather's sucrose flotation to be superior to F-E or phosphate buffered saline-ether (PBS-Ether) sedimentation (Ma, 1984; McNabb *et al.,* 1985; Zierdt, 1984), whereas others preferred F-E sedimentation technique (Casemore *et al.,* 1985b; Tzipori, 1988).

The objective of this study was to determine the comparative efficiency of six commonly used techniques, namely MZN, A-P, MAb, $ZnSO_4$ and sucrose flotation, and F-E sedimentation, for detection of *Cryptosporidium* oocysts in cat faecal material.

3.1.2. MATERIALS AND METHODS.

SOURCE OF FAECAL MATERIALS.

Twenty five grammes of fresh coccidia-free cat faecal specimens were used in this study to compare efficiencies of MZN, A-P, and FITC-labelled MAb techniques for detection of *Cryptosporidium* oocysts. In a parallel study, faecal specimens from 20 *Cryptosporidium*-negative cats were employed for comparison of three oocyst concentration methods for detection of *Cryptosporidium* oocysts. In both studies, faecal samples were spiked with known numbers of purified *C. parvum* oocysts.

OOCYST SOURCE AND PURIFICATION.

Purified *C. parvum* oocysts were obtained from experimentally infected newborn lambs. Faecal materials from the lambs were concentrated by acid flocculation (Blewett and Wright, personal communication), then purified by sucrose density gradient (Woodmansee, 1987). The total number of oocysts was determined using an improved-Neubauer haemocytometer, following a tenfold dilution of 100 µl of purified oocysts, in 900 µl of 0.16% malachite green and 0.1% SDS.

INOCULATION OF CAT FAECAL SPECIMENS WITH PURIFIED *CRYPTOSPORIDIUM* OOCYSTS FOR ANALYSIS OF STAINING TECHNIQUES.

Twenty five grammes of cat faecal specimens were diluted with 75 ml of distilled water in a 500 ml beaker, then thoroughly mixed by breaking solid particles until

a homogeneous suspension was obtained. The suspension was then filtered through a tea strainer, poured into two 50 ml centrifuge tubes and spun at 400xg for 5 minutes. The supernatant was discarded and the faecal paste in the two centrifuge tubes was resuspended in 50 ml of distilled water. After thorough mixing 9 ml were withdrawn and introduced into a universal container marked 'A', and another 4 ml into each of nine similar containers, marked serially 'B' to 'J'.

To the 9 ml of faecal suspension in the universal container 'A', 1 ml of 1.7 x 10^8/ml purified cervine derived *C. parvum* oocysts were added and thoroughly mixed using a vortex stirrer (Heidolf REAX 2000), to make a suspension of 1.7 x 10^7/ml *Cryptosporidium* oocysts. A twofold serial dilution was performed by drawing 4 ml from the 9 ml of the spiked cat faecal suspension in 'A', into 'B', and again 4 ml from 'B' into 'C' and so on to 'J'. Following serial dilution of the known number of *C. parvum* oocysts in the faecal suspensions, the expected number of oocysts in each sample was calculated as shown in Table 3.1.1. From each of the spiked faecal samples, oocysts were counted using an improved-Neubauer haemocytometer, after a 1 to 10 dilution with 0.16% malachite green solution and 0.1% SDS.

Ten microlitre (µl) aliquots from each of the spiked faecal samples were smeared on 1 cm^2 marked areas on glass slides. Fixation and staining procedures were performed as described earlier (Chapter 2). Three negative control smears were prepared from uninoculated faecal suspension from the original sample and stained with one of the three techniques.

MZN stained smears were examined was done under bright field microscopy whereas a fluorescence microscope equipped with an FITC-filter was

used for the examination of smears stained with A-P and MAb techniques. In each case a total of 50 fields were examined at x400 magnification and oocysts present in 10 positive fields were enumerated. The procedure was performed twice using different cat faecal specimens. A total of five smears were prepared for each of the three staining techniques employed, three smears for the first sample and two for the second.

INOCULATION OF CAT FAECAL SPECIMENS WITH PURIFIED *CRYPTOSPORIDIUM* OOCYSTS FOR ASSESSMENT OF THE EFFICIENCY OF CONCENTRATION TECHNIQUES.

Zinc sulphate flotation ($ZnSO_4$) (Iseki, 1979), sucrose flotation (Snyder, 1988), and F-E sedimentation (Casemore *et al.*, 1985b) were evaluated for their efficiency in the recovery of *C. parvum* oocysts from 20 cat faecal specimens. From each sample, 3 g of cat faeces were emulsified in 6 ml, and made to 9 ml in distilled water. The suspension was then thoroughly mixed and 1 ml of 8.1 x 10^7/ml of purified *C. parvum* oocysts was added to give a final oocyst count of 8.1 x 10^6/ml. For each of the three concentration techniques, one millilitre of the spiked sample was used and the number of oocysts was counted using an improved-Neubauer haemocytometer (Blewett, 1988). Uninoculated faecal suspension was used as a negative control. The procedure was performed twice using different cat faecal specimens. Four haemocytometer counts were made for each of the three concentration techniques.

3.1.3. RESULTS.

OOCYST STAINING TECHNIQUES.

Analysis using an improved-Neubauer haemocytometer indicated that there was no significant difference between the number of oocysts counted in the spiked samples and the theoretical count, following serial doubling dilution of *Cryptosporidium* oocysts. However, no oocyst was detected in sample 'J' which was spiked with 3.3 x 10^4/ml oocysts using Neubauer haemocytometer (Table 3.1.1). *Cryptosporidium* oocysts appeared as pink to red round bodies, against a green background in smears stained with MZN technique. Several oocysts were stained faintly and some had distorted morphology, which made detection difficult.

Although no oocyst was found in the negative control smears, several acid-fast bodies were encountered but these differed from *Cryptosporidium* oocysts. With A-P staining, *Cryptosporidium* oocysts appeared as apple-green round bodies against a dark background. Some oocysts were found to be faintly stained and some had distorted morphologies. No oocyst was observed in the negative control smears. Oocysts were readily detected in smears stained with FITC-labelled MAb and appeared as round to ovoid in shape with an apple-green fluorescence against a dark background. The fluorescence was more intense around the oocyst walls and along the suture line. Non-oocyst materials were also seen fluorescing but these had no clearly defined morphology. No oocyst was found in the negative control smears.

Table 3.1.2, shows both total and average numbers of oocysts counted in

10 fields using the three techniques for oocyst staining. There was a significant difference ($p<0.05$) between the three techniques used for staining Cryptosporidium oocysts, with FITC-labelled MAb detecting more oocysts than A-P and MZN. Oocysts were readily detected in samples with 1.0 x 10^6/ml oocysts or more in MZN and A-P stained smears. Very few oocysts were detected in smears prepared from samples with 1.0 x 10^5/ml or less. There was a good correlation between the number of oocysts detected in stained smears and the total oocyst counts in the original samples ($r=0.98$).

Sample	Expected No. of oocysts/ml	Observed No. of oocysts/ml
A	1.7 x 10^7	1.5 x 10^7
B	8.5 x 10^6	7.4 x 10^6
C	4.3 x 10^6	3.6 x 10^6
D	2.1 x 10^6	1.5 x 10^6
E	1.1 x 10^6	1.2 x 10^6
F	5.3 x 10^5	4.5 x 10^5
G	2.6 x 10^5	3.5 x 10^5
H	1.3 x 10^5	1.5 x 10^5
I	6.6 x 10^4	1.0 x 10^5
J	3.3 x 10^4	-

Table 3.1.1. Oocysts counts in spiked faecal specimen using an improve-Neubauer haemocytometer.

Sample	No. oocysts	Average No. in 10 fields MZN	A-P	MAb
A	1.7×10^7/ml	34 **(338)**	32 **(318)**	37 **(373)**
B	8.5×10^6/ml	20 **(199)**	18 **(180)**	27 **(271)**
C	4.3×10^6/ml	9 **(91)**	11 **(113)**	13 **(131)**
D	2.1×10^6/ml	8 **(75)**	6 **(56)**	9 **(91)**
E	1.0×10^6/ml	4 **(39)**	5 **(47)**	6 **(63)**
F	5.5×10^5/ml	1 **(13)**	4 **(41)**	5 **(54)**
G	2.3×10^5/ml	1 **(10)**	2 **(23)**	3 **(32)**
H	1.1×10^5/ml	1 **(7)**	1 **(14)**	2 **(19)**
I	6.6×10^4/ml	1 **(5)**	1 **(11)**	1 **(12)**
J	3.3×10^4/ml	1 **(4)**	1 **(4)**	1 **(11)**

Values are means of five measurements.

( ) = Total number of oocysts in all 10 fields.

Table 3.1.2. Mean number of *Cryptosporidium* oocysts counted in 10 fields using three staining techniques.

OOCYST CONCENTRATION TECHNIQUES.

During oocyst concentration with $ZnSO_4$ and sucrose flotation techniques, faecal debris and fat materials were also found mixed with the oocysts on the surface of the solutions. With F-E sedimentation, fat and other faecal debris were suspended in the ether layer, while *Cryptosporidium* oocysts and other particles were pelleted to the bottom of the containers. Of the three techniques used for oocyst concentration, F-E sedimentation was found to concentrate significantly higher numbers of oocysts ($p < 0.05$) as compared to $ZnSO_4$ and sucrose flotation techniques (Table 3.1.3). The highest numbers of oocysts were recovered by F-E

sedimentation, whereas the lowest were by $ZnSO_4$ flotation. However, in seven samples, sucrose flotation technique appeared to concentrate more oocysts than F-E sedimentation (Table 3.1.3). Table 3.1.4 provides a summary of average numbers and percent of oocysts concentrated by each method.

Sample	$ZnSO_4$	Sucrose	F-E
1	1.0×10^6 (12.3)	2.60×10^6 (32)	4.85×10^6 (60.4)
2	9.5×10^5 (11.7)	2.68×10^6 (33.3)	4.06×10^6 (50.6)
3	5.2×10^5 (6.4)	2.15×10^6 (27.2)	2.75×10^6 (34.6)
4	6.6×10^5 (7.4)	2.00×10^6 (24.7)	2.76×10^6 (34.6)
5	1.1×10^6 (13.6)	2.13×10^6 (26)	2.86×10^6 (35.8)
6	9.9×10^5 (12.2)	2.62×10^6 (32)	2.55×10^6 (32)
7	6.7×10^5 (8.3)	2.95×10^6 (36)	2.22×10^6 (27.2)
8	9.1×10^5 (11.2)	2.77×10^6 (33.3)	2.50×10^6 (31)
9	1.2×10^6 (14.8)	2.90×10^6 (35.8)	2.32×10^6 (28.4)
10	8.7×10^5 (10.7)	2.58×10^6 (32)	3.05×10^6 (38.3)
11	9.3×10^5 (11.5)	2.60×10^6 (32)	3.12×10^6 (38.3)
12	1.2×10^6 (14.8)	3.08×10^6 (38.3)	3.28×10^6 (40.7)
13	8.3×10^5 (10.2)	2.84×10^6 (34.6)	3.09×10^6 (38.3)
14	7.2×10^5 (8.9)	2.71×10^6 (33.3)	4.06×10^6 (50.6)
15	1.1×10^6 (13.6)	3.13×10^6 (38.3)	1.88×10^6 (23.5)
16	4.2×10^5 (5.2)	2.81×10^6 (34.6)	1.83×10^6 (22.2)
17	1.4×10^6 (17.3)	2.85×10^6 (35.8)	3.95×10^6 (48)
18	7.9×10^5 (9.7)	2.46×10^6 (31)	3.93×10^6 (48)
19	7.0×10^5 (8.6)	2.47×10^6 (31)	2.56×10^6 (32)
20	9.0×10^5 (11)	2.69×10^6 (33.3)	2.49×10^6 (31)

Values are means of four measuments.

( ) = percent of oocysts recovered from the original sample.

Table 3.1.3. Number and percent of *Cryptosporidium* oocysts per millilitre recovered from inoculated faecal samples using the three techniques for oocyst concentration.

Technique	Average No. of oocysts/ml	Average % of oocysts recovered
$ZnSO_4$	8.9×10^5	10.98% (5.2-17.3)
sucrose	2.7×10^6	33.33% 27.7-38.3)
F-E	3.0×10^6	37.03% (22.2-60.4)

( ) = Ranges of the percent of oocysts recovered from the original sample.

Table 3.1.4. Average number and percent of oocysts concentrated by each technique.

Of the 8.1×10^6/ml *Cryptosporidium* oocyst in the spiked cat faecal homogenates, F-E sedimentation concentrated an average of 3.0×10^6/ml (37%) whereas sucrose and $ZnSO_4$ flotation techniques concentrated 2.7×10^6/ml (33%) and 8.9×10^5/ml (11%), respectively.

3.1.4. DISCUSSION.

The results of this study suggest that, *C. parvum* oocysts can be readily detected in cat faecal specimens using MZN, A-P, and FITC-labelled MAb, particularly in samples containing 1.0×10^6/ml oocysts or more. It was also demonstrated that the number of oocysts which can be detected in faecal smears correspond with

the concentration of oocysts in the original sample. In diarrhoeic animals, detection of five or more oocysts per field in an ordinary MZN stained direct smear is an indication of severe infection with *Cryptosporidium* (Angus, 1987), and oocyst concentration techniques are rarely required. In the current study, more than five oocysts per field were detected using MZN stained smears prepared from samples with over 1.0 x 10^6/ml oocysts. Comparison of the three oocyst staining techniques revealed that FITC-labelled MAb is more efficient than MZN and A-P techniques. Monoclonal antibody techniques have been found to be more sensitive and specific, compared to conventional tinctorial techniques for detection of *Cryptosporidium* oocysts (McLauchlin *et al.*, 1987; Casemore, 1989; Snyder, 1988). In water samples however, several oocyst-like materials have been detected using FITC-labelled MAb; probably these structures cross-reacted with epitopes of *Cryptosporidium* (Smith *et al.*, 1989). In the current study, several non-*Cryptosporidium* structures were also encountered in smears stained with MAb technique, but differed in size and morphology from *Cryptosporidium* oocysts.

Detection of oocysts was relatively more difficult in MZN and A-P staining, particularly when samples contained fewer than 2.3 x 10^5/ml oocysts. This suggests that these two techniques are not sensitive enough to detect low numbers of *Cryptosporidium* oocysts in cat faecal specimens. However, some workers prefer screening with MZN and A-P staining followed by MAb technique (McLauchlin *et al.*, 1987). The results of this study have also revealed that the number of *Cryptosporidium* oocysts in cat faecal specimens, which can also be an indication of the severity of infection can be estimated indirectly in stained faecal smears.

In the study of oocyst concentration methods, F-E sedimentation was found to be the most efficient technique, compared to $ZnSO_4$ and sucrose flotation. In previous studies, some workers found sucrose flotation superior to F-E sedimentation (Ma, 1984; McNabb *et al.*, 1985; Zierdt, 1984), while others preferred F-E or PBS-ether sedimentation (Casemore *et al.*, 1985b; Tzipori, 1988). The F-E sedimentation technique was originally developed for detection of nematode and ascarid eggs in faecal specimens (Allen and Ridley, 1970). Currently, the technique has been modified for concentration of *Cryptosporidium* oocysts in animal and human faecal specimens (Casemore *et al.*, 1985b; Smith *et al.*, 1989). One advantage of F-E sedimentation technique is the facility to extract lipids from faecal specimens, hence dispersing oocysts in aqueous phase (Waldman, Tzipori and Forsyth, 1986). Because of this fact the technique is regarded as more suitable for concentration of parasites in faecal samples from the carnivorous species (O'Donoghue, 1985).

In the present study, the sucrose flotation method recovered more oocysts than F-E sedimentation in only seven out of 20 samples. The variations in the number of oocysts concentrated could be attributed to the technique used and/or the type of faecal specimen. The presence of variable amounts of faecal debris and fat materials recovered along with *Cryptosporidium* oocysts in sucrose and $ZnSO_4$ flotation techniques probably contributed to the lower oocyst counts using these two techniques compared to F-E sedimentation. One advantage of the F-E sedimentation technique is that fewer confusing fat and hair particles are pelleted, thus making microscopic analysis of the pellet and oocyst identification easier.

Oocyst flotation methods have been used commonly for diagnosis of

Cryptosporidium infection (Iseki, 1979; Monticello *et al.*, 1987; Arai *et al.*, 1990). However, in several cases the techniques failed to detect any oocyst in faecal specimens from cats found positive by histological or stained ileal smear examination (Monticello *et al.*, 1987; Arai *et al.*, 1990). Failure to detect oocysts in cat faecal specimens could be attributed to either low numbers of oocysts excreted in cat faeces and/or inefficient detection methods (Monticello *et al.*, 1987). In one study, of 19 confirmed positive cases, oocysts were detected in faecal specimens of only 13 by sucrose flotation and eight by modified cold type Kinyoun's acid-fast staining on ileal smears (Arai *et al.*, 1990). These findings indicate that no single technique should be relied upon for definitive diagnosis of cryptosporidiosis in cats by faecal examination (Arai *et al.*, 1990). The present study has shown that both F-E sedimentation and sucrose flotation methods appear to be useful for detection of *Cryptosporidium* oocysts in cat faecal specimens. Despite the fact that MZN and A-P were not efficient in detecting low numbers of oocysts, the two techniques can still be helpful for screening for oocysts in cat faeces and MAb techniques should be used for confirmation of the diagnosis.

CHAPTER III.

EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN CATS.

SECTION 2. A SURVEY OF THE PREVALENCE OF *CRYPTOSPORIDIUM* INFECTION IN CATS IN THE GLASGOW AREA.

3.2.1. INTRODUCTION.

The epidemiology of *Cryptosporidium* has been studied in the developed and developing countries and the infection has been found to be quite common in children and immunocompromised individuals (Fayer and Ungar, 1986; Current, 1989). Though infection has been described in diarrhoeic and non-diarrhoeic cats (Poonacha and Pippin, 1982; Iseki, 1979; Bennett *et al.,* 1985; Monticello *et al.,* 1987; Koch *et al.,* 1983), little is known on the prevalence of infection in this animal host (Egger *et al.,* 1990). So far, only two cases have been documented involving *Cryptosporidium* and feline leukaemia virus (FeLV) infection, an immunosuppressive retrovirus in cats (Monticello *et al.,* 1987; Goodwin and Barsanti, 1990). Recently, feline immunodeficiency virus (FIV), another immunosuppressive retrovirus has been reported in cats (Pedersen *et al.,* 1987). FIV is antigenically distinguishable from FeLV and HIV (human immunodeficiency virus) but has several features in common with HIV (Onions, 1988). However, no association between FIV and *Cryptosporidium* has been established.

The objective of this study was to determine the prevalence of *Cryptosporidium* infection in cats in the Glasgow area and its association with diarrhoea and immunosuppressive viral conditions in cats.

3.2.2. MATERIALS AND METHODS.

SAMPLE COLLECTION AND EXAMINATION.

In this study, 138 domestic, 99 feral and 57 farm cats from Glasgow and the surrounding area were examined for *Cryptosporidium* infection. Prior to euthanasia all feral and some farm cats were anaesthesized by intramuscular injection of 0.5 ml ketamine ("Ketaset", C-Vet Limited) to facilitate clinical examination. Blood samples were taken from these cats at the point of euthanasia. All cats were briefly examined for physical abnormalities and an estimation of the age of the feral and farm cats was made from examination of the teeth (Chapter 2). Faecal specimens from the cats, obtained either while hospitalised at the Glasgow University Veterinary Hospital or during necropsy, were screened for *Cryptosporidium* oocysts using MZN, A-P and sucrose flotation and F-E sedimentation methods. FITC-labelled MAb (Northumbria Biologicals Ltd.) was used for confirmation of the diagnosis.

Blood samples collected from some of the domestic, feral and farm cats were submitted for routine haematological and biochemical analysis to the respective laboratories at the Glasgow University Veterinary School (GUVS). FeLV antigen and FIV antibody tests were carried out using an enzyme immunoassay (PetChek, Idexx Systems, Portland, Maine 04101 U.S.A) according

to the manufacturer's directions. Feral and farm cats were euthanased by administration of an intracardiac injection of pentobarbitone sodium (200mg/ml) ("Euthatal" RMB), and necropsy examination was performed. Faecal samples found to be positive for cryptosporidiosis and/or diarrhoea were all submitted for routine parasitological and bacteriological examination.

POST MORTEM EXAMINATION.

During necropsy, the stomach and intestine were opened and examined for macroscopic lesions and parasites, and faecal materials were gently collected from colonic lumen using a wooden applicator stick, placed in clean, marked plastic pots and preserved in equal volume of 2.5% (w/v) $K_2Cr_2O_7$ solution. Two impression smears were prepared from each of the following sites: stomach, duodenum, jejunum, ileum, caecum, colon and rectum, by gently pressing marked glass slides onto the mucosal surfaces. The smears were air-dried and fixed with methanol for 5 minutes. Sections of the stomach, duodenum, jejunum, ileum, caecum, colon and rectum, were preserved in 10% buffered neutral formalin (BNF) and 3% glutaraldehyde, for histology and SEM, respectively (Chapter 2). Epithelial mucosal impression smears were stained with the MZN technique as described earlier (Chapter 2).

3.2.3. RESULTS.

PREVALENCE OF *CRYPTOSPORIDIUM* INFECTION IN VARIOUS GROUPS OF CATS.

Domestic cats.

Of the 138 domestic cats examined, seven (5.1%) were positive for *Cryptosporidium* infection (Table 3.2.1), four of which were adults and three were kittens. Twenty adult domestic cats had diarrhoea, but only one was positive for *Cryptosporidium* infection. Of the 17 domestic kittens with diarrhoea, none was infected with *Cryptosporidium.*

Feral cats.

Twelve out of 99 (12.1%) feral cats were positive for *Cryptosporidium* infection. Of the positive feral cats, 11 were kittens, three of which had diarrhoea, while only one was an adult cat without any signs of diarrhoea. Fourteen feral cats had diarrhoea.

Farm cats.

Seven of the 57 (12.3%) farm cats were positive for *Cryptosporidium* infection. Of the positive cats, four were obtained from one farm (Farm A), two from another farm (Farm B) and the remaining one from third farm (Farm C) (Appendix 3.2.3). Three of the positive farm cats were kittens and four were adults; one adult cat had diarrhoea.

