Cryptosporidiosis in man, domestic animals and birds: a review[1]

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Cryptosporidia (Apicomplexa: order Eucoccidiorida, suborder Eimeriorina) are coccidian parasites which can infect many species of mammal, bird and reptile (Levine 1973). Their small size (oocysts 4–4.5 μm; endogenous stages 2–6 μm) and moderate excretion rate make them difficult to detect in faeces or tissue sections, thus they may be overlooked in routine examinations of smears or tissues at low magnifications. Characteristically the endogenous stages are found adhering by specialized attachment zones to the microvillous borders of enterocytes, often in both small and large intestines. Each is enclosed in a parasitiphorous vacuole, the outer covering of which is derived from the enterocyte cell membrane (Bird & Smith 1980). Adaptation of the specialized attachment zone probably allows the parasite to derive nourishment from the host cell (Hampton & Rosario 1966, Bird & Smith 1980).

The organism is not exclusively found in the gut: cryptosporidia have been observed in the glands of the stomach (mice–Tyzzer 1910; snakes–Brownstein *et al.* 1977; foals–Snyder *et al.* 1978), respiratory epithelium (turkeys–Hoerr *et al.* 1978; chickens–Dhillon *et al.* 1981), bile duct (rhesus monkey–Kovatch & White 1972; foal–Snyder *et al.* 1978), pancreatic duct and gallbladder (rhesus monkey–Kovatch & White 1972) and the tonsillar region (man–Webster *et al.* 1980).

An important feature which distinguishes Cryptosporidium from most other coccidia is lack of species specificity (Tzipori *et al.* 1980*a*, Moon & Bemrick 1981). The results of cross-transmission experiments (see below), using isolates from farm animals and human patients, suggested to Tzipori *et al.* (1980*a*) that Cryptosporidium might be a single-species genus. If so, the domestic species may be a reservoir of infection for susceptible human individuals.

Life cycle and morphology of Cryptosporidium

The life cycle of the parasite is direct, and is essentially similar to other coccidia of the family Eimeriidae, although it is not always clear whether sporulation takes place outside or within the host. Until recently, the precise nature of the infective stage(s) in faeces was the subject of controversy. Tyzzer (1912) had described structures in mouse faeces which he identified as 'oocysts containing 4 naked sporozoites'. The existence of an oocyst stage was disputed by Vetterling *et al.* (1971), who failed to find oocysts in the faeces of guinea pigs infected with Cryptosporidium. This situation was partly resolved by Pohlenz *et al.* (1978) and Iseki (1979), who found sporulating oocysts attached to the gut wall and in the faeces of infected calves and cats, respectively. In a recent ultrastructural study in mice, Brändler (1982) identified an oocyst stage with a multilaminar wall, attached to enterocytes in the small and large intestine. This author found that sporulation occurred *in situ,* and a 'sporocyst' containing 4 sporozoites enclosed in a bilaminar membrane was released and shed in the faeces. In livestock, faecal stages are easily demonstrated by simple staining procedures (Figure 1A) and can be isolated and concentrated by flotation methods (see below under Diagnosis), while structures resembling the 'sporocysts' of Brändler (1982) have been found by electron microscopy in the faeces of calves with cryptosporidiosis (E W Gray, personal communication; Figure 1B). The authors of recent papers on cryptosporidiosis, including the author of this review, have found it convenient to refer to these structures collectively as

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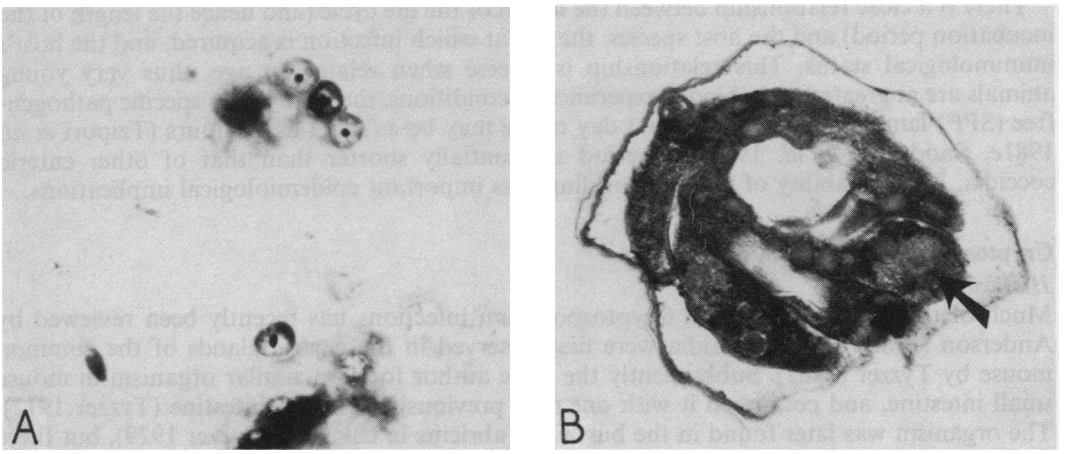