Type of cat	Number examined	Number positive	Percent positive
domestic	138	7	5.1
feral	99	12	12.1
farm	57	7	12.3
Total	**294**	**26**	**8.8**

Table 3.2.1. Summary of cases of cats examined for *Cryptosporidium* infection.

PREVALENCE OF *CRYPTOSPORIDIUM* INFECTION BY AGE, TYPE OF CAT AND ITS ASSOCIATION WITH DIARRHOEA AND FELINE RETROVIRUS INFECTIONS.

From the total of 294 cats examined, 26 (8.8%) were positive for *Cryptosporidium* infection. There was a higher rate of infection in kittens than in adult cats ($p > 0.01$). Comparison of the three groups of cats revealed a significantly higher prevalence of infection in farm and feral than domestic cats ($p > 0.05$). However, there was no significant difference in the prevalence of *Cryptosporidium* infection between farm and feral cats. There was no association between *Cryptosporidium* infection and the gender of the cats, presence of diarrhoea, FeLV or FIV infections ($p < 0.05$).

OOCYST DETECTION AND MORPHOLOGY.

Direct smear staining.

Cryptosporidium oocysts appeared as spherical to ovoid bodies, with pink to red staining against a green background with MZN (Figure 3.2.1), and apple-green fluorescence against a dark background with A-P staining (Figure 3.2.2). It was slightly difficult to detect oocysts in stained direct faecal smears; in most cases only one or two oocysts would be seen after scanning through many fields under microscopy at x400 magnification (Figure 3.2.3). Using direct smear staining, oocysts were detected in three out of seven positive samples from the domestic cats, four out of 12 feral cats and all seven positive samples from the farm cats (Appendix 3.2.4). The numbers and sizes of the oocysts detected are given in Appendix 3.2.5. With the exception of two samples (M17 and F22), the mean oocyst size was 5.0 x 4.3 µm, while some oocysts in sample M17 and F22 measured approximately 7.4 x 6.5 µm and 6.0 x 5.4 µm, respectively. The oocysts from the farm cat (F22) were used subsequently for experimental transmission in newborn lambs (Chapter 4). Because of the low numbers of oocysts in most of the samples, numerical quantification using an improved-Neubauer haemocytometer was not possible, but indirect estimation of concentrated samples suggested that most specimens had 10^4 or less oocysts per gramme of faeces. However, up to 10^5 oocysts per gramme faeces were counted using haemocytometer in samples from farm cats.

The FITC-labelled MAb was used for confirmation of the diagnosis, and with this technique *Cryptosporidium* oocysts appeared as apple-green against

dark background, with an intensely fluorescing suture. Results of MAb staining are summarized in Appendix 3.2.4.

Oocyst concentration methods.

With the sucrose flotation method, oocysts were recovered from the flotation solution together with some hair particles and fat globules. Staining of oocysts in the samples concentrated by sucrose flotation was difficult, and more often examination of wet smears under phase-contrast microscopy was necessary for the identification of oocysts. With phase-contrast microscopy the oocysts appeared as refractile round bodies with slightly dull internal structures. Using sucrose flotation, *Cryptosporidium* oocysts were detected in four out of seven samples from domestic cats, nine of the positive feral cats and three of the farm cats. Using the F-E sedimentation technique, *Cryptosporidium* oocysts were detected in the sediment together with some debris, but there were less fat and hair particles present in the samples. Using this technique, it was possible to stain oocysts with conventional techniques such as A-P or MZN, thus facilitating detection of the oocysts. Two to five oocysts could be seen per field in the concentrated samples, and oocysts were detected in six domestic, 11 feral and three farm cats by this technique (Appendix 3.2.4).

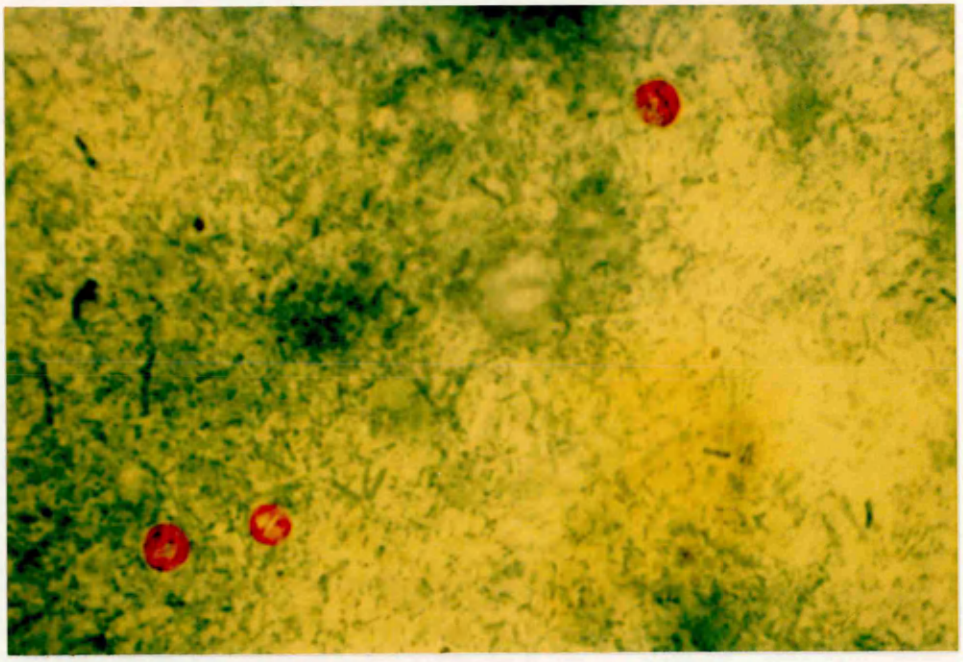

Figure 3.2.1. MZN-stained *Cryptosporidium* oocysts from a feral cat (M36). Magnification x1000 (oil immersion).

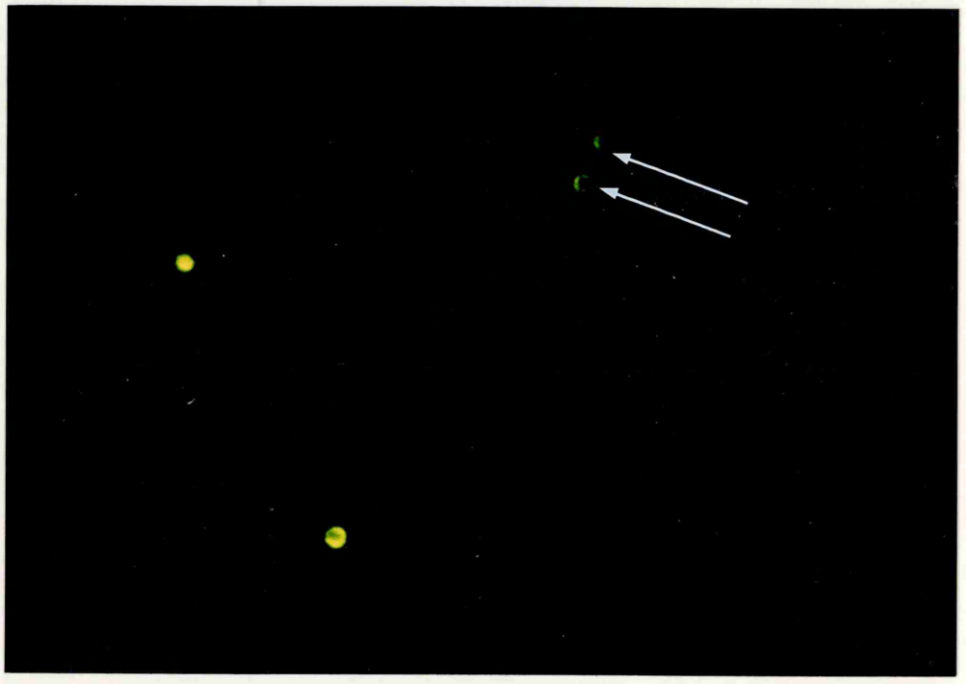

Figure 3.2.2. *Cryptosporidium* oocysts from a farm cat (F22) stained with A-P technique. Note small and large (arrow) oocysts. Magnification x400 under fluorescence microscopy with FITC filters.

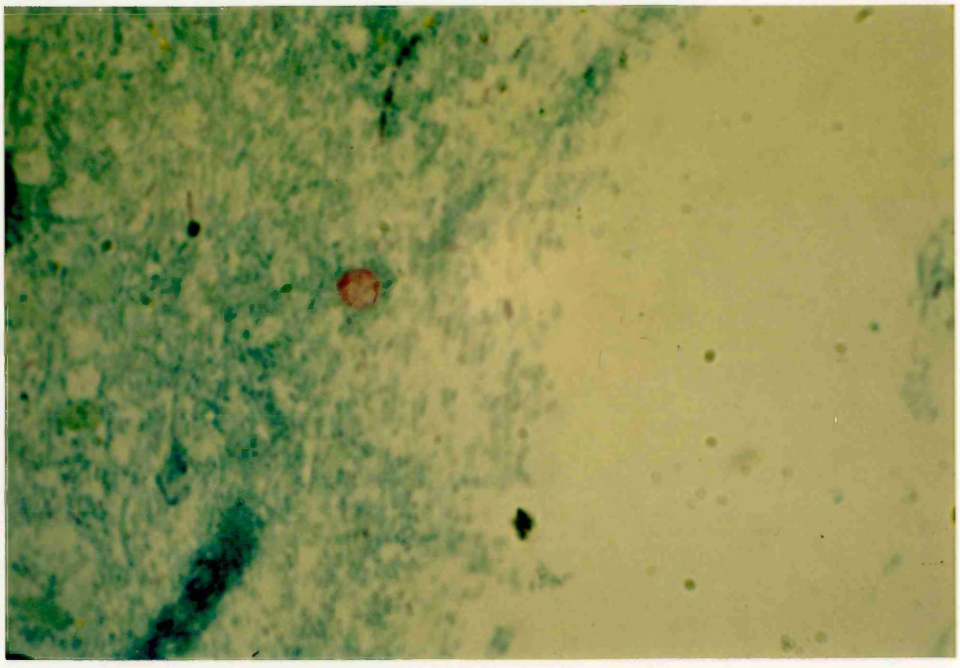

Figure 3.2.3. MZN-stained *Cryptosporidium* oocyst in a direct faecal smear from a domestic cat. Magnification x1000 (oil immersion).

POST-MORTEM EXAMINATION.

There were no significant necropsy findings specific for *Cryptosporidium* infection in any of the positive cats examined. However, hyperaemia of the serosa and enlargement of the mesenteric lymph nodes were the most prominent pathological features found in the positive cats. Numerous round worms (*Toxocara*) were found in the stomach and the intestines of 67 cats. At times, portions of long grasses were seen in the intestines of the feral and farm cats.

Cryptosporidium oocysts were readily detected from stained intestinal mucosal impression smears (Figures 3.2.4 and 3.2.5). In some cases up to six oocysts were seen in the smears especially those of the ileum and the caecum. Oocysts were detected in impression smears in five samples from domestic and 10 from feral cats. The staining characteristics of the oocysts were similar to those detected in direct smears and stained with similar techniques. Desquamated epithelial cells and Figures 3.2.4 and 3.2.5. some inflammatory cells were also found in the smears. However, it was not difficult to distinguish *Cryptosporidium* oocysts from the cells due to their size and staining characteristics. Oocysts were more frequently detected in the impression smears from the ileum and the caecum, but in one case (A18) oocysts were also found in the stomach and the duodenum. In another case (M17), oocysts were also detected in the rectal mucosal impression smears.

Histologically, endogenous stages of *Cryptosporidium* appeared as small, spherical to ovoid bodies, ranging in size from 1 to 4 μm on the microvillus regions of the epithelial cells (Figure 3.2.6). Some organisms were found at the tips of the villi, and others in the crypts. Occasionally, the organisms were

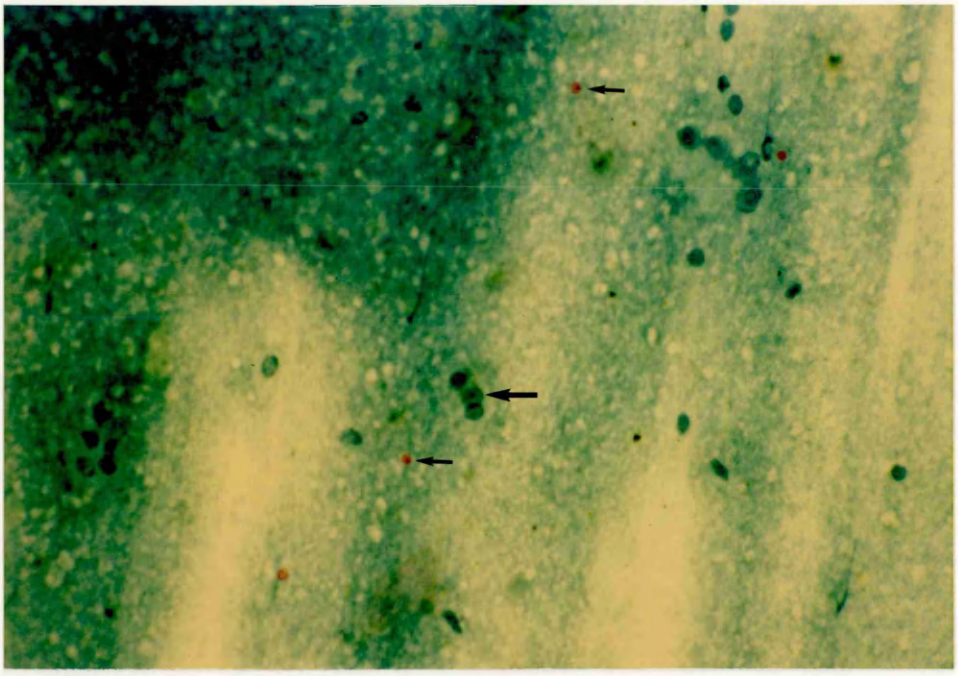

Figure 3.2.4. MZN-stained ileal impression smear from a domestic cat (M4). *Cryptosporidium* stains red (arrow) and desquamated cells pick the colour of a counter stain (thicker arrow). Magnification x250.

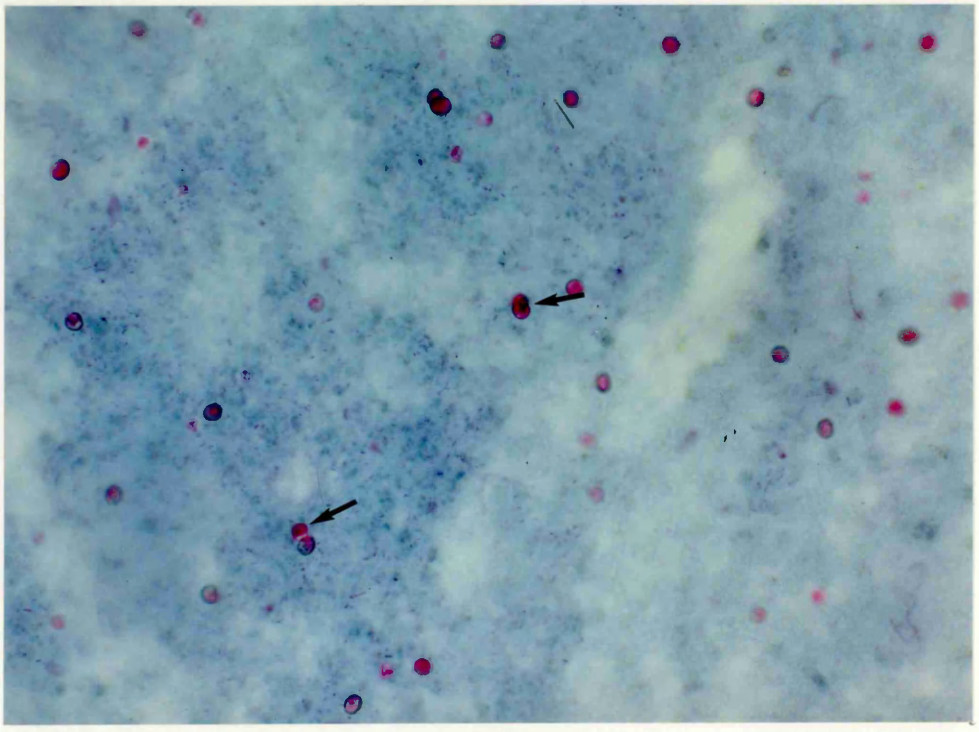

Figure 3.2.5. MZN-stained ileal impression smear from a farm cat (F22). Note thick and thin-walled oocysts (arrows). The background is purple due to prolonged counterstaining. Magnification x400.

detected in the lumen either free or attached to desquamated cells. The main sites in which the endogenous stages of *Cryptosporidium* were seen, included the jejunum, ileum and caecum. No organisms were found in sections of the stomach, duodenum, colon or rectum of the infected cats. In most cases very few organisms were found, and often it was difficult to make a conclusive decision due to autolysis of the epithelial mucosae. Several sections had to be examined before finding evidence of infection. Where sections were of good diagnostic quality, histological changes associated with *Cryptosporidium* infection included degeneration of epithelial cells, sometimes leading to loss of normal epithelial architecture and infiltration of the lamina propria with inflammatory cells, including plasma cells, lymphocytes and occasionally globule leucocytes. Only eight positive cases were detected histologically, one from the domestic cats, seven from the feral cats and none from the farm cats (Appendix 3.2.4).

RESULTS OF SCANNING ELECTRON MICROSCOPICAL (SEM) EXAMINATION.

It was difficult to detect *Cryptosporidium* under SEM due to extensive degeneration of epithelial mucosa. In only three out of 16 sections examined, were organisms resembling *Cryptosporidium* found; they were either free or attached to the epithelial cells. Moreover, the number of organisms detected was also very low; occasionally two or three parasites were seen. In the positive samples, *Cryptosporidium* appeared as round bodies attached to the epithelial cells (Figure 3.2.7); however, in some cases the presence of mucus impaired the detection of the parasite.

CASES OF FIV AND FELV INFECTIONS.

Of the 185 (73 domestic, 89 feral and 23 farm cats) animals examined, nine domestic cats were positive for FIV antibody and two for FeLV antigen using ELISA tests. Two of the FIV positive domestic cats were also infected with *Cryptosporidium* (Table 3.2.2), and one (M4) had chronic diarrhoea, gingivitis, anaemia, and low albumin and high globulin levels. The haematocrit value for this cat was 19.6%, and total red blood cell count (RBC-count) was 4,400/mm^3. Normal values for haematocrit and RBC count for cats are 30-45%, and 5-10,000/mm^3, respectively (Prasse and Mahaffey, 1987). None of the feral cats with either FIV or FeLV had *Cryptosporidium* infection (Table 3.2.2). One farm cat was FIV positive but had no evidence of *Cryptosporidium* infection.

Age	Number			Diarrhoea			FIV/FeLV		
(mths)	dom	feral	farm	dom	feral	farm	dom	feral	farm
>6	4(100)	1 (43)	4(48)	1(20)	0 (4)	0(5)	2(7)	0(7)	0(1)
≤6	3(38)	11(56)	3 (9)	0(17)	3(10)	0(0)	0(2)	0(1)	0(0)
Total	**7(138)**	**12(99)**	**7(57)**	**1(37)**	**3(14)**	**0(5)**	**2(9)**	**0(8)**	**0(1)**

(mths) = months; **dom** = domestic

Table 3.2.2. Number of domestic and feral cats positive for *Cryptosporidium* infection by age and association with diarrhoea and infection with FIV and FeLV.

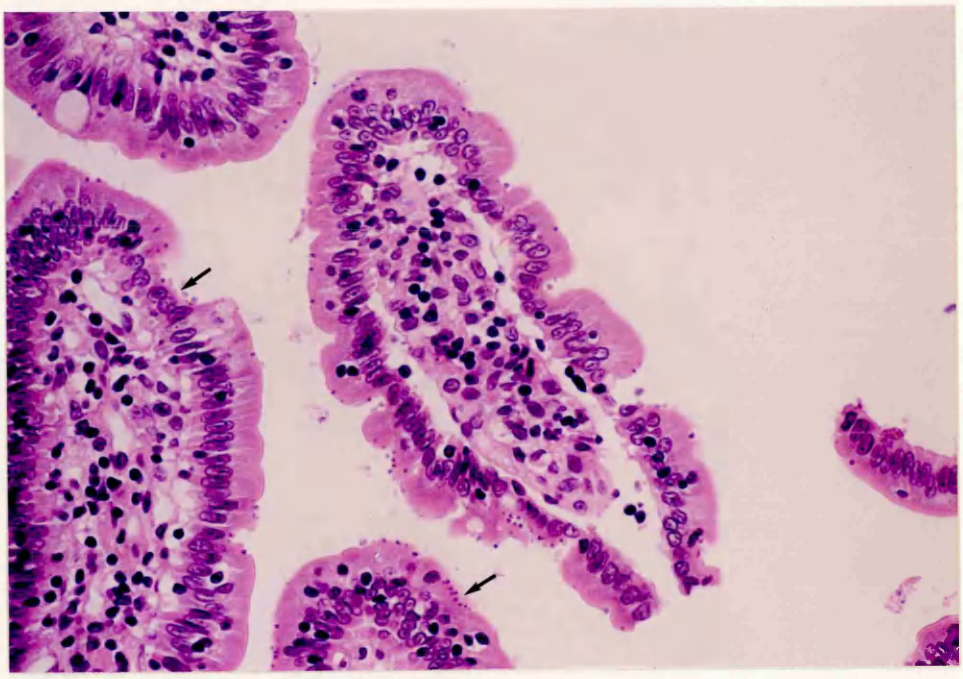

Figure 3.2.6. Section of jejunum of a domestic cat (M4) showing endogenous stages of cryptosporidia (arrow). Haematoxylene and eosin. Magnification x450.

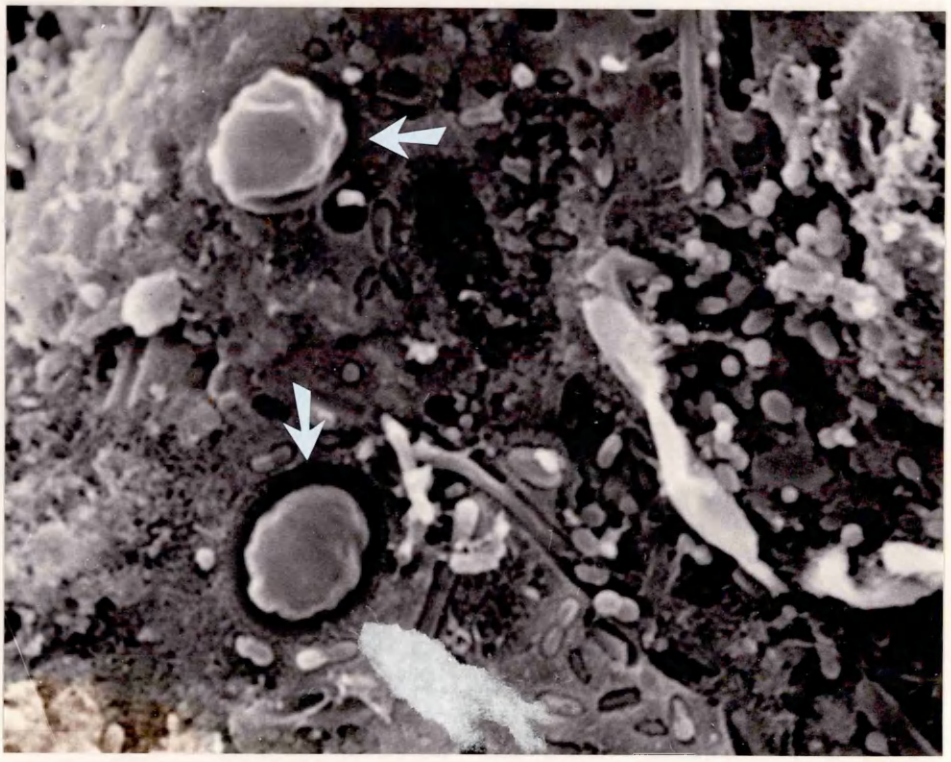

Figure 3.2.7. Endogenous stages of cryptosporidia (arrow) in the ileum of a feral cat (M36) as seen under SEM. Magnification x2500.

RESULTS OF ROUTINE BACTERIOLOGY AND PARASITOLOGY.

The results of bacteriological examination showed that 48 of the screened faecal samples were positive for ß-haemolytic *Escherichia coli*, 17 of the samples were from diarrhoeic cats. ß-haemolytic *Streptococcus* sp. was identified in 21 faecal samples 14 of which were from domestic cats, five from feral cats and two from farm cats. *Campylobacter* sp. was detected in faecal specimens from one diarrhoeic domestic cat, six feral cats, one of which also had diarrhoea, and two non-diarrhoeic farm cats. *Salmonella* sp. occurred in only a single case involving a diarrhoeic domestic cat. Appendix 3.2.6 shows the occurrence of *Cryptosporidium* with other enteropathogens in cat faecal specimens.

Parasitological examination of faecal specimens showed *Toxocara cati* eggs in 14 samples from domestic cats, 34 from feral cats and 19 from farm cats. Nine samples from domestic cats, six from feral and one from a farm cat found with *T. cati* came from diarrhoeic animals. Two cases of tapeworm infestation (*Spirometra* spp.) were recorded in one domestic and a feral cat with no diarrhoea. Among the coccidian parasites of the cat, *Isospora felis* oocysts were detected in faecal material from seven domestic and 13 feral cats, whereas *I. rivolta* was found in only one domestic cat and six feral cats, respectively. Of seven domestic cats with *I. felis*, five were kittens (less than 6 month old) and two were adults, whereas of the feral cats, eight were kittens and four adults. *I. rivolta* oocysts were found in only one domestic kitten, but were present in four feral kittens and two adult feral cats. Oocysts of *I. felis* and *I. rivolta* stained pink to red against a green background with MZN (Figures 3.2.8. and 3.2.9), fluoresced apple-green against a dark background with the A-P technique and ranged in size from 33 to 51 µm by 21 to 39 µm and 18 to 27 µm by 15 to 21 µm,

respectively. The oocysts sporulated after exposure to air for 2 days, and contained two sporocysts. Oocysts measuring 11 to 13 µm by 8 to 12 µm, with similar appearance under MZN and A-P staining were also seen in faecal specimens from one domestic cat, three feral and two farm cats (Figures 3.2.10 and 3.2.11). The size of these oocysts resembled those of *T. gondii*, *Sarcocystis* sp., *Besnoitia* sp. and *Hammondia* sp. (Lindsay and Blagburn, 1991). The *Cryptosporidium* oocysts were easily distinguished from other coccidian parasites by virtue of their small size. The significance of these parasites in relation to *Cryptosporidium* or diarrhoea in affected cats could not be established.

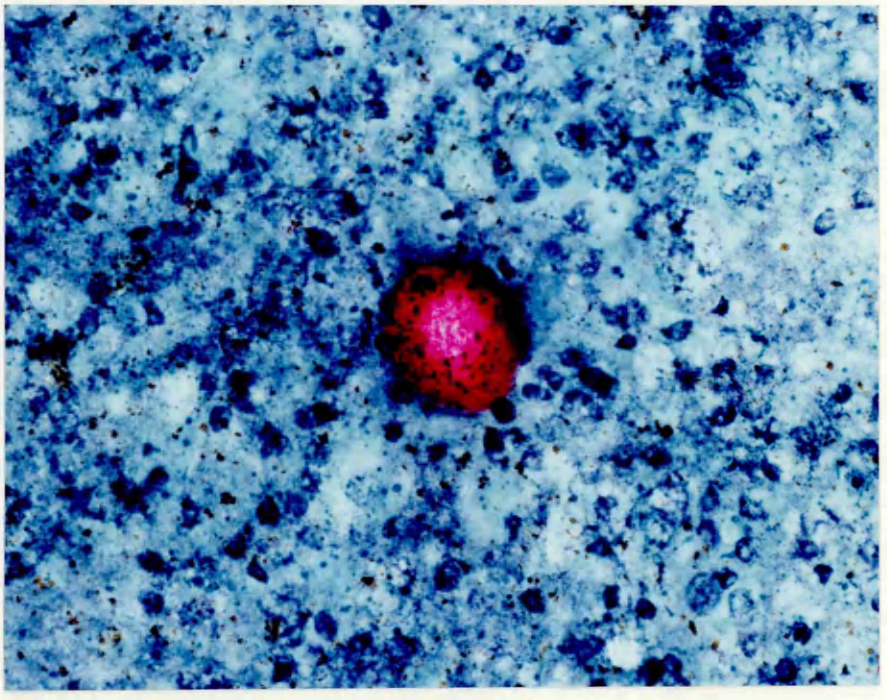

Figure 3.2.8. MZN-stained *I. felis* oocysts. Magnification x250.

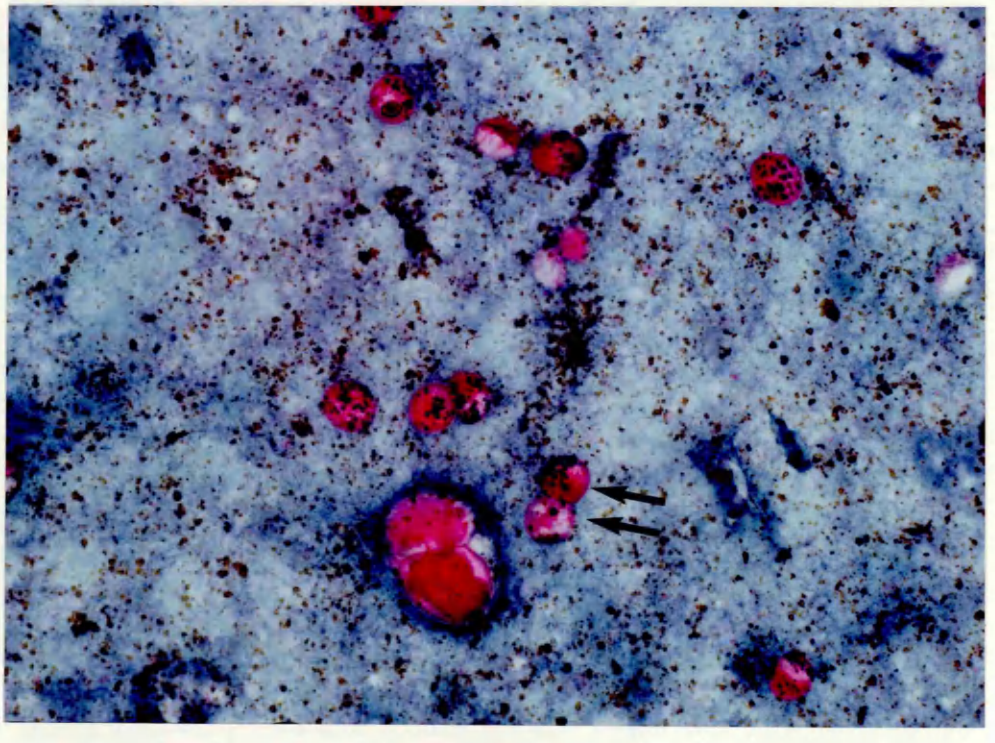

Figure 3.2.9. MZN-staining of sporulated *I. rivolta* oocyst together with oocysts resembling *T. gondii* (arrow). Magnification x400.

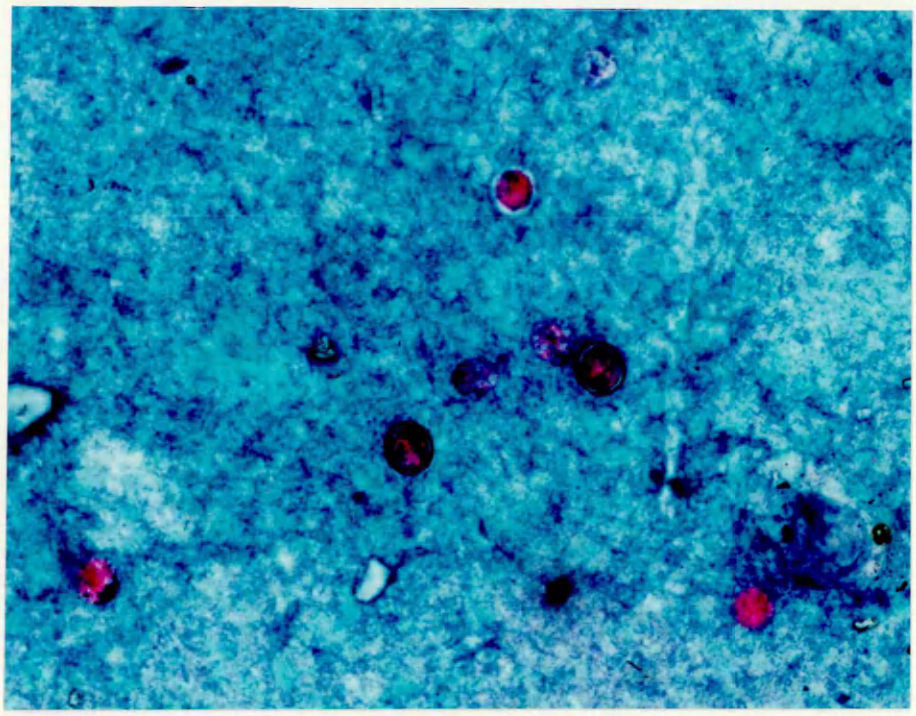

Figure 3.2.10. MZN-stained coccidian oocysts similar to *T. gondii.*

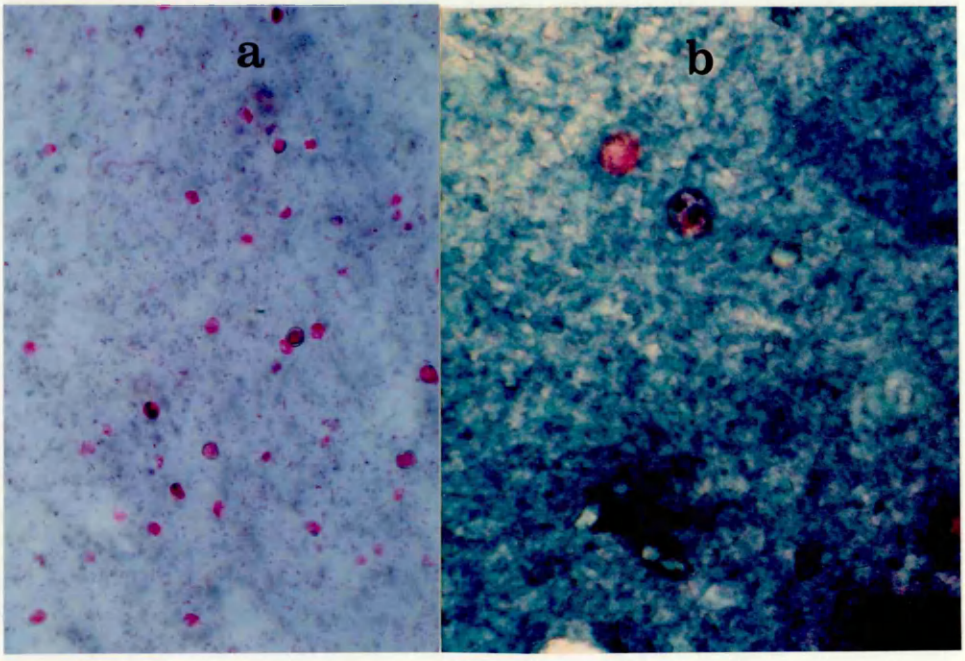

Figure 3.2.11. *Cryptosporidium* oocysts (a) compared with other coccidian parasites in MZN-stained smear. Magnification x400.

3.2.4. DISCUSSION.

This study has shown that *Cryptosporidium* is relatively common among cat populations in the Glasgow area. About 8.8% of the total cats examined were infected with *Cryptosporidium.* The prevalence of *Cryptosporidium* infection in feral and farm cats was higher than that in domestic cats, probably due to the fact that *Cryptosporidium* oocysts are widely spread in the environment, especially on farms where infection is very common in newborn calves (Fayer and Ungar, 1986). In addition, feral and farm cats move freely in the environment compared with domestic cats, increasing the chance of contact with contaminated materials. *Cryptosporidium* oocysts were detected in cats from three out of eight farms visited; however, on one farm only one cat was examined which was negative for infection. Oocysts were also detected in faeces from calves and dogs on a farm in which the cats were positive for *Cryptosporidium* infection. Of the cats examined, infection was found to be more common in kittens than in adults. *Cryptosporidium* infection was not associated with gender, nor did it appear to be associated with diarrhoea, the latter being complicated by the presence of other potential enteropathogens. Similar results have been reported by other workers (Arai *et al.,* 1990; Uga *et al.,* 1989); these workers reported a prevalence of 3.8% and 3.9% respectively, in cats in Japan.

In this study, it was found that diagnosis of *Cryptosporidium* infection by direct smear staining is not easy due to the low numbers of oocysts excreted in cat faeces. It is possible that many of the positive cats examined were either in the early, or convalescence stages of infection at which times only a few oocysts are shed. However, it was easier to detect oocysts in samples from farm cats, which were shedding relatively higher numbers of oocysts than feral and

domestic cats. It is likely that this is because farm cats are more frequently infected with *Cryptosporidium* oocysts in the potentially highly contaminated farm environment. It has also been demonstrated that F-E sedimentation is a more suitable technique for concentration of *Cryptosporidium* oocysts in cat faecal specimens, probably due to the ability of ether to dissolve any fat present in the faecal specimens. Sucrose flotation was also successful in concentrating oocysts in cat faeces but the subsequent staining of oocysts recovered by this technique was more difficult. Similar findings have been documented elsewhere (Casemore *et al.*, 1985b). Poor staining of *Cryptosporidium* oocysts following sucrose flotation might result from the formation of a layer of sucrose on the smears leading to minimal contact between the stains and the oocysts.

Examination of stained impression smears obtained at necropsy from the ileum was found to be more rewarding than histological and SEM procedures, as the former permitted ready detection of oocysts unaffected by the autolysis of the epithelial cells which hampers histological methods (Angus, 1987). It also permitted detection of thin-walled oocysts which could not be found in stained faecal smears. As in other animal species, the commonest site for infection was the ileum, though infection was also found in the jejunum, and the caecum. Using impression smears, oocysts were found in the stomach, duodenum, jejunum, caecum and rectum. The finding of *Cryptosporidium* oocysts in the impression smears from the stomach was of interest, especially as the sizes of the oocysts were different from that of *C. muris*, the only species known to infect the gastric epithelium (Tyzzer, 1907). However, the presence of oocysts in the stomach could have been the result of contamination during preparation of the smears, or following backflush of the intestinal contents. In this study, SEM was

used for screening some intestinal sections from cats positive for *Cryptosporidium* infection. It was disappointing that only three of the 16 sections revealed *Cryptosporidium* and this could probably be attributed to the extensive degeneration of epithelial cells and presence of mucus on the epithelial surface. From these findings, it is recommended that SEM should be used for examination of materials recovered from animals immediately after they are euthanased, following adequate washing of the tissues.

The mean size of oocysts was 5.0 x 4.3 µm, similar to *C. parvum* (Current, 1985). However, oocysts measuring 7.4 x 6.5 µm were found in one of the feral cats. The size of these oocysts is similar to *C. muris* which measures 7.4 x 5.6 µm (Current, 1989). Nevertheless, *C. muris* mainly affects the stomach or abomasum of infected animals (Tyzzer, 1910; Anderson, 1987; Pospischil *et al.,* 1987), but in this study of cats no oocysts of that size nor endogenous stages were found in the stomach. However, oocysts measuring 6.0 x 5.4 µm were detected in a farm cat. The sizes of these oocysts are similar to those of *C. baileyi* which mainly cause respiratory infections in broiler chickens (Current *et al.,* 1986). These oocysts stained with MZN and A-P, and were used in subsequent experimental transmission studies in newborn lambs and mice (chapter 4).

In this study, histological changes were limited to enlargement of regional lymph nodes and hyperaemia of the serosa, but these lesions are not specific for *Cryptosporidium* infection. Numerous roundworms, *Isospora* spp. oocysts, and a number of bacteria were also found in the affected animals, and any of these might have contributed to the pathological findings.

Two of the FIV positive domestic cats were also infected with

Cryptosporidium. One of these had severe anaemia, chronic diarrhoea and gingivitis. Although there was no significant association between FIV and *Cryptosporidium,* infection appeared to be much more intense than in FIV-negative cats in terms of the numbers of endogenous stages of the parasite encountered on histological examination. To date, two cases of *Cryptosporidium* infection have been reported in association with FeLV infection (Monticello *et al.,* 1987; Goodwin and Barsanti, 1990). The results of this study suggest that the prevalence of *Cryptosporidium* infection is determined more by exposure to the parasite than to the immune status of the cats. However, infection may be more severe in immunodeficient animals. Although enteric parasites and bacteria were detected together with *Cryptosporidium,* any significance of this co-existence was not established.

The finding of *Isospora* oocysts in cat faeces was interesting as the oocysts were acid fast and exhibited similar staining properties to *Cryptosporidium* with conventional staining techniques. However, it was not difficult to distinguish between *Cryptosporidium* and *Isospora* oocysts on the basis of oocyst sizes. Oocysts resembling *T. gondii, Sarcocystis, Besnoitia* or *Hammondia* were also found in cat faecal specimens but due to the similarity of oocysts of these parasites it was not possible to identify the species. However, oocysts from one domestic cat B38 and two farm cats F14 and F52 were much more similar to *T. gondii* and *Sarcocystis* than to *Besnoitia* and *Hammondia* measuring appromately 11 x 8 μm (Lindsay and Blagburn, 1991). On the other hand, oocysts typical of *Sarcocystis* sp. were seen already sporulated in MZN stained ileal smears from sample F52 (Lindsay and Blagburn, 1991). In several epidemiological surveys, the prevalence of *T. gondii* oocysts in cat faeces was found to be low (Dubey,

1973; Christie, Dubey and Pappas, 1976; Gethings, Stephens, Wills, Howard, Balfour, Wright and Morgan, 1987). In the present study, only six out of 294 samples (2%) had oocysts resembling *T. gondii* but they also could have been *Sarcocystis*, *Besnoitia* or *Hammondia*, suggesting a low prevalence of *T. gondii* oocysts. The detection of coccidial oocysts was enhanced by staining with MZN or A-P, a finding which indicates that most coccidian parasites of the cat are acid-fast, a feature shared with *Cryptosporidium.* Oocyst staining techniques with MZN or A-P could therefore, be used for diagnosis of not only *Cryptosporidium* but also other coccidia.

It would be interesting to have been able to compare the results of this study with a detailed serological survey in order to determine the number of cats infected or previously infected with *Cryptosporidium* in the same area. This is addressed to some extent in the next section.

CHAPTER III.

EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN CATS.

SECTION 3. DETECTION OF SPECIFIC ANTI-*CRYPTOSPORIDIUM* IgG, IgM, AND IgA ANTIBODIES IN CATS SERA USING AN INDIRECT IMMUNOFLUORESCENCE ANTIBODY TEST (IFA).

3.3.1. INTRODUCTION.

Surveys on *Cryptosporidium* infection in cats have mainly been focused at the detection of oocysts in faecal specimens (Iseki, 1979; Arai *et al.,* 1990). Only one seroepidemiological survey of *Cryptosporidium* infection in 23 cats has been reported (Tzipori and Campbell, 1981). In human and other animal species, indirect immunofluorescence antibody test (IFA) and enzyme linked immunoassays (ELISA) have been used for detection of specific antibodies to *Cryptosporidium* (Tzipori and Campbell, 1981; Koch *et al.,* 1985; Campbell and Current, 1983; Ungar *et al.,* 1986; Casemore, 1987; Van Opdenbosch and Wellemans, 1985). In seroepidemiological studies, cryostat sections from the intestine of infected animals and oocysts from natural and experimentally infected animals have been used as antigens in IFA techniques (Tzipori and Campbell, 1981; Campbell and Current, 1983; Williams, 1987). The use of sporozoites as antigens in serological surveys to improve levels of specific anti-*Cryptosporidium* antibody titres in IFA techniques has been suggested (Casemore, 1987).

Currently, *in-vitro* excystation of *Cryptosporidium* oocysts enables the use

of sporozoites and oocysts as antigens in serological surveys, and an IFA test utilizing sporozoites and oocysts as antigens has been employed for detection of specific faecal IgA, and serum IgG and IgM in experimentally infected lambs (Hill *et al.,* 1990). The following study utilized a similar IFA assay for detection of specific anti-*Cryptosporidium* IgG, IgM and IgA antibodies in serum samples from cats in the Glasgow area.

3.3.2. MATERIALS AND METHODS.

Sera from sick and healthy domestic, feral, and specific pathogen free (SPF) cats were used in the study. Blood sampling was performed as mentioned earlier. However, not all samples were taken from cats which had also been screened for *Cryptosporidium* oocysts. Some of the sera were obtained from the serum bank of the Feline Virus Unit. The serum samples were tested for specific anti-*Cryptosporidium* IgG, IgM and IgA antibodies using an IFA assay employing *C. parvum* sporozoites and oocysts as antigens (Hill *et al.,* 1990).

EXCYSTATION OF OOCYSTS.

Oocyst excystation was performed according to the method of Blewett and Wright (personal communication). Four hundred microlitres of a suspension containing 6.5 x 10^7 *C. parvum* oocysts per ml were added to 500 µl of 1% Trypsin in HBSS (pH 3), in a 1.5 ml microcentrifuge tube. The sample was well mixed and incubated for 1 hour at 37°C in a water bath, then centrifuged for 5 minutes at 400xg. The supernatant was discarded and the pellet resuspended in 900 µl of HBSS and 100 µl of 2.2% sodium bicarbonate and 100 µl of sodium deoxycholate (BDH Chemicals Ltd.). The sample was incubated for 40 minutes

at 37°C in a water bath, followed by centrifugation for 5 minutes at 400xg, the supernatant discarded and the pellet resuspended in 1.5 ml of 0.1% bovine serum albumin (BSA) in PBS (pH 7.4). Excystation was checked using light and phase-contrast microscopy. This method gives 80% excystation (Blewett and Wright, personal communication).

ANTIGEN PREPARATION FOR IFA TECHNIQUE.

One drop of excysted oocyst sample was applied to each well of either 12 or 15 well microscope slides and aspirated after a few seconds, leaving behind the sporozoites, unexcysted oocysts and empty oocysts on the wells. The slides were then air-dried and fixed with acetone (BDH Chemicals Ltd.) for 10 minutes. Following fixation the slides were air-dried, wrapped in clean tissue papers and preserved at -20°C until used.

DETERMINATION OF THE OPTIMAL WORKING DILUTION OF THE ANTISERA AND ANTIBODY TEST PROCEDURE.

Serum from cat A18, positive for *Cryptosporidium* infection, was serially double diluted with from 1:10 to its end point, using PBS (pH 7.4) (Oxoid Limited, Hampshire, England) in a microtitre tray (Sero-Wel, Sterilin Limited, Hounslow, England). Aliquots were transferred to appropriate wells of the previously prepared IFA slides and for each dilution of test serum four wells were used. The slides were incubated for 40 minutes at 37°C in a moist chamber, then washed with PBS for 30 minutes and air-dried. Serially double diluted (1:10 to 1:80) FITC-conjugated goat anti-cat IgG antiserum (whole molecule) (Sigma

Chemical Co. Limited, Poole, England) was used and different dilutions of the antiserum were applied to cover each reaction site, then incubated as before. Following incubation, the slides were again washed with PBS for 30 minutes, air-dried and mounted with cover slips using 50% glycerol in PBS. A similar procedure was followed for IgM (Binding Site Limited, Edgbaston, England) and IgA (Serotec Limited, Kidlington, England) antisera. Slides were examined under a fluorescence microscope equipped with FITC filters, at x250 and x400 magnifications.

Using the same procedure, 258 serum samples from various groups of cats were tested for the presence of anti-*Cryptosporidium* antibodies using 1:10 and 1:20 dilutions of the sera with PBS (pH 7.4). Sera from 8 SPF kittens were used as a negative control. For each sample two dilutions of the serum were used (1:10 and 1:20) and the antisera were used at 1:40 dilution. The sera were tested for the presence of specific anti-*Cryptosporidium* IgG, IgM and IgA.

3.3.3. RESULTS.

Serial dilution of the sample from cat A18 showed that 1:40 was the optimal working dilution for all the specific anti-cat antisera isotypes (Appendix 3.3.5.). At this dilution, moderate fluorescence of the antigens was observed, with up to 1:80 dilution of the serum sample. Slightly more intense fluorescence was observed with IgG antibody than IgM and IgA, at 1:10 and 1:20 dilutions of the serum sample.

PREVALENCE OF SPECIFIC ANTI-*CRYPTOSPORIDIUM* ANTIBODIES IN CATS.

Specific anti-*Cryptosporidium* IgG, IgM, and IgA antibodies were detected in 192 (74%), 84 (32%) and 67 (26%), respectively, in sera from 258 cats examined (Table 3.3.1). The prevalence of specific antibodies to *Cryptosporidium* appeared to be higher in adult cats than kittens ($p<0.05$) (Table 3.3.2). Figure 3.3.1 shows the prevalence of different classes of specific antibodies to *Cryptosporidium* in various groups of cats examined. The prevalence of IgM and IgA antibody classes was higher in sick than healthy domestic cats (Table 3.3.1). All 8 SPF kittens were negative for anti-*Cryptosporidium* antibodies at 1:10 and 1:20 dilutions.

Specific IgG, IgM and IgA antibodies were detected in various combinations in test sera from the cats. Of the 258 sera examined, 54 had all three antibody classes (IgG, IgM, IgA), whereas, IgM and IgA antibodies were present in only one sample. Specific IgG antibody was detected together with IgM antibody in 27, and with IgA antibody in 11 serum samples, respectively. One hundred out of 192 antibody positive serum samples contained IgG isotype antibodies, whereas specific IgM antibody alone was found in only two samples, and only one sample was positive for IgA antibody alone.

Type of cat	Number	Number Positive (%) IgG	IgM	IgA
sick domestic	104	80 (77)	42 (40)	37 (35)
healthy domestic	100	83 (83)	24 (24)	18 (18)
feral	46	29 (63)	18 (39)	12 (26)
SPF	8	0	0	0
Total	258	192 (74)	84 (32)	67 (26)

Dilution 1:10

Table 3.3.1. IFA test of sera from different groups of cats

Age (months)	Total Number	Number positive IgG	IgM	IgA
>6	210	166	75	58
≤6	40	26	9	9
Total	250	192	84	67

>6 = more than 6 months of age
≤6 = less than 6 months of age

Table 3.3.2. Age prevalence of specific antibodies to *Cryptosporidium* in cats

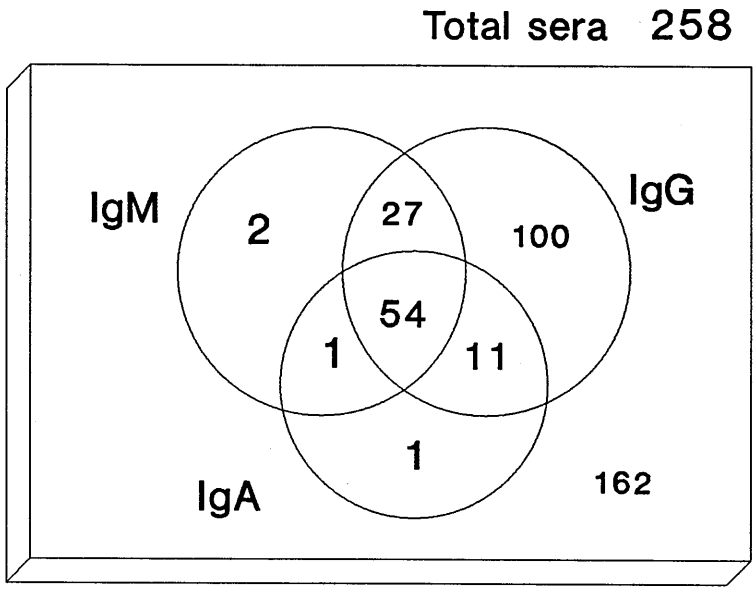

Figure 3.3.1. Occurrence of different classes of specific anti-*Cryptosporidium* antibody in cat sera.

ANTIBODY TITRES OF SELECTED IFA POSITIVE SERA.

Table 3.3.3 shows antibody titres in selected IFA positive samples, including four samples from cats which were shedding oocysts in faeces. The lowest titre for IgG antibody was 1:20 whilst for IgM and IgA antibody was 1:10. Sera from cats with oocysts in their faeces had all three classes of antibodies present. Nevertheless, the antibody titres were lower compared to sera from cats negative for oocysts but positive for specific IgG, IgM and IgA antibodies (Table 3.3.3).

	Antibody titres			
Sample	IgG	IgM	IgA	Oocysts in faeces
M17	20	20	10	P
M32	40	10	20	P
M140	40	20	10	P
A18	80	20	10	P
A19	40	10	10	N
M16	160	80	40	N
M31	20	0	0	N
B50	40	40	0	N
M121	320	10	40	N
M122	160	160	80	N

P = Positive; N = Negative

Table 3.3.3. Antibody titres of selected sera from cats positive and negative for faecal oocysts

REACTIVITY OF SPECIFIC ANTIBODIES TO SPOROZOITE OR OOCYST ANTIGENS.

In the IFA positive samples, sporozoite antigens were easily detected using fluorescence microscopy especially in sera tested for IgG antibodies (Figure 3.3.2). In some of the positive samples, fluorescence of the apical (blunt) end of the sporozoites was more intense than the rest of the sporozoite. Occasionally, a small intensely fluorescing dot appeared near the terminal end of the sporozoite, presumably the sporozoite nucleus. Although the IgG isotype response was against sporozoites predominantly, IgG specific fluorescence of oocyst walls was also observed but the number and intensity of fluorescing oocysts was less than that in IgM and IgA positive samples. In some cases, a suture line was prominent on the oocyst wall and at times one or two excysting sporozoites were seen to be caught up at the suture.

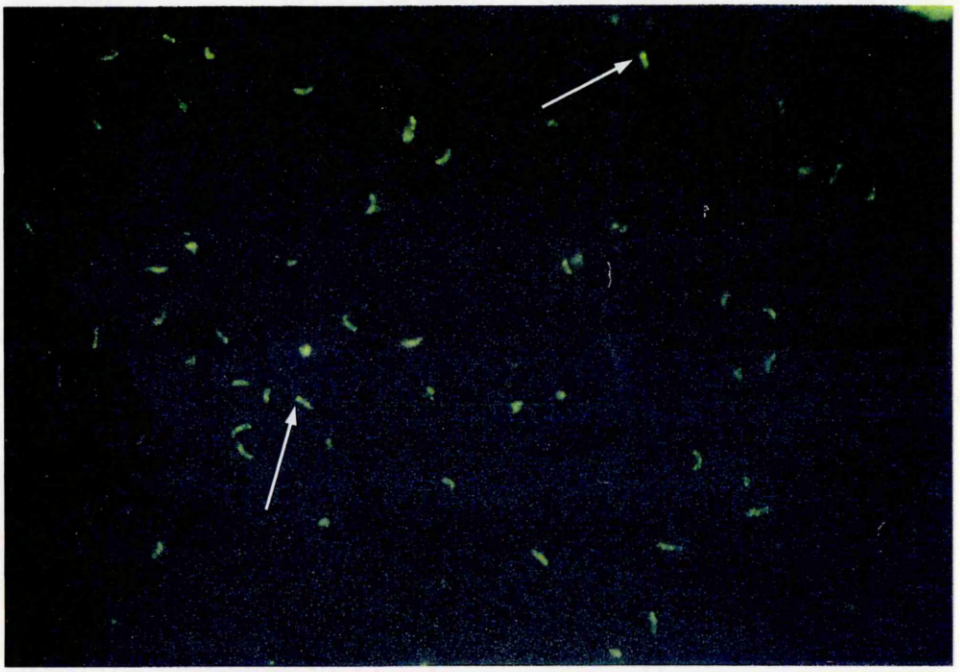

Figure 3.3.2. IFA positive sample showing *C. parvum* sporozoites fluorescing under fluorescence microscopy with FITC-filters. The serum tested contained anti-*Cryptosporidium* IgG antibody. Note the slightly more intense fluorescence at the anterior portion of the sporozoites (arrows). Magnification x400.

3.3.4. DISCUSSION.

The current study indicates that 74% (192 out of 258) of sera tested for specific antibodies came from cats which had been infected at some time with *Cryptosporidium*. However, 39% (100 out of 258) of the samples tested contained only IgG antibodies. It has been reported that specific IgG antibodies appear later on in infection than IgM and IgA, and persist for a long period in the circulation and may indicate a previous exposure to the parasite (Casemore, 1987; Ungar *et al.,* 1988). On the other hand, 35% (92 out of 258) serum samples from domestic cats were positive for either IgM and/or IgA antibodies suggesting a more recent infection (Ungar *et al.,* 1988; Hill *et al.,* 1990), and were present both in cats found positive for *Cryptosporidium* oocysts by faecal examination and those which were negative. These findings suggest that detection of oocysts in faecal samples is not a very reliable method for diagnosis of cryptosporidiosis in animals which shed oocysts intermittently. The duration for oocyst shedding in cats infected with *Cryptosporidium* is between 7 to 10 days, but oocysts could be detected for up to 5 months post infection (Iseki, 1979). However, the precise times for the appearance and duration of anti-*Cryptosporidium* antibodies in cats infected with *Cryptosporidium* are not known. In the current study, it is possible that faecal samples from the cats were obtained either before or after the patent period. In lambs, specific IgM and IgA antibodies start to appear in serum by day 6 post-infection, and reach a peak by day 16 post infection (Hill *et al.,* 1990). Specific IgG antibodies rise slowly and persist longer than IgM and IgA (Ungar *et al.,* 1988; Hill *et al.,* 1990). The only other previous seroepidemiological survey reported a prevalence of 87% (20 out

23) of cats examined for specific anti-*Cryptosporidium* IgG antibodies (Tzipori and Campbell, 1981).

There was no sex or age prevalence of specific antibodies to *Cryptosporidium* in cats examined in the present study. However, the majority of the samples were obtained from cats of more than two months of age; ages of the feral cats were not accurately known. Studies involving the detection of oocysts in faecal samples have indicated a higher prevalence of cryptosporidiosis in kittens (Arai *et al.*, 1990), children and neonatal animals (Fayer and Ungar, 1986). Sick domestic cats had a higher prevalence of specific IgM and IgA antibodies than healthy domestic cats ($p<0.05$), presumably indicating recent infections, however, only a twofold increase in titre was measured.

The pronounced polar fluorescence of the sporozoites in the IFA positive samples was probably a result of reactivity of the antibodies to apical complex antigens (Smith, personal communication). Arrowood *et al.* (1989), reported similar findings using an IgM monoclonal antibody reacted with air-dried sporozoites. In the current study, the IgG antibody response was more marked than either the IgM or the IgA antibody response, and sporozoites were more reactive than oocysts. The higher reactivity of specific anti-*Cryptosporidium* antibodies to sporozoites is probably due to the presence of recycling sporozoites through the generation of thin-walled oocysts (Mead *et al.*, 1988). Previous serological studies using human sera, indicated a pronounced IgM and IgA response to *Cryptosporidium*. However, these assays utilized only oocyst antigens (Casemore, 1987). In the current study, the use of oocysts which had undergone the excystation process enabled detection of both sporozoites and excysted and unexcysted oocysts.

All eight sera from SPF kittens were negative for specific anti-*Cryptosporidium* antibodies at 1:10 dilution. The fact that anti-*Cryptosporidium* antibodies were detected in both *Cryptosporidium* positive and negative cats suggests that the test is more sensitive than oocyst detection methods. Specificity of the test was difficult to determine but the use of purified sporozoite and oocyst antigens eliminated possible interference with other organisms. Moreover, no report has indicated cross-reactivity between *Cryptosporidium* antigens and antibodies to other organisms (Ungar *et al.*, 1988).

The IFA assay used in this study utilized a deer isolate of *C. parvum*, passaged in lambs. This fact supports the findings that *Cryptosporidium* isolates from various animal species share common antigens. In view of the presence of animals with subclinical infections and short periods of oocyst shedding, the detection of specific IgG and IgM and/or IgA antibodies in sera from cats could be helpful in the diagnosis of cryptosporidiosis. However, further studies are required to determine the times for the appearance and persistence of specific antibodies in cat sera using similar techniques.

CHAPTER IV.

INFECTIVITY OF *CRYPTOSPORIDIUM* ISOLATE FROM A FARM CAT IN LAMBS AND MICE.

SECTION 1. EXPERIMENTAL TRANSMISSION OF *CRYPTOSPORIDIUM* SPP. FROM A FARM CAT TO LAMBS.

4.1.1. INTRODUCTION.

In the preceding chapter, *Cryptosporidium* oocysts were detected in the faeces of domestic, feral and farm cats. In the present study, *Cryptosporidium* oocysts recovered from a farm cat (F22) were passaged in newborn lambs. Studies on the experimental transmission of a *Cryptosporidium* isolate from domestic cats has been performed only in cats, mice, guinea pigs (Iseki, 1979; Asahi *et al.,* 1991) and dogs (Asahi *et al.,* 1991). There are no previous reports of attempts to infect farm animals with *Cryptosporidium* isolates from cats.

The objectives of the following study were to determine the infectivity of a *Cryptosporidium* isolate from a farm cat for newborn lambs.

4.1.2. MATERIALS AND METHODS.

SOURCE OF ANIMALS.

Two male Scottish Blackface x Suffolk, and one Finnish-Landrace lambs were used. The lambs were separated from their dams when they were only one day old, fitted with harnesses and plastic bags (Figure 4.1.1) for faecal collection and

kept isolated in sterilized boxes. Faecal samples were collected daily and examined for *Cryptosporidium* oocysts for between 4 and 7 days prior to experimental infection to ensure that natural infection was not present.

OOCYST SOURCE AND INOCULATION.

Cryptosporidium oocysts from a farm cat were concentrated by the sucrose flotation method and quantified using an improved-Neubauer haemocytometer prior to inoculation of the animals. The first lamb (F2398) Scottish Blackface x Suffolk was inoculated with 10^5 oocysts at 8 days of age, whereas lamb (V596) a Finnish-Landrace was inoculated with 10^4 oocysts at 5 days of age. The remaining lamb V655 was uninoculated and kept as a negative control. Each lamb was kept in a separate box which had been cleaned and sprayed with 35% ammonium hydroxide (SG 0.88) and 10% hydrogen peroxide solutions (H_2O_2). The lambs were bottle-fed twice daily with 400 to 500 ml of semi-pasteurised milk. Faecal samples continued to be collected daily in the attached plastic bags and examined for *Cryptosporidium* oocysts using MZN and A-P techniques. The lambs were also examined for diarrhoea and monitored for 18 to 21 days post-infection (PI). They were then euthanased using pentobarbitone sodium (Euthatal RMB Animal Health) and immediately necropsied. Sections from the alimentary tract, including abomasum, duodenum, jejunum, ileum, caecum and colon were taken, preserved in 10% formol-saline and processed for histological examination. Similar sections were preserved in glutaraldehyde solution and processed for SEM as described in Chapter 2. Impression smears were also prepared from similar sites and stained using the MZN and A-P techniques and examined for *Cryptosporidium* oocysts.

Figure 4.1.1. A Finnish-Landrace lamb (V596) used for experimental transmission of *Cryptosporidium* sp. oocysts from a farm cat.

4.1.3. RESULTS.

Cryptosporidium oocysts were detected in the two experimentally-infected lambs, F2398 and V596, on day 3 and 10 PI, respectively. Quantitative detection of *Cryptosporidium* oocysts with an improved-Neubauer haemocytometer was not possible in the diluted faeces of lamb F2398 before day 9 PI (Table 4.1.1). The number of *Cryptosporidium* oocysts detected in stained smears for lamb F2398 ranged from one to two oocysts per high power field from day 3 to 7 PI, then increased to three to four oocysts per field from day 9 to 12 PI (Table 4.1.1). Peak oocyst shedding occurred on day 9 and 10 PI with total oocyst output of 2.2 x 10^6 and 2.9 x 10^6, respectively, using the haemocytometer and three to four oocysts per field in A-P stained smears. The number of oocysts excreted per gramme of faeces per day ranged between 1 x 10^5 to 2 x 10^6, with the higher oocyst count on day 15 PI corresponding with two to five oocysts per field in A-P stained smears. At the termination of the experiment on day 18 PI, only one to four oocysts per field were detected in the stained smears.

Lamb V596 started shedding oocysts 7 days later than lamb F2398, on day 10 PI (Table 4.1.2). The number of oocysts counted using haemocytometer ranged between 10^6 to 10^7 per gramme faeces during the first 6 days of oocyst excretion. However, no oocyst was detected by means of the haemocytometer prior to day 10 PI or after day 15 PI. The total number of oocysts excreted daily ranged from 10^7 to 10^8. With A-P staining, four to 11 oocysts were detected per high power field on day 10 to 13 PI, which then dropped to two to three per field between day 14 and 17 PI. Nevertheless, on days 18 and 19 PI, the number of oocysts observed in stained smears increased to four to seven per field and

dropped again to one to two oocysts per field until the termination of the experiment at day 21 PI (Table 4.1.2).

Day PI	Faecal weight (g)	Oocysts/field small	Oocysts/field large	Oocyst/gramme faeces
0	15.8	N	N	N
1	1.7	N	N	N
2	1.9	N	N	N
3	5.5	N	1+-	N
4	6.0	N	1-2	N
5	11.4	1-2	1-2	N
6	13.6	1-2	1-2	N
7	17.5	1-2	1-2	N
8	7.6	1-2	N	N
9	12.3	1-2	3-4	1.8×10^5
10	4.4	1-2	3-4	6.6×10^5
11	7.1	1-2	3-4	1.3×10^5
12	2.3	N	3-4	N
13	9.3	N	1-2	N
14	4.5	1-2	1-2	N
15	0.46	N	2-5	2.4×10^6
16	3.18	1-2	1-4	2.2×10^5
17	0.42	1-2	1-4	1.3×10^5
18	5.6	1-2	1-4	1.3×10^5

N = no oocyst detected; +- = inconclusive

Table 4.1.1. Daily faecal weight and number of *Cryptosporidium* oocysts obtained in experimentally-infected lamb F2398.

Day PI	Faecal weight (g)	Oocysts/field small	Oocysts/field large	Oocyst/gramme faeces
0	32.5	N	N	N
1	44.7	N	N	N
2	23.3	N	N	N
3	13.2	N	N	N
4	2.1	N	N	N
5	5.0	N	N	N
6	32.8	N	N	N
7	11.5	N	N	N
8	6.3	N	N	N
9	10.4	N	N	N
10	17.7	4-6	1-2	3.3×10^6
11	46.3	7-11	1-2	1.6×10^7
12	46.9	5-9	1-2	1.4×10^7
13	34.5	4-8	1-2	1.0×10^7
14	34.3	2-4	1-2	9.5×10^6
15	42.2	1-2	2-3	2.8×10^6
16	19.8	N	2-3	N
17	36.7	N	2-3	N
18	25.0	N	4-6	N
19	60.7	N	5-7	N
20	35.3	N	1-2	N
21	24.5	N	1-2	N

N = no oocyst detected

Table 4.1.2. Daily faecal weight and number of *Cryptosporidium* oocysts obtained in experimentally-infected lamb V596.

In both the experimentally-infected lambs, two sizes of oocysts were identified. The first type was small with a mean oocyst size of 4.8 x 4.4 μm (4.5-5.0 x 3.6-4.8 μm), whereas, the other type was larger measuring approximately 6.0 x 5.0 μm (5.4-6.0 x 4.5-5.7 μm) (Appendix 4.1.1.). The larger oocysts were detected from day 3 PI in lamb F2398 in A-P stained smears, whereas, the small oocysts were observed from day 5 PI. Both small and large oocysts appeared in small numbers in stained faecal smears of lamb F2398, ranging from one to two oocysts per field of view for the small type, and one to four for the large oocysts (Table 4.1.1.). In contrast, the majority of oocysts detected in faecal specimens of lamb V596 in the first 6 days of oocyst shedding were small, but large oocysts were observed in small numbers from day 10 PI to the termination of the experiment at day 21 PI.

Clinically, the infected lambs remained in good health and throughout the experiments the lambs produced pasty and occasionally hard pellet-like faeces. However, slightly loose faeces were observed on days 9, 10 and 11 PI for lamb F2398 and from day 9 to 12 PI for V596. The control lamb was normal and also produced pasty or hard, pellet-like faeces throughout the experiment. At necropsy, there were no significant gross pathological lesions found in the alimentary tract. Endogenous stages of *Cryptosporidium* were detected in both epithelial border of the ileum, in histological and SEM sections (Figures 4.1.2 and 4.1.3). From impression smears stained with MZN and A-P, oocysts were detected in the jejunum, ileum, caecum and colon. Oocysts were not detected in the abomasum of any of the lambs. The number of oocysts per field was higher in the ileum, followed by the colon, with both MZN and A-P techniques (Table 4.1.3). Two sizes of oocysts (small and large) were observed in all the positive

impression smears, similar in size to those found in the direct faecal smears from the lambs and the farm cat.

Site	Number of oocysts per field	
	MZN	A-P
abomasum	N	N
duodenum	N	1-2
jejunum	1-2	N
ileum	4-6	7-9
caecum	1-2	2-4
colon	2-4	4-6

magnification x 400; N = no oocyst detected

Table 4.1.3. *Cryptosporidium* oocyst detection in impression smears made from six anatomical sites of the GI tract of an experimentally-infected lamb (F2398).

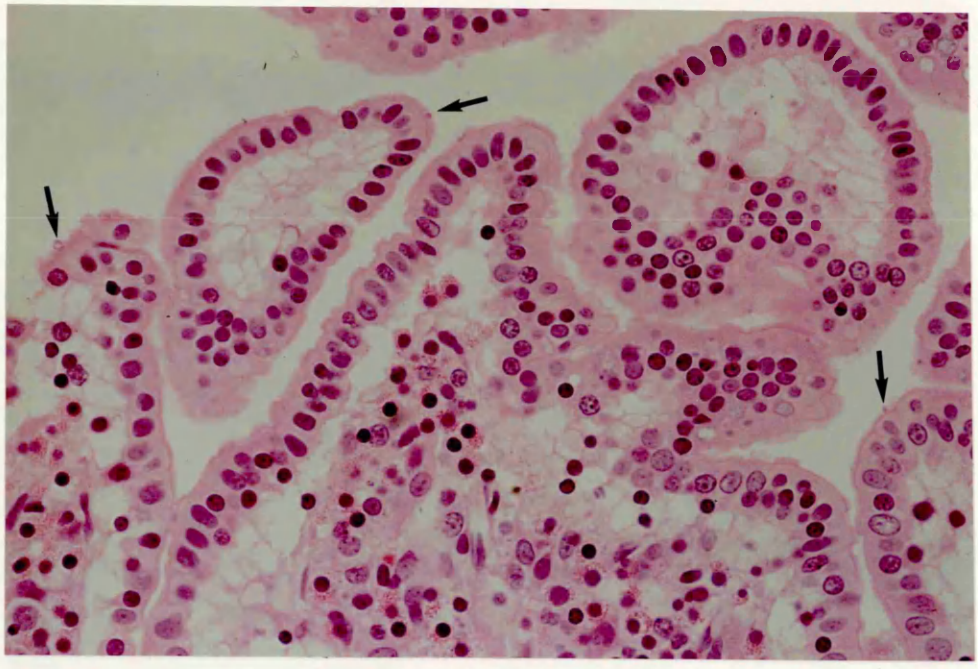

Figure 4.1.2. Histological section from the ileum of experimentally-infected lamb showing endogenous stages of *Cryptosporidium.*

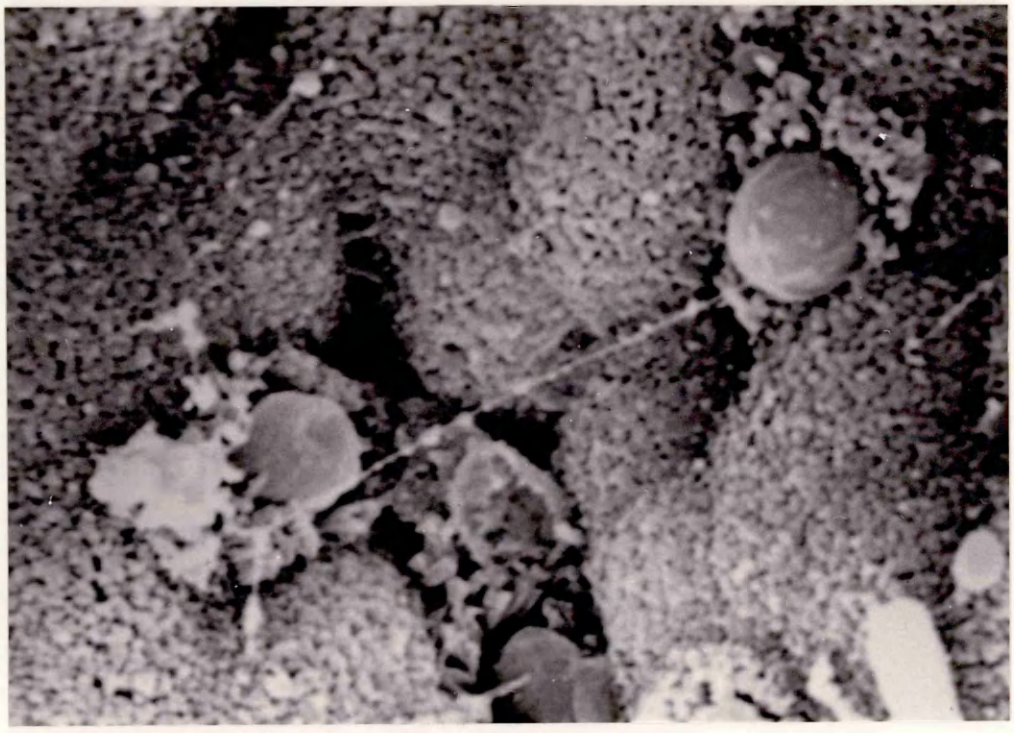

Figure 4.1.3. Endogenous stages of *Cryptosporidium* in the ileum of the lamb under SEM.

4.1.4. DISCUSSION.

In this study, it has been demonstrated that *Cryptosporidium* spp. isolated from a farm cat could produce infection in newborn lambs. Although diarrhoea was not observed in any of the lambs, the consistency of faeces changed slightly from hard to soft just before and during oocyst excretion. This could possibly be due to intestinal malfunctioning resulting from *Cryptosporidium* infection. The prepatent periods were different for the two lambs; the first lamb (F2398) began to shed oocysts on day 3 PI, while the second lamb (V596) did not start to shed oocysts until day 10 PI. This difference might be related to the fact that the number of oocysts inoculated into each lamb also differed, lamb F2398 received 10^5 oocysts, whereas, lamb V596 received 10^4 oocysts. The reason for the different doses of inocula was because the total number of oocysts available was limited. In similar studies using a cervine isolate, prepatent periods were extended as the number of oocysts used in the inocula was reduced, but the final oocyst output remained constant (Blewett and Wright, unpublished findings).

Daily oocyst output was quite low in the first lamb (F2398) compared with lamb V596, with peak oocyst outputs of 1.2×10^6 and 7.4×10^8, respectively. This was possibly due to the fact that lamb V596 was infected at a younger age (5 days old) compared to F2398 (8 days old). Previous studies have demonstrated that younger animals more often succumb to severe infections with *Cryptosporidium* accompanied by a higher oocyst output (Current, 1990). In similar experiments, using a cervine isolate, daily oocyst output ranged between 10^8 to 10^9, accompanied by watery diarrhoea (Blewett and Wright, unpublished findings). The shedding pattern of lamb V596 resembled that of a *C. parvum* isolate, with a large number of oocysts during the first days of oocyst shedding

(Hill *et al.*, 1990), although in this case there was only a slight change of faecal consistency from hard to soft with no diarrhoea.

Interestingly, lamb F2398 shed low numbers of both small and large types of oocyst, whereas, lamb V596 excreted more small oocysts in a manner typical of *C. parvum*. The likelihood of extraneous infection occurring in any of the lambs was minimal. The lambs were obtained from well monitored, closed breeding stock at the Moredun Research Institute, and the isolation units were intensely cleaned and sprayed before the introduction of the lambs, with 35% ammonium hydroxide solution and 10% H_2O_2, which are known to kill *Cryptosporidium* oocysts (Blewett, 1988). Moreover, the lambs were examined for *Cryptosporidium* oocysts for 5 consecutive days prior to inoculation, and none of the lambs was found to be infected. The fact that no infection was found in the control lamb ruled out the possibility of contamination.

As in other studies, the ileum was the main site of infection. Neither oocysts nor endogenous stages of *Cryptosporidium* were detected in the abomasum, and such findings eliminate the possibility of infection with *C. muris*, which mainly infects the stomach of mice (Tyzzer, 1907) and abomasum of the ruminants (Anderson, 1987; Pospischil *et al.*, 1987). Both small and large variants of oocysts stained with the FITC-labelled MAb (Northumbria Biologicals Ltd.) confirming that they were cryptosporidia.

The findings of this study have therefore, demonstrated that *Cryptosporidium* isolate from farm cats can be transmitted to lambs, suggesting that farm cats are possible reservoirs of *Cryptosporidium* for farm animals. Since infection was also found in faecal samples taken from two dogs and calves on the same farm as the infected cat (F22), it is possible that these animals became

infected from the same source. Oocysts found in one dog were similar to the two variants found in the farm cat and later in the experimentally infected lambs.

In this study, two sizes of *Cryptosporidium* oocysts were observed in the faecal specimens from the experimentally-infected lambs. The small oocysts resembled *C. parvum* in size, morphology and staining characteristics (Iseki, 1979; Uga *et al.*, 1989; Smith *et al.*, 1989; Asahi *et al.*, 1991). The large oocysts described in the present study have never been documented before in cats or other mammalian hosts but have similar morphology and staining characteristics as *C. parvum*. Both small and large oocysts stained with FITC-labelled MAb (Northumbria Biologicals Ltd.). In reptiles, oocysts ranging in size from 5.3 to 6.3 by 4.2 to 5.7 μm and 5.2 to 8.8 by 4.4 to 6.2 μm have been reported in lizards (Ostrovska and Paperna, 1990) and snakes (Upton *et al.*, 1989), respectively. *C. baileyi* and *C. meleagridis*, the species affecting avian hosts have oocysts measuring 5.6-6.3 x 4.5-4.8 μm and 4.5-6.0 x 4.2-5.3 mm, respectively (Current *et al.*, 1986; Upton *et al.*, 1989). Only one report of cross infection of *C. baileyi* to mammalian host has been documented in immunocompromised human working on a poultry farm (Ditrich *et al.*, 1991). Hitherto, there has been no reports of cross-transmission of *Cryptosporidium* from avian or reptilian species to cats or livestock. The large oocysts observed in the present study are possibly one of the isolates of *C. parvum*, or just merely represented an upper range of oocyst size of *C. parvum*, though little evidence is available to support either of these two possibilities. Further investigations beyond the scope of this thesis are required to resolve this question.

CHAPTER IV.

INFECTIVITY OF *CRYPTOSPORIDIUM* ISOLATE FROM A FARM CAT IN LAMBS AND MICE.

SECTION 2. EXPERIMENTAL TRANSMISSION OF *CRYPTOSPORIDIUM* SPP. ISOLATED FROM A FARM CAT TO NEWBORN MICE

4.2.1. INTRODUCTION.

Previous studies on experimental transmission using *Cryptosporidium* oocysts recovered from domestic cats have failed to cause infection in mice, guinea pigs (Iseki, 1979; Asahi *et al.*, 1991), rats or dogs (Asahi *et al*, 1991). In the preceding section, *Cryptosporidium* spp. isolated from a farm cat led to patent infection in newborn lambs. Oocysts originally obtained from the farm cat and later from the lambs were used in the present experiment in an attempt to establish infection mice.

The objective of this study was to determine infectivity of *Cryptosporidium* oocysts from cat to mice, and cat oocysts passaged in lambs to mice.

4.2.2. MATERIALS AND METHODS.

Four litters each of ten 2 day old mice were used in this study. The first two litters were inoculated orally with partially purified oocysts, using a 1 ml syringe and a 23 gauge needle. Each mouse was given 100 µl containing 10^3 *Cryptosporidium* oocysts from the farm cat. The other two litters were

inoculated orally with 100 µl containing 10^4 oocysts from the lambs. Following inoculation with *Cryptosporidium* oocysts, all mice were returned to their dams for a period of 7 days, then euthanased using chloroform (BDH chemicals Ltd.). Sections from the stomach, duodenum, jejunum, ileum, caecum and colon were removed and placed in separate containers containing 2 ml of distilled water. The tissues were homogenized using Ultra-Turrax-T25 homogenizer (IKA® Labortechnik (Janke and Kunkel GMBH & Co. G7813 Tausen, Germany), and washed three times by centrifugation in deionised water at 400xg for 10 minutes (Current, 1990). Examination for *Cryptosporidium* oocysts was performed in wet preparations with 0.16% malachite green and 0.1% SDS, and in MZN, A-P and FITC-labelled MAb stained smears. An additional five mice were kept as uninoculated controls in each experiment.

4.2.3. RESULTS.

On day 7 PI, oocysts were not detected in any of the homogenized section from any of the mice inoculated with *Cryptosporidium* isolate from the farm cat. Infection was, however, found in the homogenized sections taken from both litters of mice infected with *Cryptosporidium* oocysts obtained from the two lambs (F2398 and V596). Only one mouse out of 20 was negative using an improved-Neubauer haemocytometer and oocyst staining methods (Table 4.2.1). The mean number of oocysts recovered from the intestinal homogenates was 4.0 x 10^5/ml and two distinct sizes of oocysts were seen, measuring approximately 4.8 x 4.5 µm and 6.0 x 5.4 µm for small and large forms, respectively. Interestingly, groups of seven to 30 oocysts were seen per field in some fields in

MZN-stained smears comprising of oocysts with more or less similar dimensions. However, in some other fields both small and large oocysts could be seen together but in varying proportions (Figure 4.2.1). In most cases, the large oocysts predominated. The sizes and morphology of these two types of oocysts were similar to those found in the experimentally infected lambs and the farm cat (Table 4.2.2).

Sample	No. oocysts/ml.	No oocysts/field
1	8.7×10^5	6-10
2	1.5×10^5	4-12
3	5.6×10^5	7-16
4	9.5×10^5	6-25
5	4.0×10^5	5-15
6	9.0×10^5	7-19
7	3.8×10^5	4-10
8	5.8×10^5	2-7
9	6.0×10^5	5-12
10	5.0×10^5	3-14
11	1.5×10^5	2-14
12	N	2-5
13	1.5×10^5	4-6
14	N	N
15	1.3×10^5	8-15
16	1.0×10^5	1-2

N = no oocyst detected

Table 4.2.1. Number of *Cryptosporidium* oocysts detected in experimentally-infected mice using improved-Neubauer haemocytometer and oocyst staining methods.

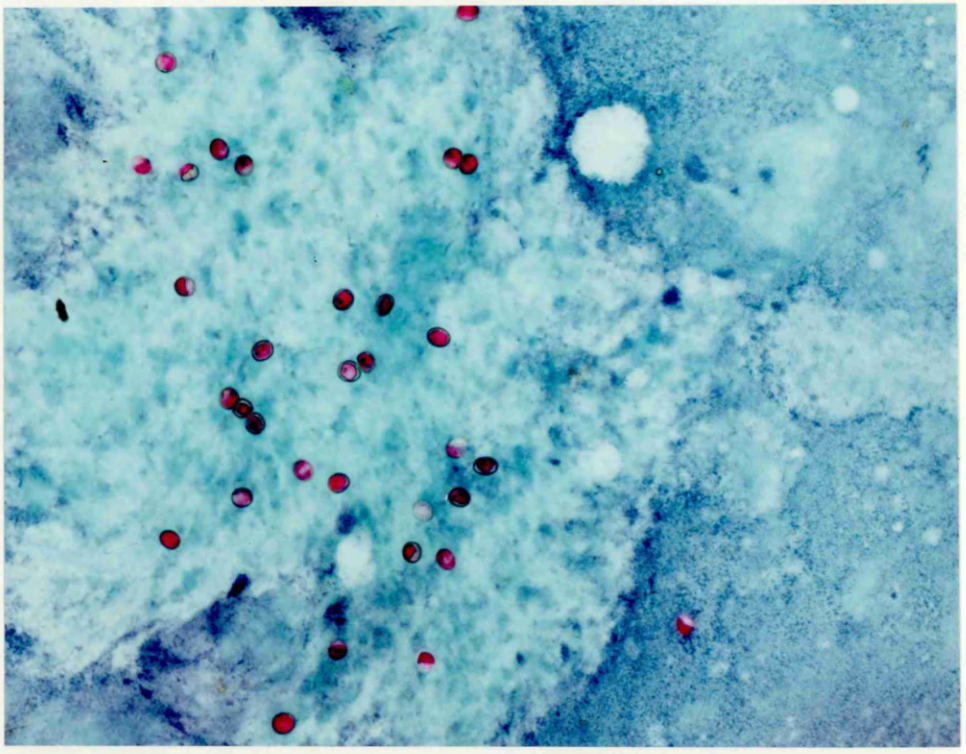

Figure 4.2.1. MZN-stained smear showing *Cryptosporidium* oocysts in the homogenized intestine from the experimentally-infected mice.

	Oocysts from mice[2]		*C. parvum* isolates	
No.	Small	Large	Calf	Cervine
1	4.8 x 4.3	6.0 x 5.4	4.8 x 4.8	4.6 x 4.2
2	5.0 x 4.8	6.0 x 6.0	4.8 x 4.6	4.8 x 4.0
3	4.8 x 4.5	6.0 x 5.4	4.9 x 4.2	4.8 x 4.2
4	4.8 x 4.6	6.0 x 4.8	4.8 x 4.2	4.8 x 4.5
5	4.8 x 4.8	6.0 x 5.4	4.9 x 4.8	4.2 x 3.6
6	4.8 x 4.5	6.0 x 5.8	5.0 x 4.8	4.8 x 4.6
7	4.9 x 4.8	6.0 x 4.8	4.9 x 4.8	5.0 x 4.6
8	5.0 x 4.2	5.7 x 4.8	5.0 x 4.2	4.2 x 4.2
9	4.8 x 4.8	6.0 x 5.0	5.0 x 4.2	4.8 x 4.0
10	4.8 x 3.6	6.0 x 5.4	4.8 x 4.8	4.8 x 4.2
11	4.8 x 4.8	5.9 x 5.4	5.0 x 4.6	4.8 x 4.2
12	5.0 x 4.8	6.0 x 5.4	4.8 x 4.2	4.8 x 4.5
13	4.8 x 4.2	5.4 x 4.8	4.8 x 3.6	4.8 x 3.6
14	4.8 x 4.2	5.4 x 4.2	4.8 x 4.2	4.8 x 4.0
15	4.8 x 4.2	6.0 x 5.4	5.0 x 4.2	4.2 x 4.2
16	5.0 x 4.8	6.0 x 5.0	4.6 x 3.8	4.8 x 4.2
17	4.8 x 4.2	6.0 x 4.2	4.8 x 4.8	4.8 x 4.4
18	4.8 x 4.8	6.0 x 4.2	4.8 x 4.2	5.0 x 4.8
19	4.8 x 4.8	6.0 x 6.0	4.8 x 4.8	4.8 x 4.8
20	4.8 x 3.6	6.0 x 5.4	4.8 x 4.2	4.8 x 4.6
Mean	4.9 x 4.5	5.9 x 5.2	4.9 x 4.4	4.7 x 4.3

[1]in micrometer (μm).
[2]experimentally infected using *Cryptosporidium* oocysts originally isolated from a farm cat and passaged in a lamb.

Table 4.2.2. Comparison of sizes[1] of *Cryptosporidium* oocysts detected in the experimentally-infected mice with *C. parvum* isolates from a naturally-infected calf and deer (cervine).

The sizes of oocysts from the mice were compared with those from naturally infected calf and deer isolates (Table 4.2.2). In all smears prepared from the homogenized tissues of mice, more of the larger oocysts were detected than the small oocysts. There was great variation in the number of oocysts detected per microscope field (Table 4.2.1), with up to 30 oocysts being detected, whereas in some fields only one or two oocysts were seen.

4.2.4. DISCUSSION.

Despite the fact that direct infection using oocysts from the farm cat failed to induce infection in any of the mice, oocysts from the same source which were passaged in lambs were shown to be infective to mice. This lack of infection in mice directly infected with oocysts from the farm cat was probably due to the low number of oocysts in the inoculum, but prolonged storage of oocysts prior to inoculation may have been a major contributing factor. In the experimental transmission of *Cryptosporidium* oocysts from the cat into the lambs, about 10^5 and 10^4 oocysts were used, whereas using the same materials each mouse was inoculated with 10^3 oocysts. The lambs were infected one month after recovery of the oocysts from the farm cat, whereas experimental transmission study in mice was performed 5 months later. Nevertheless, about 10^4 oocysts recovered from the experimentally infected lambs were infective to mice. Previous studies have indicated that infectivity of *Cryptosporidium* oocysts can be lost after 2 to 6 months storage at 4°C (Tzipori, 1983).

In several studies, *Cryptosporidium* isolates from naturally infected cats failed to induce infection in mice (Iseki, 1979; Asahi *et al.,* 1991). In the present

study, indirect transmission in mice was successful using a *Cryptosporidium* isolate from a farm cat. It is possible that *Cryptosporidium* isolates detected in cats in Japan were different from the one used in this study. It is possible that the isolate used in this study originated from farm animals, since infection was also found in dogs and cattle on the farm. In two studies, *Cryptosporidium* isolates from cattle were shown to be infective to cats, although no clinical signs were observed (Pavlasek, 1983b; Augustin-Bichl *et al.,* 1984). The findings of this study showed that *Cryptosporidium* can be passed from farm cats to lambs, and from the lambs to mice, suggesting a wide potential spread of the parasite to various animal hosts in the farm environment. These results also suggest that farm cats can be reservoirs of *Cryptosporidium* and capable of spreading infection to other susceptible hosts.

The occurrence of the two forms of oocysts in the intestine homogenates of mice were consistent with the findings in the previous experimental transmission when oocysts from the farm cat were used to infect lambs. Occasionally, several oocysts with similar sizes were found in the same microscope field at x400 magnification. Due to the presence of recycling merozoites and autoinfection, numerous oocysts can develop from a single oocyst causing infection at a particular site. This being the case, the presence of oocysts with mean size of 6.0 x 5.0 μm indicates that infection emanated from a distinct oocyst of similar dimensions. These findings are also supported by the fact that oocysts of the small form were also found in some fields without the occurrence of the large form. However, as explained earlier there were other fields in which both forms of oocysts were found together.

The small forms of oocyst found in mice, lambs and the farm cat were

similar to those found in naturally-infected calves, deer, and humans, typical of *C. parvum*. The ranges of the size of *C. parvum* isolates as reported by other workers are 4.8-5.4 x 4.2-5.4 µm and 4.6-5.5 x 4.4-5.4 µm in calves (Upton and Current, 1985), and 4.8-5.6 x 4.2-5.5 µm, 4.8-5.6 x 4.4-5.5 µm, 4.8-5.7 x 4.2-5.6 µm, 4.6-5.6 x 4.2-5.6 µm in humans (Current and Reese, 1986). No oocyst of any *C. parvum* isolate has a maximum diameter of 6.0 µm. The fact that oocysts were not found in any of the homogenized sections of the stomach of the mice ruled out the possibility of infection with *C. muris*. There were similar findings in the experiment using the lamb, in which oocysts were not found in the abomasum. Some workers have reported several isolates of *Cryptosporidium* recovered from reptiles in which there were differences in oocyst size, suggesting the presence of several subspecies of *Cryptosporidium* in this host. Nevertheless, there is no enough evidence to implicate the large form of *Cryptosporidium* consistently detected in this study as a distinct species, or subspecies.

CHAPTER V.

GENERAL DISCUSSION.

In this study it has been shown that *Cryptosporidium* infection is relatively common among cats in the Glasgow area and affects domestic, feral and farm cats. This survey has also indicated that the prevalence of *Cryptosporidium* infection is higher in kittens than adults, and in feral and farm cats than in domestic cats. However, no association has been established between *Cryptosporidium* infection and diarrhoea, FIV or FeLV infections. Previous studies in Japan have also shown a higher prevalence of *Cryptosporidium* infection in kittens than in adult cats and also that diarrhoea was not common feature (Arai *et al.,* 1990). However, in England, Bennett *et al.* (1985) detected *Cryptosporidium* oocysts in three kittens of which two had diarrhoea. The higher prevalence of *Cryptosporidium* infection in kittens is possibly due to the patially-developed immune system which fails to confer adequate protection. Human surveys have also shown that *Cryptosporidium* infection is much more common in young children than adults, and that newborn rodents, calves and lambs are highly susceptible to oral challenge with oocysts (Zu, Fang, Fayer and Guerrant, 1992). Other studies have demonstrated that calves experimentally-infected with *Cryptosporidium* oocysts at 1 week or 4 weeks of age produce lower antibody titres to *Cryptosporidium* compared to calves infected at 3 months, suggesting that there is some degree of immunological tolerance to the parasite in newborn animals (Harp *et al.,* 1990). In the present study, relatively

more adult cats were screened for *Cryptosporidium* infection than kittens. This could be attributed to the fact that the domestic cat section of the survey was a veterinary hospital referral population, the greater majority of which were adult cats suffering from chronic diseases.

All the *Cryptosporidium* positive feral cats described in this study were captured in and around factories, whereas six out of the seven positive farm cat were obtained from two farms in which young calves were also infected with *Cryptosporidium* and had developed diarrhoea. *Cryptosporidium* oocysts were detected in faecal specimens from one cat on another farm where there were no known cases of cryptosporidiosis in the livestock. The finding that more positive cats were on farms which also had infected calves indicated that cross-infection among animal species had probably occurred on those premises. These observations suggest that *Cryptosporidium* oocysts are common in the farm environment with a consequent higher risk of naive animals becoming infected. Nevertheless, on one farm, where calves were severely affected by cryptosporidial-associated diarrhoea, no oocysts were found in any of the four cats examined. However, the detection techniques used may have been inadequate and insensitive since only rectal and colonic swabs were obtained from these four cats. The prevalence of *Cryptosporidium* infection in farm cats reported in this study might have been higher had it been possible to obtain more adequate faecal specimens from all of the farm cats. However, it is well known that surveys of enteric infections in farm cats are rendered difficult by the free movement of these animals throughout the premises, hampering the location and collection of faeces (Gethings *et al.*, 1987).

Despite the fact that in this study the prevalence of *Cryptosporidium* was

not associated with FIV or FeLV infections, it remains possible that immunosuppressed cats could succumb to severe cryptosporidial infection associated with chronic diarrhoea. Indeed, previous reports have shown that cats immunosuppressed due to FeLV infection became severely affected by *Cryptosporidium* and developed chronic diarrhoea (Monticello *et al.,* 1987; Goodwin and Barsanti, 1990). However, the role of *Cryptosporidium* infection in the pathogenesis of diarrhoea in cats and other mammalian species is not clearly understood (Zu *et al.,* 1992). FIV-positive cats might be a suitable experimental model for comparative study of the pathogenesis of *Cryptosporidium* infection in HIV-positive humans because of the marked similarity of the two retroviruses (Onions, 1988).

In this study, diarrhoea was not a common feature in cats positive for *Cryptosporidium* infection and similar findings have been reported by previous workers (Iseki, 1979; Uga *et al.,* 1989; Arai *et al.,* 1990; Asahi *et al.,* 1991). Of the five cats with *Cryptosporidium* infection which had diarrhoea, three were kittens and two were adult cats, one of which was FIV-positive. Three kittens of less than 10 days of age were positive for *Cryptosporidium* infection but none had diarrhoea. With incubation periods of 5 to 7 days (Iseki, 1979) or 9 to 11 days (Asahi *et al.,* 1991), it is very likely that the kittens could have been sampled during the early stages of oocyst shedding prior to the onset of diarrhoea. It is possible that cryptosporidial-associated diarrhoea may be common in kittens at certain ages and also in immunosuppressed cats, but this requires further investigation.

The prevalence of 8.8% of *Cryptosporidium* infection found in this study is higher than the prevalence rates of 3.9% and 3.8% reported after similar surveys

in Hyogo Prefecture and Tokyo, respectively, in Japan (Uga *et al.,* 1989; Arai *et al.,* 1990). The relatively higher prevalence of *Cryptosporidium* infection found in the present study is probably a result of the screening of feral and farm cats in addition to domestic cats. The two prevalence studies of *Cryptosporidium* infection in Japan were limited to domestic cats presented by their owners for euthanasia at the Animal Administration Office in Hyogo or Tokyo Metropolitan Animal Protection Centre in Tokyo Metropolitan District (Uga *et al.,* 1989; Arai *et al.,* 1990). Furthermore, it is possible that the diagnostic methods used in this study were more sensitive than those used by Uga et al. (1989) and Arai *et al.* (1990). In the present study, in addition to the examination of *Cryptosporidium* oocysts in MZN stained impression smears from euthanased cats, F-E sedimentation and sucrose flotation followed by MZN and A-P staining were used, whereas only sucrose flotation with examination under phase-contrast microscopy (Uga *et al.,* 1989) and sucrose flotation combined with oocyst staining techniques and ileal smear staining (Arai *et al.,* 1990) were used in the Japanese surveys of feline *Cryptosporidium* infection. Indeed, one group of workers concluded that sucrose flotation or ileal smear staining with Kinyoun's acid-fast method alone were not fully sensitive for detection of *Cryptosporidium* oocysts in cats (Arai *et al.,* 1990).

No single technique proved wholly reliable for diagnosis of *Cryptosporidium* infection in cats in this study. Although $ZnSO_4$ flotation was used by Iseki (1979) for detection of *Cryptosporidium* oocysts in cat faecal specimens, this technique was less efficient than sucrose flotation and F-E sedimentation. In the current study, *Cryptosporidium* oocysts were not detected in five samples from positive cats by sucrose flotation, whereas F-E

sedimentation failed to detect oocysts in only one known positive case. It was also demonstrated that more oocysts were recovered using F-E sedimentation than by sucrose flotation in cat faecal specimens spiked with equal numbers of oocysts (3.1.3.2), indicating that F-E sedimentation is more sensitive than sucrose flotation for concentration of *Cryptosporidium* oocysts in cat faecal specimens. Specific monoclonal antibodies are known to be highly sensitive for detection of *Cryptosporidium* oocysts (McLauchlin *et al.,* 1987; Smith *et al.,* 1989) but the technique is relatively expensive and only used for confirmation of diagnosis (Smith *et al.,* 1989).

Detection of endogenous developmental stages of *Cryptosporidium* was relatively difficult by histological and SEM examination due to rapid autolysis of intestinal tissues. It has been recommended that for improved histological quality, intestinal tissues should be fixed within 20 minutes of death of the animal (Angus, 1990). In previous observations, Giemsa-stained impression smears from ileal sections were used for detection of endogenous stages and oocysts from calf cadavers with *Cryptosporidium* infection (Pohlenz *et al.*, 1978a). In this study, *Cryptosporidium* oocysts were demonstrated in MZN-stained impression smears from the intestine of the cats, and greater numbers of oocysts were seen in the ileum and the caecum.

The size of oocysts detected in the present study was similar to that of *C. parvum* isolates, although oocysts resembling those of *C. muris* were detected in one cat. However, autolysis and low numbers of oocysts present prevented the establishment of conclusive evidence of *C. muris* infection. Nevertheless, previous experimental transmission studies have revealed that *C. muris* can infect cats and the parasite appears to be well adapted to this host species (Iseki *et al.,*

1989). *Cryptosporidium* oocysts measuring 6.0 x 5.4 μm were found in one farm cat in this study. Although the size of these oocysts was different from those of other *Cryptosporidium* isolates reported in cats (Iseki, 1979; Uga *et al.,* 1989; Arai *et al.,* 1990; Asahi *et al.,* 1991) and other mammalian hosts (Tyzzer, 1912; Upton and Current, 1985; Current and Reese, 1986), the parasite showed no host-specificity and caused infection in the small intestine similar to *C. parvum.* With the existing evidence, this isolate was probably a variant of *C. parvum* with larger oocysts. *Cryptosporidium* species designated as *C. curyi* with oocysts measuring 32.3 x 31.1 μm have been described in cats (Ogassawara *et al.,* 1986). However, the very large size of these oocysts together with findings based solely on oocyst morphology with no evidence of endogenous developmental stages arouses suspicion about the veracity of this claim.

Numerical quantification of concentrated oocysts showed that farm cats were shedding relatively more oocysts when compared with domestic and feral cats. None of the cats with *Cryptosporidium* infection excreted over 10^6 oocysts/g faeces even when diarrhoeic. The number of oocysts detected per field at x400 magnification ranged between one and three, corresponding to 10^3 or 10^4 oocysts/g faeces. Shedding patterns of *Cryptosporidium* oocysts in experimentally-infected cats have been described but extremely low numbers of oocysts were excreted daily (Iseki, 1979). In another study, several peaks of oocyst shedding were observed in cats inoculated orally with 10^5 oocysts, with maximum numbers of 10^3 to 10^4 oocysts excreted (Asahi *et al.,* 1991). By contrast, over 10^7 oocysts/g faeces are commonly found in lambs and calves with *Cryptosporidium* infection associated with diarrhoea (Angus, 1990). In experimentally-infected cats, it was reported that shedding of low numbers of

oocysts persisted for over 5 months post-infection (Iseki, 1979; Asahi *et al.,* 1991) and that immunosuppression following corticosteroid administration promoted increased oocyst shedding (Asahi *et al.,* 1991). The fact that *Cryptosporidium* is a potential zoonotic parasite, and that asymptomatic infection is common in cats, highlights the importance of cats in the transmission of *Cryptosporidium* infection to owners, particularly children and immunodeficient individuals.

Although the prevalence of *Cryptosporidium* infection was found to be 8.8% by detection of oocysts in faecal specimens, 74% of serum samples from the cats in the same area had specific antibodies to *Cryptosporidium*. Of the sero-positive cats, 39% had predominantly anti-*Cryptosporidium* IgG antibody, indicating previous exposure to the parasite (Casemore, 1987; Ungar *et al.,* 1988), whereas specific anti-*Cryptosporidium* IgM and IgA were present in 35% of sera from cats suggesting recent infection (Ungar *et al.,* 1988; Hill *et al.,* 1990). In humans with *Cryptosporidium* infection, specific IgM antibody was found to persist for up to 12 months from the time of initial detection (Ungar *et al.,* 1988). Such long-term follow-up of sero-positive cases in the current study was not possible, as most of the cats were either euthanased or not re-presented. The presence of specific anti-*Cryptosporidium* antibodies in cats which were also shedding oocysts suggests that humoral response does not necessarily confer protection against *Cryptosporidium* infection. In HIV-positive human patients, the presence of anti-*Cryptosporidium* IgM and IgG antibodies does not alter the course of chronic cryptosporidiosis (Fayer *et al.,* 1990b). The exact time for the appearance and duration of specific antibodies to *Cryptosporidium* in cats has never been determined.

Finally, in experimental transmission studies it has been demonstrated

that *Cryptosporidium* spp. oocysts recovered from farm cats can cause infection in newborn lambs. Although direct transmission of *Cryptosporidium* oocysts from cat to mice was not successful, oocysts recovered from newborn lambs experimentally-infected with oocysts from the cat were found to be infective to mice. Failure to infect mice directly with oocysts from the cat probably resulted from prolonged storage of the oocysts. Viability of *Cryptosporidium* oocysts may be lost from 2 to 6 months at 4°C in potassium dichromate solution but in some cases oocysts remain viable for up to 9 months, when stored under similar conditions (Tzipori, 1983). Iseki (1979) and Asahi *et al.* (1991) failed to induce infection in mice and other laboratory species using *Cryptosporidium* oocysts from domestic cats, indicating differences in pathogenicity between *Cryptosporidium* isolates used in the studies in Japan and those described in the present study. The presence of two types of oocysts in faeces and ileal smears of the experimentally-infected lambs and mice, similar to those found in the cat, does indicate that these parasites are apparently *C. parvum* isolates. Therefore, the present study has provided further evidence of the existence of several cryptosporidial isolates among mammalian species. There is adequate information to indicate that cats are susceptible to *Cryptosporidium* isolates from humans (Current *et al.,* 1983) and calves (Augustin-Bichl *et al.,* 1984; Pavlasek, 1985) which complicates attempts to recover a cat-specific isolate specific for the cat.

Other coccidian parasites were also detected in cat faecal specimens by means of the various techniques used for demonstration of *Cryptosporidium* oocysts. Although it was not possible to distinguish morphologically between oocysts of *T. gondii* and those of *Sarcocystis*, *Besnoitia* or *Hammondia,* F-E

sedimentation followed by MZN staining may still be useful for screening for coccidian parasites in cat faeces with such results being confirmed by serological or histopathological examination.

It is concluded that *Cryptosporidium* infection is common in cats in the Glasgow area with a higher rate of infection in kittens, feral and farm cats, and therefore, these animals may be regarded a potential reservoir of infection for other animals and humans. Further epidemiological surveys of *Cryptosporidium* infection in cats should be conducted using F-E sedimentation followed by staining with MZN or A-P, with sucrose flotation as an alternative. Serologically, determination of specific anti-*Cryptosporidium* IgG, IgM and IgA is imperative and should be performed simultaneously with oocyst detection, while in autolysed tissues, MZN-stained impression smears are recommended for detection of oocysts.

APPENDIX 3.1.1.

Total numbers of oocysts counted in 10 fields in MZN-stained faecal smears.

Sample	C1	C2	C3	C4	C5	MEAN	STDEV
A	321	264	300	331	475	338	80
B	165	190	217	253	169	199	36
C	56	75	89	105	132	91	28
D	65	82	80	52	95	75	16
E	27	37	38	44	47	39	7
F	12	13	12	14	13	13	1
G	12	9	10	9	12	10	1
H	8	4	8	6	7	7	2
I	4	2	5	6	9	5	3
J	6	4	2	4	4	4	1

C = counts;

APPENDIX 3.1.2.

Total numbers of oocysts counted in 10 fields in A-P stained faecal smears.

Sample	C1	C2	C3	C4	C5	MEAN	STDEV
A	301	295	311	319	363	318	27
B	208	166	178	198	148	180	24
C	116	113	104	108	122	113	7
D	60	56	55	62	48	56	5
E	48	46	52	47	43	47	3
F	46	43	49	39	27	41	8
G	21	24	28	26	15	23	5
H	18	11	13	16	12	14	3
I	11	13	9	11	12	11	1
J	4	4	2	6	5	4	1

C = counts;

APPENDIX 3.1.3.

Total numbers of oocysts counted in 10 fields in faecal smears stained with FITC-labelled MAb.

Sample	C1	C2	C3	C4	C5	MEAN	STDEV
A	463	382	357	376	288	373	63
B	267	265	278	277	269	271	6
C	127	138	137	122	132	131	7
D	84	94	95	91	92	91	4
E	63	62	65	57	69	63	4
F	56	53	55	56	49	54	3
G	28	32	35	31	35	32	3
H	18	18	16	21	22	19	2
I	12	13	12	10	14	12	1
J	11	12	10	12	11	11	1

C = counts;

APPENDIX 3.1.4.

Oocyst counts with Neubaeuer haemocytometer in the two analyses in samples concentrated with zinc sulphate flotation.

	A		B			
Sample	**C1**	**C2**	**C1**	**C2**	**Mean**	**STDEV**
1	32	45	70	57	51.0 (1.0×10^6)	16.2686
2	46	49	53	50	49.5 (9.5×10^5)	2.8868
3	24	26	28	26	26.0 (5.2×10^5)	1.6330
4	29	30	37	36	33.0 (6.6×10^5)	4.0825
5	39	42	68	65	53.5 (1.1×10^6)	15.1107
6	45	47	54	52	49.5 (9.9×10^5)	4.2032
7	41	35	26	32	33.5 (6.7×10^5)	6.2450
8	60	55	31	36	45.5 (9.1×10^5)	14.1539
9	71	48	44	67	57.5 (1.2×10^6)	13.4784
10	52	44	35	43	43.5 (8.7×10^5)	6.9522
11	61	48	32	45	46.5 (9.3×10^5)	11.9024
12	79	68	40	51	59.5 (1.2×10^6)	17.3686
13	45	43	38	40	41.5 (8.3×10^5)	3.1091
14	42	37	30	35	36.0 (7.2×10^5)	4.9666
15	49	52	65	62	57.0 (1.1×10^6)	7.7028
16	84	63	57	78	70.5 (4.2×10^5)	12.6095
17	34	32	45	47	39.5 (1.4×10^6)	7.5939
18	49	37	21	33	35.0 (7.9×10^5)	11.5470
19	55	39	35	51	45.0 (7.0×10^5)	9.5219
20	30	28	12	14	21.0 (9.0×10^5)	9.3095

Values are average numbers of oocysts counted in two chambers of the haemocytometer.
Dilution x2.
( ) = mean number of oocysts per ml.; **A** = Analysis 1; **B** = Analysis 2; **C** = Oocyst count; **STDEV** = Standard deviation.

APPENDIX 3.1.5.

Oocyst counts with Neubaeuer haemocytometer in the two analyses in samples concentrated with sucrose flotation.

	A		B				
Sample	C1	C2	C1	C2	Mean		STDEV
1	123	147	135	125	132.50	(2.60×10^6)	11.0000
2	145	123	129	139	134.00	(2.68×10^6)	9.8658
3	90	125	131	84	107.50	(2.15×10^6)	23.9235
4	112	88	122	78	100.00	(2.00×10^6)	20.4613
5	101	112	126	87	106.50	(2.13×10^6)	16.5429
6	132	130	143	119	131.00	(2.62×10^6)	9.8319
7	149	146	135	160	147.50	(2.95×10^6)	10.2794
8	129	148	134	143	138.50	(2.77×10^6)	8.5829
9	141	149	123	167	145.00	(2.90×10^6)	18.2574
10	139	119	152	106	129.00	(2.58×10^6)	20.4776
11	127	133	125	135	130.00	(2.60×10^6)	4.7610
12	245	63	133	175	154.00	(3.08×10^6)	76.2540
13	185	99	127	157	142.00	(2.84×10^6)	37.1842
14	170	101	138	146	138.75	(2.71×10^6)	28.6051
15	161	152	144	127	146.00	(3.13×10^6)	14.4453
16	91	190	109	172	140.50	(2.81×10^6)	47.9062
17	132	153	137	148	142.50	(2.85×10^6)	9.6782
18	130	116	121	125	123.00	(2.46×10^6)	5.9442
19	119	128	134	113	123.50	(2.47×10^6)	9.3274
20	104	165	123	146	134.50	(2.69×10^6)	26.6145

Values are average numbers of oocysts counted in two chambers of the haemocytometer.
Dilution x2.
( ) = mean number of oocysts per ml.; **A** = Analysis 1; **B** = Analysis 2; **C** = Oocyst count; **STDEV** = Standard deviation.

APPENDIX 3.1.6.

Oocyst counts with Neubaeuer haemocytometer in the two analyses in samples concentrated with formol-ether sedimentation technique.

	A		B				
Sample	C1	C2	C1	C2	Mean		STDEV
1	260	173	225	312	242.5	(4.85×10^6)	58.518
2	220	189	186	217	203.0	(4.06×10^6)	17.981
3	96	123	179	152	137.5	(2.75×10^6)	35.893
4	184	154	92	122	138.0	(2.76×10^6)	39.766
5	200	137	86	149	143.0	(2.86×10^6)	46.797
6	140	132	115	123	127.5	(2.55×10^6)	10.847
7	120	138	102	84	111.0	(2.22×10^6)	23.238
8	136	125	114	125	125.0	(2.50×10^6)	8.981
9	142	122	90	110	116.0	(2.32×10^6)	21.787
10	167	165	138	140	152.5	(3.05×10^6)	15.631
11	111	121	201	191	156.0	(3.12×10^6)	46.547
12	181	157	147	171	164.0	(3.28×10^6)	15.011
13	90	198	219	111	154.5	(3.09×10^6)	63.522
14	97	231	309	175	203.0	(4.06×10^6)	89.517
15	89	105	99	83	94.0	(1.88×10^6)	9.866
16	74	130	109	53	91.5	(1.83×10^6)	34.530
17	77	69	318	326	197.5	(3.95×10^6)	43.834
18	183	168	210	225	196.5	(3.93×10^6)	25.749
19	126	111	130	145	128.0	(2.56×10^6)	13.976
20	72	123	177	126	124.5	(2.49×10^6)	42.884

Values are average numbers of oocysts counted in two chambers of the haemocytometer.
Dilution x2.
( ) = mean number of oocysts per ml.; **A** = Analysis 1; **B** = Analysis 2; **C** = Oocyst count; **STDEV** = Standard deviation.

APPENDIX 3.2.1.

A survey of *Cryptosporidium* infection in domestic cats

Case No.	Sex	Age	Diarr	Crypto	FeLV	FIV	Other parasites
M1	M	>6	N	N	N	N	
M2	F	>6	P	N	N	N	*
M3	F	≤6	P	N	N	N	*E. coli*
M4	M	>6	P	P	N	P	*
M10	F	≤6	N	N	N	N	
M11	M	>6	P	N	P	N	*
M13	M	>6	N	N	N	N	
M26	M	≤6	P	N	N	P	*Salmonella/E. coli*
M29	M	>6	P	N	N	P	*E. coli*
M30	F	≤6	P	N	N	N	*Streptococcus* sp.
M33	F	>6	P	N	N	N	*T. cati/ Streptococcus* sp.
M35	F	>6	P	N	N	N	*
M38	F	≤6	P	N	N	N	*Streptococcus* sp.
M39	F	≤6	P	N	N	N	*Streptococcus* sp.
M40	M	≤6	P	N	N	N	*Streptococcus* sp.
M41	F	>6	P	N	N	N	*T. cati/ Streptococcus* sp.
M42	F	>6	P	N	N	N	*T. cati/ Streptococcus* sp.
M43	M	>6	P	N	N	N	*E. coli/ Streptococcus* sp.
M44	F	≤6	P	N	N	N	*
M49	F	>6	N	N	N	N	*Lactobacillus*
M50	F	>6	N	N	N	N	*E. coli/ Streptococcus* sp.
M51	M	>6	N	N	N	N	
M52	M	>6	N	N	N	N	*E. coli*
M53	M	≤6	P	N	N	N	*
M55	F	>6	N	N	N	N	
M56	F	>6	P	N	N	N	*
M57	F	>6	N	N	N	N	
M58	F	>6	N	N	N	N	
M59	F	>6	P	N	N	N	*
M60	F	>6	P	N	N	N	*
M92	F	≤6	P	N	N	N	*
M93	F	>6	N	N	N	N	*Streptococcus* sp.
M94	F	>6	N	N	N	N	*Streptococcus* sp.
M95	F	>6	N	N	N	N	*T. cati/ E. coli*
M96	M	>6	N	N	N	N	*Streptococcus* sp.
M97	M	>6	N	N	N	N	*E. coli*
M98	M	>6	N	N	N	N	*E. coli*
M99	F	>6	N	N	N	N	*Streptococcus* sp.

APPENDIX 3.2.1. CONTINUED.

Case No.	Sex	Age	Diarr	Crypto	FeLV	FIV	Other parasites
M104	F	≤6	N	N	N	N	
M113	F	>6	P	N	N	N	*T. cati/ E.coli*
M119	M	>6	P	N	N	N	*
M120	M	≤6	P	N	N	N	*
M121	M	>6	N	N	N	P	
M122	F	>6	N	N	N	N	*T. cati*
M123	M	>6	N	N	N	P	
M124	F	>6	P	N	N	N	*
M126	M	>6	N	N	N	P	
M127	M	>6	P	N	N	N	*T. cati/**
M128	M	>6	N	N	N	N	
M136	F	>6	N	N	N	N	
M137	F	>6	N	N	N	N	
M138	M	>6	P	N	N	N	*E. coli*
M139	F	>6	P	N	N	N	*E. coli*
M140	M	>6	N	P	N	P	*T. cati/Spirometra/**
M141	F	≤6	N	N	N	N	*T. cati/I. felis*
M142	M	≤6	P	N	N	N	*T. cati/**
M143	F	≤6	N	N	N	P	
M144	M	>6	N	N	N	N	
M145	F	>6	N	P	N	N	*
M146	M	>6	N	N	N	N	
M147	F	>6	N	N	N	N	
M148	F	>6	N	N	N	N	
M149	M	>6	N	N	N	N	
M150	M	>6	N	N	N	N	
M151	F	>6	N	P	N	N	*I. felis/**
M152	M	>6	N	N	N	N	
M153	F	>6	P	N	N	N	*E. coli*
M154	F	>6	N	N	N	N	
M155	M	≤6	N	P	N	N	
M156	M	>6	N	N	N	N	
M157	M	>6	N	N	N	N	
M158	F	>6	N	N	N	N	
M159	M	>6	N	N	N	N	
B1	F	>6	N	N	ND	ND	
B2	M	>6	N	N	ND	ND	
B3	F	>6	N	N	ND	ND	
B4	M	>6	N	N	ND	ND	
B5	F	>6	N	N	ND	ND	
B6	F	>6	N	N	ND	ND	
B7	M	>6	N	N	ND	ND	
B8	F	>6	N	N	ND	ND	
B9	M	>6	N	N	ND	ND	
B10	M	>6	N	N	ND	ND	
B11	M	>6	N	N	ND	ND	

APPENDIX 3.2.1. CONTINUED.

Case No.	Sex	Age	Diarr	Crypto	FeLV	FIV	Other parasites
B12	M	>6	N	N	ND	ND	
B13	M	>6	N	N	ND	ND	
B14	F	>6	P	N	ND	ND	*
B15	M	≤6	P	N	ND	ND	*I. felis*/*
B16	F	>6	N	N	ND	ND	
B17	F	>6	N	N	ND	ND	
B18	F	>6	N	N	ND	ND	
B19	M	>6	N	N	ND	ND	
B20	F	≤6	N	P	ND	ND	*T. cati/I. felis*/*
B21	M	≤6	N	N	ND	ND	
B22	F	>6	N	N	ND	ND	
B23	F	>6	N	N	ND	ND	
B24	F	>6	N	N	ND	ND	
B25	M	>6	N	N	ND	ND	
B26	F	>6	N	N	ND	ND	
B27	M	>6	N	N	ND	ND	
B28	F	>6	N	N	ND	ND	*I. felis*
B29	F	>6	N	N	ND	ND	
B30	M	>6	N	N	ND	ND	
B31	F	>6	N	N	ND	ND	
B32	M	>6	N	N	ND	ND	
B33	F	>6	N	N	ND	ND	
B34	F	>6	N	N	ND	ND	
B35	F	>6	N	N	ND	ND	
B36	F	>6	N	N	ND	ND	
B37	F	>6	N	N	ND	ND	
B38	F	>6	N	N	ND	ND	*T. gondii?/ Sarcocystis* sp.?
B39	M	≤6	N	N	ND	ND	
B40	M	≤6	N	N	ND	ND	
B41	F	≤6	N	N	ND	ND	
B42	F	≤6	N	N	ND	ND	
B43	M	>6	N	N	ND	ND	
B44	F	≤6	N	N	ND	ND	
B45	M	>6	N	N	ND	ND	
B46	M	>6	N	N	ND	ND	
B47	M	>6	N	N	ND	ND	
B48	M	>6	N	N	ND	ND	
B49	M	≤6	N	N	ND	ND	
B50	M	>6	N	N	ND	ND	
B51	F	>6	N	N	ND	ND	
B52	M	>6	N	N	ND	ND	
B53	F	≤6	P	N	ND	ND	*Campylobacter*
B54	M	≤6	N	N	ND	ND	
B55	F	≤6	N	P	ND	ND	*I. felis/I. rivolta*/*
B56	F	≤6	N	N	ND	ND	*T. cati*

APPENDIX 3.2.1. CONTINUED.

Case No.	Sex	Age	Diarr	Crypto	FeLV	FIV	Other parasites
B57	F	≤6	N	N	ND	ND	*T. cati*
B58	M	≤6	N	N	ND	ND	
B59	F	≤6	N	N	ND	ND	*T. cati*
B60	M	≤6	P	N	ND	ND	*
B61	F	≤6	P	N	ND	ND	*T. cati*/*
B62	F	≤6	P	N	ND	ND	*T. cati*/*
B63	F	≤6	P	N	ND	ND	*T. cati*/*I. felis*/*
B64	F	≤6	N	N	ND	ND	
B65	F	≤6	N	N	ND	ND	

APPENDIX 3.2.2.

A survey of *Cryptosporidium* infection in feral cats

Case No.	Sex	Age	Diarr	Crypto	FeLV	FIV	Other parasites
M16	F	>6	N	N	N	P	*T. cati*
M17	M	≤6	N	P	N	N	*T. cati*/*
M20	M	≤6	N	N	N	N	
M21	F	≤6	N	N	N	N	
M22	F	≤6	N	N	N	N	
M23	F	≤6	N	N	N	N	
M24	M	≤6	N	N	N	N	*T. cati*
M27	F	>6	N	N	N	N	
M28	F	>6	N	N	N	N	
M31	M	>6	N	N	N	N	*T. cati/ Spirometra*
M32	M	>6	N	P	N	N	*T. cati*/*
M36	F	≤6	N	P	N	N	*T. cati/ E. coli Campylobacter*
M37	M	≤6	N	P	N	N	*T. cati/ E. coli*
M46	F	>6	N	N	N	N	*E. coli*
M47	F	>6	N	N	N	N	
M61	M	≤6	N	N	N	N	
M62	F	≤6	N	P	N	N	*T. cati*
M63	F	≤6	P	N	N	N	*Campylobacter*
M64	M	≤6	N	N	N	N	*I. rivolta*
M65	F	≤6	N	N	N	N	
M66	M	≤6	N	N	N	N	
M67	M	≤6	P	N	N	N	*T. cati/ E. coli/ I. felis*
M68	M	≤6	N	N	N	N	
M69	M	≤6	P	N	N	N	*E. coli*
M70	F	≤6	P	N	N	N	*E. coli*
M71	F	≤6	P	N	N	N	*E. coli/T. cati*
M74	F	≤6	N	N	N	N	
M75	M	≤6	N	N	N	N	*T. cati*
M76	F	>6	P	N	N	N	*T. cati*/*
M77	F	≤6	N	N	N	N	*T. cati*
M78	F	≤6	N	N	N	N	
M79	F	≤6	N	N	N	N	*I. felis/ T. gondii*?, *Hammondia* sp.?, *Besnoitia* sp.? *Sarcocystis* sp.?
M80	M	≤6	P	P	N	N	*T.cati/I.felis*/*
M81	F	≤6	P	P	N	N	*T. cati*/*
M82	F	≤6	N	N	N	N	*I. felis*
M83	M	≤6	N	N	N	N	*I. felis*

APPENDIX 3.2.2. CONTINUED.

Case No.	Sex	Age	Diarr	Crypto	FeLV	FIV	Other parasites
M84	M	≤6	N	N	N	N	
M85	M	≤6	N	N	N	N	*T. cati/I. rivolta*
M86	F	>6	N	N	N	N	*T. cati*
M87	M	>6	N	N	N	N	*Campylobacter/ E. coli*
M88	M	≤6	N	N	N	N	*T. gondii*?, *Hammondia*?, *Besnoitia*?, *Sarcocystis*?
M89	M	≤6	N	N	N	N	*T. cati/ T. gondii*?, *Hammondia*?, *Besnoitia*?, *Sarcocystis*?
M90	M	>6	N	N	N	N	*E. coli*
M91	F	>6	N	N	N	P	*E. coli*
M100	F	>6	N	N	N	N	*E. coli*
M101	M	>6	N	N	N	N	*E. coli/I. felis*
M102	M	≤6	N	N	N	N	*E. coli/I. felis*
M103	F	≤6	N	N	N	N	*E. coli/I. rivolta*
M105	F	≤6	N	N	N	N	*Streptococcus*
M106	M	>6	N	N	N	N	*Streptococcus*
M108	M	>6	N	N	N	N	
M109	F	≤6	N	N	N	N	
M110	F	≤6	P	N	P	N	*
M111	F	≤6	N	N	N	N	
M112	M	≤6	N	N	N	N	*E. coli*
M132	M	>6	P	N	N		*I. felis/**
M133	F	>6	P	N	N		*I. rivolta/**
M134	F	>6	P	N	N	N	*I. rivolta/**
A1	F	>6	N	N	N	N	*E. coli*
A2	M	>6	N	N	N	N	*T. cati/I. felis Campylobacter*
A3	F	>6	N	N	N	N	*T. cati/E. coli I. felis*
A4	M	>6	N	N	N	N	*Campylobacter*
A5	M	≤6	P	N	N	N	*E. coli*
A6	M	≤6	N	N	N	N	*Campylobacter/ E. coli*
A7	M	≤6	N	N	N	N	*E. coli*
A8	M	>6	N	N	N	N	*E. coli/ T. cati*
A9	M	>6	N	N	N	P	*E. coli/ T. cati Streptococcus*
A10	M	>6	N	N	N	P	*E. coli/ T. cati Streptococcus*
A11	F	>6	N	N	N	P	*E. coli/ T. cati*
A12	F	>6	N	N	N	N	*Streptococcus/ E. coli*
A13	F	>6	N	N	N	P	

APPENDIX 3.2.2. CONTINUED.

Case No.	Sex	Age	Diarr	Crypto	FeLV	FIV	Other parasites
A14	M	>6	N	N	N	N	
A15	F	>6	N	N	N	N	
A16	M	>6	N	N	N	N	*T. cati*
A17	M	≤6	N	N	N	N	
A18	F	≤6	N	P	N	N	*T. cati/I. felis*
A19	M	>6	N	N	N	N	
A20	F	>6	N	N	N	N	*T. cati*
A21	M	>6	N	N	N	P	
A22	F	>6	N	N	N	N	
A23	F	>6	N	N	N	N	*T. cati*
A24	F	>6	N	N	N	N	*T. cati*
A25	F	>6	N	N	N	N	
A26	M	>6	N	N	N	N	*T. cati*
A27	M	>6	N	N	N	N	*T. cati*
A28	M	≤6	N	N	N	N	*T. cati/I. felis*
A29	M	≤6	N	N	N	N	*I. felis*
A30	F	>6	N	N	N	N	*T. cati*
A31	F	>6	N	N	N	N	*T. cati*
A32	M	≤6	N	N	ND	ND	*I. rivolta*
A33	M	≤6	N	N	ND	ND	
A34	F	≤6	N	P	ND	ND	*
A35	M	≤6	N	N	ND	ND	
A36	F	≤6	N	N	ND	ND	
A37	M	≤6	N	P	ND	ND	*
A38	M	≤6	N	N	ND	ND	
A39	F	≤6	N	P	ND	ND	*
A40	F	≤6	N	N	ND	ND	
A41	F	≤6	P	P	ND	ND	*T. cati/E. coli*

APPENDIX 3.2.3.

A survey of *Cryptosporidium* infection in farm cats

Case No.	Sex	Age	Diarr	Crypto	FeLV/FIV	Farm	Other parasites
F1	M	>6	N	N	N	A	*Campylobacter/ E. coli*
F2	M	≤6	N	P	N	A	*E. coli/T. cati*
F3	F	≤6	N	P	N	A	*Campylobacter E. coli/ T. cati Staphylococcus*
F4	M	≤6	N	N	N	A	*E. coli/ Staphylococcus*
F5	M	≤6	N	P	N	A	*Campylobacter Staphylococcus T. cati*
F6	M	>6	N	P	N	A	*E. coli Campylobacter Staphylococcus*
F7	M	>6	N	N	N	B	
F8	F	>6	P	N	N	B	*E. coli Streptococcus*
F9	F	>6	N	N	N	B	
F10	NS	>6	N	N	ND	B	*T. cati*
F11	NS	>6	N	N	ND	B	*T. cati*
F12	NS	>6	N	N	ND	B	
F13	NS	>6	N	N	ND	B	*T. cati*
F14	NS	>6	N	P	ND	B	*T. gondii?, Sarcocystis?/**
F15	NS	>6	N	N	ND	B	
F16	NS	>6	N	N	ND	B	*T. cati*
F17	NS	>6	N	N	ND	B	*T. cati*
F18	F	>6	N	N	ND	C	*T. cati*
F19	F	>6	N	P	N	D	*T. cati/**
F20	M	>6	N	N	P	D	
F21	F	>6	N	N	N	D	*T. cati*
F22	F	>6	N	P	N	D	*
F23	M	>6	N	N	N	D	
F24	M	>6	N	N	N	D	
F25	M	>6	N	N	N	D	
F26	M	>6	N	N	N	D	
F27	M	>6	N	N	N	E	
F28	M	>6	N	N	N	E	
F29	F	>6	P	N	N	E	*T. cati/E. coli*
F30	F	>6	P	N	N	E	*E. coli*
F31	F	>6	P	N	N	E	*E. coli*
F32	F	>6	N	N	N	E	
F33	F	≤6	N	N	ND	E	

APPENDIX 3.2.3. CONTINUED.

Case No.	Sex	Age	Diarr	Crypto	FeLV/FIV	Farm	Other parasites
F34	F	≤6	N	N	ND	E	
F35	M	≤6	N	N	ND	E	
F36	F	≤6	N	N	ND	E	*T. cati*
F37	F	≤6	N	N	ND	E	*T. cati*
F38	M	>6	N	N	ND	F	
F39	F	>6	N	N	ND	F	*T. cati*
F40	M	>6	N	N	ND	F	*T. cati*
F41	F	>6	N	N	ND	F	
F42	F	>6	N	N	ND	G	
F43	F	>6	N	N	ND	G	
F44	M	>6	N	N	ND	G	
F45	M	>6	N	N	ND	G	
F46	F	>6	N	N	ND	G	
F47	NS	>6	N	N	ND	G	
F48	F	>6	N	N	ND	G	
F49	MN	>6	N	N	ND	H	
F50	F	>6	N	N	ND	H	
F51	MN	>6	N	N	ND	H	
F52	F	>6	N	N	ND	H	*T. cati* *Sarcocystis?*
F53	F	>6	N	N	ND	H	*T. cati*
F54	M	>6	N	N	ND	H	
F55	F	>6	N	N	ND	H	
F56	M	>6	N	N	ND	H	
F57	MN	>6	P	N	ND	H	*T. cati/E. coli* *Streptococcus*

Age in months; Crypto = *Cryptosporidium*; Diarr = Diarrhoea; M = Male; F = Female; N = Negative; P = Positive; ND = Not done; NS = Not seen; * = non pathogenic bacteria

APPENDIX 3.2.4.

Summary of results; animal details and diagnostic techniques.

Ref.	Sex	Age	Animal Type	Diarr	MZN/A-P	MAb	Conc.	Touch smear	Hist.
M4	M	>6	Dom	P	N	I	ND	P	P
M140	M	>6	Dom	N	N	ND	P	P	N
M145	F	>6	Dom	N	N	P	P	N	N
M151	F	>6	Dom	N	P	ND	P	P	N
M155	M	≤6	Dom	N	P	ND	P	P	N
B20	F	≤6	Dom	N	P	ND	P	P	N
B55	F	≤6	Dom	N	N	P	P	N	N
M17	M	≤6	Fer	N	P	I	P	P	P
M32	M	>6	Fer	N	P	ND	P	P	P
M36	F	≤6	Fer	P	N	P	N	P	P
M37	M	≤6	Fer	P	N	P	P	P	P
M62	F	≤6	Fer	N	P	P	P	P	N
M80	M	≤6	Fer	P	N	P	P	P	P
M81	F	≤6	Fer	P	N	P	P	P	P
A18	F	≤6	Fer	N	P	P	P	P	P
A34	F	≤6	Fer	N	N	P	P	I	N
A37	M	≤6	Fer	N	P	P	P	P	N
A39	F	≤6	Fer	N	N	P	P	I	N
A41	F	≤6	Fer	P	N	P	P	P	N
F2	M	≤6	Farm	N	P	ND	ND	P	N
F3	F	≤6	Farm	N	P	ND	ND	P	N
F5	M	≤6	Farm	N	P	ND	ND	P	N
F6	M	>6	Farm	N	P	ND	ND	P	N
F14	F	>6	Farm	N	P	P	P	P	N
F19	F	>6	Farm	P	P	P	P	P	N
F22	F	>6	Farm	N	P	P	P	P	N

Ref. = Case number; Age in months; **Conc.** = Oocyst concentration with F-E or sucrose flotation; **MAb** = FITC-labelled monoclonal antibody technique; **M** = Male; **F** = Female; **Dom** = Domestic cat; **Fer** = Feral cat; **P** = Positive; **N** = Negative; **ND** = Not done; **I** = inconclusive

APPENDIX 3.2.5.

Number and size of *Cryptosporidium* sp. oocysts in cat faecal smears stained by MZN technique

Sample	Type	No. oocyst per field	Size (µm)	Range (mean)
M17	Feral	1-2	8.8 x 5.7 6.5 x 6.2 7.4 x 6.5 7.1 x 5.8 7.5 x 5.2	6.2-8.8 x 5.2-6.5 (7.4 x 6.5)
M36	Feral	1-2	5.8 x 5.2 4.9 x 4.4 5.5 x 4.6 5.8 x 4.3 4.1 x 3.2 4.1 x 3.5 5.2 x 4.1	4.1-5.8 x 3.2-5.2 (4.9 x 4.3)
M37	Feral	1-2	5.8 x 4.7 5.9 x 4.7 5.9 x 5.5 5.9 x 4.2 5.5 x 5.2 5.8 x 5.5 5.5 x 4.4 5.2 x 4.1 5.5 x 3.9 5.3 x 4.1	5.2-5.9 x 3.9-5.5 (5.6 x 4.6)
A18	Feral	1-3	5.8 x 4.5 5.8 x 5.2 4.3 x 3.3 5.8 x 4.5 5.8 x 4.6 5.8 x 4.6 4.9 x 4.0 5.0 x 4.0 5.3 x 4.7 5.0 x 4.1 5.3 x 4.7 4.7 x 3.6	4.3-5.8 x 3.3-5.2 (5.3 x 4.3)

APPENDIX 3.2.5. CONTINUED.

Sample	Type	No. oocyst per field	Size (µm)	Range (mean)
M80	Feral	1-2	5.8 x 5.5 5.5 x 4.6 4.9 x 4.1 5.3 x 3.8 5.8 x 5.2 4.9 x 4.0 5.6 x 3.6 5.3 x 4.9 3.5 x 3.3 5.5 x 4.0 5.2 x 4.4	3.5-5.8 x 3.3-5.5 (5.2 x 4.3)
M81	Feral	1-2	4.7 x 4.1 5.0 x 3.5 5.0 x 3.5 5.2 x 4.9 5.3 x 3.8 4.7 x 4.1 5.9 x 4.1 5.5 x 3.8 4.1 x 4.1 5.3 x 4.4	4.1-5.9 x 3.5-4.9 5.1 x 4.0
M4	Dom	1-3	5.5 x 3.6 5.6 x 5.2 5.7 x 4.5 4.1 x 3.7 5.2 x 4.4 4.8 x 4.2 5.5 x 4.5 5.2 x 4.0 4.9 x 4.5 5.5 x 4.5	4.1-5.7 x 3.6-5.2 (5.2 x 4.3)
F22	Farm	1-3	4.8 x 4.2 5.4 x 5.4 4.5 x 4.2 4.5 x 3.6 5.4 x 3.6 4.8 x 4.2 4.5 x 4.8 4.5 x 4.2 4.6 x 3.6 4.8 x 4.8	4.5-5.4 x 3.6-5.4 (4.8 x 4.3)

APPENDIX 3.2.5. CONTINUED.

Sample	Type	No. oocyst per field	Size (µm)	Range (mean)
F22	Farm	1-2	6.0 x 5.4 6.0 x 6.0 6.0 x 4.5 6.0 x 5.8 6.0 x 6.0 6.0 x 5.1 6.0 x 5.2 6.0 x 3.3 6.0 x 5.8 5.8 x 4.2	5.8-6.0 x 4.2-6.0 (6.0 x 5.1)

Dom = Domestic.

APPENDIX 3.2.6.

Other parasites detected in addition to *Cryptosporidium*

Case No.	Animal Type	Diarr	Parasites	Bacteria
M4	Dom	P	N	
M140	Dom	N	*T. cati*	
M145	Dom	N	N	
M151	Dom	N	*Isospora*	
M155	Dom	N	N	
B20	Dom	N	*T. cati*	
B55	Dom	N	*Isospora*	
M17	Feral	N	*T. cati*	
M32	Feral	N	*T. cati*	
M36	Feral	P	*T. cati*	*E. coli* *Campylobacter* sp.
M37	Feral	P	*T. cati*	*E. coli*
M62	Feral	N	*T. cati*	
M80	Feral	P	*T. cati* *Isospora*	*E. coli*
M81	Feral	P	*T. cati*	*E. coli*
A18	Feral	N	*T. cati*	
A34	Feral	N	N	
A37	Feral	N	N	
A39	Feral	N	N	
A41	Feral	P	*T. cati*	*E. coli*
F2	Farm	N	*T. cati*	*E. coli*
F3	Farm	N	*T. cati*	*E. coli/* *Campylobacter* sp.
F5	Farm	N	*T. cati*	*Staphylococcus* sp./ *Campylobacter sp.*
F6	Farm	N	N	*Campylobacter* sp./ *Staphylococcus* sp.
F14	Farm	N	*T. cati*	
F19	Farm	P	*T. cati*	*Campylobacter* sp.
F22	Farm	N	N	
F39	Farm	N	*T. cati*	
F40	Farm	N	*T. cati*	
F52	Farm	N	*T. cati* *Sarcocystis* sp.*?*	
F57	Farm	N	*T. cati*	*E.coli/* *Streptococcus* sp.

Diarr = Diarrhoea; Dom = Domestic cat; P = Positive; N = Negative.

APPENDIX 3.2.7.

Other coccidia detected in MZN stained smears

Sample	Oocyst size (µm)	Coccidia*
M64	24 x 21	*I. rivolta*
M67	42 x 33	*I. felis*
M79	13 x 12	*T. gondii?, Sarcocystis? Hammondia?, Besnoitia?*
"	42 x 33	*I. felis*
M80	42 x 30	*I. felis*
M82	33 x 21	*I. felis*
M83	36 x 33	*I. felis*
M85	27 x 21	*I rivolta*
M88	12 x 11	*T. gondii?, Sarcocystis? Hammondia?, Besnoitia?*
M89	13 x 12	*T. gondii?, Sarcocystis? Hammondia?, Besnoitia?*
M101	42 x 33	*I. felis*
M102	39 x 27	*I. felis*
M103	18 x 16	*I. rivolta*
M132	51 x 36	*I. felis*
M133	18 x 15	*I. rivolta*
M134	21 x 19	*I. rivolta*
M141	51 x 42	*I. felis*
M151	39 x 30	*I. felis*
A2	45 x 29	*I. felis*
A3	43 x 33	*I. felis*
A18	49 x 39	*I. felis*
A28	51 x 32	*I. felis*
A29	45 x 36	*I. felis*
A32	21 x 18	*I. rivolta*
B15	42 x 39	*I. felis*
B20	38 x 33	*I. felis*
B28	51 x 33	*I. felis*
B38	11 x 9	*T. gondii?, Sarcocystis?*
B55	27 x 18	*I. rivolta*
B63	39 x 27	*I. felis*
F14	12 x 9	*T. gondii?, Sarcocystis?*

* Based on oocyst sizes (Lindsay and Blagburn, 1991).

APPENDIX 3.3.1.

Serological survey of *Cryptosporidium* infection in domestic and Pedigree cats

REF	AGE	SEX	IgG	IgM	IgA
F155A	12	M	P(64)	N	N
F162A	108	M	P(64)	N	N
F163A	24	F	P(16)	N	N
F166A	144	MN	P(32)	P	P
F167A	84	MN	P(256)	N	N
F171A	108	F	P(32)	N	N
F172A	16	MN	P(32)	N	N
F173A	3	F	N	N	N
F174A	120	MN	P(128)	N	N
F177A	120	M	P(64)	N	N
F179A	12	F	P(256)	N	N
F181A	12	M	P(160)	N	N
F188A	60	MN	P(80)	N	N
F197A	48	FN	P(320)	N	N
F198A	24	MN	N	N	N
F203A	120	M	N	P	P
F205A	36	MN	P(160)	P	P
F207A	156	F	N	N	N
F208A	12	M	P(320)	P	N
F210A	156	F	P(80)	N	N
F211A	96	F	P(40)	P	P
F215A	84	M	P	N	P
F218A	36	F	P	N	N
F220A	24	FN	P	P	P
F225A	12	FN	N	N	N
F230A	36	FN	P	P	N
F234A	60	F	N	N	N
F235A	24	MN	P	N	N
F236A	84	F	P	N	N
F238A	12	M	P	N	P
F241A	24	F	P	P	P
F244A	48	MN	P	N	N
F257A	72	F	P	N	P
F258A	12	MN	P	N	P
F260A	12	F	P	N	N
F264A	168	M	P	P	P
F266A	108	F	P	N	P
F267A	36	MN	P	N	N
F269A	96	M	P	N	N
F271A	24	M	P	N	N
F274A	72	F	N	N	N
F276A	96	FN	P	P	P
F287A	48	F	N	N	N

APPENDIX 3.3.1. CONTINUED.

REF	AGE	SEX	IgG	IgM	IgA
F288A	12	MN	N	P	N
F290A	96	M	N	N	N
F292A	72	F	P	P	P
F305A	144	M	P	P	P
F306A	144	F	P	P	P
F309A	36	M	P	N	N
F310A	144	F	P	N	N
F312A	132	MN	P	N	P
F313A	84	MN	P	N	N
F318A	156	FN	P	P	P
F319A	36	MN	P	N	N
F325A	120	MN	P	P	P
F331A	24	M	N	N	N
F332A	132	FN	N	N	N
F338A	12	M	N	N	N
F339A	24	F	N	N	P
F341A	12	MN	N	N	N
F342A	12	M	N	N	N
F326A	108	MN	N	N	N
F344A	24	M	P	P	P
F833A	72	FN	P	P	P
F863A	156	MN	P	P	N
F606A	36	F	P	P	P
F1041A	180	M	N	P	N
2480B	24	M	P	P	P
2290B	36	FN	P	P	P
2289B	84	MN	P	P	P
F887A	36	FN	P	P	P
F2375A	120	M	P	P	N
F2330A	156	FN	P	N	N
F2376B	72	F	P	N	N
F2205B	120	MN	P	P	P
F2321B	36	M	N	N	N
F2352B	36	F	P	P	N
F2266B	108	M	P	P	P
F2446B	24	MN	P	P	N
F2364B	84	MN	N	N	N
F2323B	24	F	P	P	P
F2233B	12	M	P	N	N
F2388B	24	M	N	N	N
F779A	10	F	N	N	N
F516A	36	MN	P	P	N
F544A	60	FN	N	N	N
2408	96	F	P	P	P
2421	48	MN	P	P	P
2443	60	MN	P	P	P
2444	96	MN	P	P	P
2463	2.2	F	P	N	N
2473	120	M	P	N	N

APPENDIX 3.3.1. CONTINUED.

REF	AGE	SEX	IgG	IgM	IgA
2479	96	M	P	P	P
2480	96	M	P	P	P
2482	156	M	P	N	N
2527	108	M	P	N	N
B50	>6	M	P	P	N
M112	≤6	M	P	N	N
M140	>6	M	P	N	N
109306	37	F	N	N	N
109378A	125	FN	P	P	N
109378C	125	FN	P	P	N
M121	41	MN	P	N	N
M122	27	FN	P	P	P

Age in months; M = Male; F = Female; MN = Male neutered; FN = Female neutered; P = Positive; N = Negative;

APPENDIX 3.3.2.

Serological survey of *Cryptosporidium* infection in healthy domestic cats

REF	AGE	SEX	IgG	IgM	IgA
195	12	M	P	N	N
196	12	F	P	N	N
199	66	F	P	N	N
201	5	F	P	N	N
204	6	F	P	N	N
205	13	F	P	N	N
206	36	M	P	N	N
210	12	F	P	N	N
212	72	F	P	N	N
213	36	M	P	N	N
215	8	F	P	N	N
216	15	F	P	N	N
217	12	F	P	N	N
221	18	M	P	N	N
224	60	M	P	P	N
225	60	M	P	N	N
227	18	F	P	P	N
228	24	F	P	P	N
229	24	F	P	N	N
230	18	F	P	N	N
231	12	F	P	P	N
232	6	F	P	P	P
235	ND	M	P	N	P
237	8.5	M	P	P	N
238	8	M	P	P	N
239	9	M	P	N	N
240	13	M	P	N	N
244	12	F	P	N	N
245	12	F	P	N	N
246	6	F	P	N	N
247	6	F	P	N	N
248	10	F	P	N	N
249	10	F	P	N	N
385	30	F	P	N	N
387	72	M	P	N	N
388	ND	M	P	N	N
393	4	M	N	N	N
393	5	M	P	P	N
394	4	M	P	P	P
395	60	F	P	P	P
396	18	MN	N	N	N
404	12	F	P	P	P

APPENDIX 3.3.2. CONTINUED.

REF	AGE	SEX	IgG	IgM	IgA
405	60	MN	P	P	P
408	48	M	P	N	N
409	9	F	P	N	N
411	7	M	P	N	N
412	12	M	P	N	N
413	9	F	N	N	N
418	138	F	P	N	N
419	36	M	N	N	N
421	12	F	N	N	N
422	11	F	P	N	N
423	9	F	N	N	N
425	8	M	N	N	N
435	24	F	N	N	N
447	6	M	P	N	N
541	18	M	P	N	N
542	50	F	N	N	N
543	8	F	P	N	N
544	156	FN	P	N	N
545	12	M	N	N	N
546	24	F	N	N	N
548	6	F	P	N	P
549	24	M	N	N	N
550	12	M	P	P	P
551	12	F	P	P	P
552	6	M	P	N	N
553	6	F	P	N	N
554	5	F	N	N	N
555	36	FN	N	N	N
556	72	FN	P	N	N
557	180	FN	N	N	N
558	ND	MN	P	N	N
559	84	M	N	N	N
560	9	F	P	N	N
561	18	F	P	N	N
562	18	F	P	N	N
564	6	F	P	N	N
565	7	M	P	P	P
566	7	F	P	N	N
570	5	F	P	N	N
571	7	M	P	N	P
572	8	M	P	N	P
573	7	M	P	N	N
574	7	M	P	N	N
576	7	M	N	N	N
577	7	M	P	P	P
578	7	M	P	P	P
579	7	F	P	N	P
580	24	F	P	N	N

APPENDIX 3.3.2. CONTINUED.

REF	AGE	SEX	IgG	IgM	IgA
581	5	F	P	P	P
582	6	F	P	N	N
583	5	F	P	N	N
586	30	M	P	P	P
587	5	M	P	P	P
588	5.5	M	P	N	N
589	12	M	P	P	N
590	12	M	P	P	N
591	84	F	P	P	N
592	12	M	P	P	N

REF = Reference number; Age in months; M = Male; F = Female; MN = Male neutered; FN = Female neutered; ND = Not determined; P = Positive; N = Negative.

APPENDIX 3.3.3.

Serological survey of *Cryptosporidium* infection in feral cats

REF	AGE	SEX	IgG	IgM	IgA
A1	A	F	P	N	N
A2	A	M	N	N	N
A3	A	F	P	P	P
A4	K	M	N	N	N
A6	K	M	N	N	N
A7	K	M	N	N	N
A8	K	M	P	P	P
A9	A	M	P	P	P
A10	A	M	P	N	N
A11	K	F	N	N	N
A12	A	F	P	P	P
A13	A	F	N	N	N
A14	A	M	P	P	N
A15	A	F	P	P	P
A16	A	M	N	N	N
A17	K	M	N	N	N
A18	K	F	P	P	P
A19	A	M	P	P	P
A20	A	F	N	N	N
A22	A	F	P	P	N
A23	A	F	P	P	N
A24	A	F	N	N	N
A25	A	F	P	N	N
A26	A	M	P	P	N
A27	A	M	P	P	P
A28	K	M	N	N	N
A29	K	M	P	P	P
A30	A	F	N	N	N
A31	A	F	P	P	N
M16	A	F	P	P	P
M17	K	M	P	P	P
M31	A	M	P	N	N
M32	A	M	P	P	P
M75	K	M	N	N	N
M84	K	M	N	N	N
M86	A	F	P	N	N
M87	A	M	P	N	N
M90	A	M	P	P	N
M91	A	F	P	N	N
M101	A	M	N	N	N

APPENDIX 3.3.3. CONTINUED.

REF	AGE	SEX	IgG	IgM	IgA
M105	K	F	N	N	N
M106	K	M	P	N	N
M108	A	M	P	N	N
M109	K	F	P	N	N
M110	K	F	P	N	N
M111	K	F	N	N	N

REF = Reference number; A = Adult; K = Kitten; M = Male; F = Female; P = Positive; N = Negative.

APPENDIX 3.3.4.

Serological survey of *Cryptosporidium* infection in SPF kittens.

REF	AGE	SEX	IgG	IgM	IgA
257-1	K	NS	N	N	N
257-2	K	NS	N	N	N
257-3	K	NS	N	N	N
257-4	K	NS	N	N	N
258	K	NS	N	N	N
260-1	K	NS	N	N	N
260-2	K	NS	N	N	N
260-3	K	NS	N	N	N

K = Kitten; NS = Not seen; N = Negative.

APPENDIX 3.3.5.

Determination of optimal dilutions of the antisera

Serum dilution	Antisera dilutions 1:10 IgG	1:10 IgM	1:10 IgA	1:20 IgG	1:20 IgM	1:20 IgA	1:40 IgG	1:40 IgM	1:40 IgA	1:80 IgG	1:80 IgM	1:80 IgA
1:10	+++	+++	++	+++	+++	++	++	++	++	++	++	++
1:20	+++	+++	++	+++	+++	++	++	++	++	++	+	+
1:40	++	±	±	++	±	-	++	±	-	++	-	-
1:80	++	-	-	++	-	-	+	-	-	+	-	-
1:160	+	-	-	±	-	-	-	-	-	-	-	-
1:320	-	-	-	-	-	-	-	-	-	-	-	-
1:640	-	-	-	-	-	-	-	-	-	-	-	-
1:1280	-	-	-	-	-	-	-	-	-	-	-	-
1:2560	-	-	-	-	-	-	-	-	-	-	-	-
1:5120	-	-	-	-	-	-	-	-	-	-	-	-
1:10240	-	-	-	-	-	-	-	-	-	-	-	-
1:20480	-	-	-	-	-	-	-	-	-	-	-	-

+++= intense fluorescence; ++ = moderate fluorescence; + = faint fluorescence; ± = very faint fluorescence; - = no fluorescence

APPENDIX 4.1.1.

Sizes[1] of oocysts found in two lambs experimentally-infected with *Cryptosporidium* oocysts from a farm cat.

Lamb	Small	Range (mean)	Large	Range (mean)
F2398	4.5 x 4.2	4.5-5.0 x 3.6-4.8	6.0 x 4.8	6.0-6.3 x 4.5-5.7
	4.8 x 4.5	4.8 x 4.2	6.0 x 5.1	5.9 x 4.9
	4.5 x 3.6		6.0 x 5.7	
	4.5 x 3.6		6.0 x 4.5	
	5.0 x 4.5		6.0 x 5.4	
	4.8 x 4.2		5.7 x 4.5	
	4.5 x 3.6		6.0 x 4.5	
	4.8 x 4.8		6.0 x 4.5	
	4.9 x 4.2		6.0 x 5.7	
	4.8 x 4.5		6.0 x 5.4	
	5.0 x 4.2		5.4 x 4.5	
	4.8 x 3.6		6.0 x 4.8	
	4.8 x 3.6		6.0 x 4.8	
	4.8 x 4.2		5.7 x 4.5	
	4.8 x 4.8		6.0 x 4.8	
	4.9 x 4.8		5.4 x 4.8	
	4.8 x 3.6		6.0 x 5.7	
	4.5 x 3.6		6.0 x 4.5	
	4.8 x 4.5		6.0 x 4.8	
	4.8 x 4.8		6.0 x 4.8	
V596	4.8 x 4.8	4.8-5.0 x 3.6-4.8	6.0 x 4.8	5.4-6.0 x 4.5-5.7
	4.8 x 4.8	4.8 x 4.5	6.0 x 4.8	6.0 x 5.0
	4.8 x 4.2		6.0 x 4.8	
	4.8 x 4.7		6.0 x 4.8	
	4.8 x 4.5		5.4 x 4.8	
	4.8 x 4.8		6.0 x 5.4	
	4.8 x 4.5		6.0 x 5.7	
	4.8 x 4.2		6.0 x 4.5	
	4.8 x 4.5		5.7 x 4.8	
	4.8 x 4.8		6.0 x 5.4	
	4.8 x 4.5		6.0 x 5.4	
	4.8 x 3.6		6.0 x 4.5	
	4.8 x 4.8		6.0 x 4.8	
	5.0 x 4.8		6.0 x 4.8	
	4.8 x 4.5		6.0 x 4.8	
	5.0 x 4.8		6.0 x 4.8	
	4.8 x 4.5		6.0 x 4.8	
	4.8 x 4.8		6.0 x 5.4	
	4.8 x 4.5		6.0 x 4.5	
	4.8 x 3.6		6.0 x 5.4	

[1] in micrometre (μm).

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