Figure 1. A: Cryptosporidium oocysts in bovine faeces smear, photographed using oil immersion. The deeply-stained speck in some oocysts is the residual body of the zygote; sporozoites are not stained by this method (modified Ziehl-Neelsen stain, × 1480). B: Ultrastructural appearance of Cryptosporidium oocyst/sporocyst in bovine faeces stored at 4°C for 7 days. After glutaraldehyde fixation (3% in phosphate buffer at pH 7.4) the sample was pelleted by centrifugation, which probably caused some collapse and distortion. Four sporozoites and a residual body (arrowed) can be seen (Uranyl acetate & lead citrate (Ua-PbCit), × 30000)

oocysts, as their presence in faeces is a reliable index of infection. The possibility that in some species full maturity to an oocyst stage fails to take place, and that infectivity depends on the excretion of endogenous stages, cannot be altogether excluded.

After sporulation, merogony (schizogony) and gametogony cycles take place rapidly within the host. The sporozoites invade the microvillous borders of enterocytes and the resultant trophozoites quickly differentiate to form meronts (schizonts) with 8 merozoites (Figure 2). The first schizont generation may be followed by at least one other, in which either four (Vetterling *et al.* 1971, Pohlenz *et al.* 1978) or eight (D R Snodgrass & E W Gray, unpublished data) merozoites are formed. It is not known to what extent, if any, the number of schizont generations is influenced by the species, age or immune status of the host. Merogony is followed by gametogony; microgametes from what seems to be rather a restricted generation of microgametocytes fuse with macrogametes (Figure 3) to form zygotes with specialized protective outer walls. Further development to oocysts probably takes place while the organisms are still attached to enterocytes. The morphology of the various endogenous stages in man is very well described by Bird & Smith (1980).

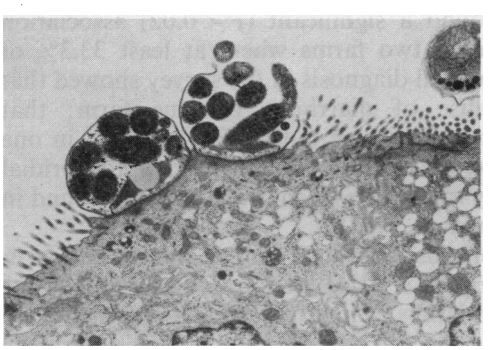

Figure 2. Mature schizonts with 8 merozoites (Ua-PbCit, × 9000)

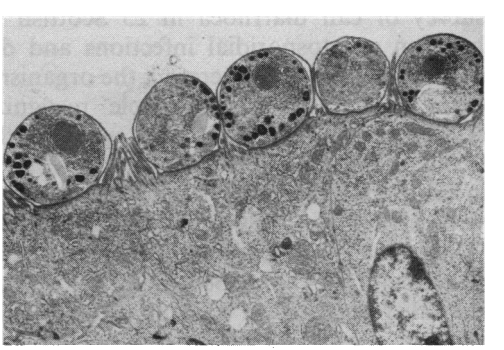

Figure 3. Macrogametes ranged along adjacent enterocytes. Polysaccharide granules are arrayed peripherally (Ua-PbCit, × 9000)

There is a close relationship between the length of the life cycle (and hence the length of the incubation period) and the host species, the age at which infection is acquired, and the host's immunological status. This relationship is inverse when related to age, thus very young animals are at greatest risk. Under experimental conditions, the life cycle in specific pathogen-free (SPF) lambs infected on the first day of life may be as short as 72 hours (Tzipori *et al.* 1981*c*, Snodgrass *et al.* 1983), a period substantially shorter than that of other enteric coccidia. This capability of Cryptosporidium has important epidemiological implications.

Cryptosporidiosis – the natural disease

Historical

Much of the early literature on Cryptosporidium infections has recently been reviewed by Anderson (1982). Cryptosporidia were first observed in the gastric glands of the common mouse by Tyzzer (1907). Subsequently the same author found a similar organism in mouse small intestine, and compared it with one seen previously in rabbit intestine (Tyzzer 1912). The organism was later found in the bursa of Fabricius in chickens (Tyzzer 1929), but there was no indication of its possible pathogenicity prior to a report associating its presence with diarrhoea in young turkeys (Slavin 1955). Important studies in guinea pigs (Hampton & Rosario 1966, Jervis *et al.* 1966, Vetterling *et al.* 1971) led to a clearer understanding of the morphology and life cycle of the parasite, though these authors' conclusions on the existence of an oocyst stage, and on the site-specificity, species-specificity and pathogenicity of the organism, have been largely superseded by more recent studies.

The natural disease in food animals and poultry

Reports of sporadic cryptosporidial infections in calves in the early 1970s were quickly followed by extensive surveys in which the organism was identified with complex outbreaks of diarrhoea involving large groups of animals under different management systems and in various localities: e.g. Canada (Morin *et al.* 1976), United States (Pohlenz *et al.* 1978, Bergeland *et al.* 1979). Hungary (Nagy *et al.* 1980), United Kingdom (Snodgrass *et al.* 1980), Denmark (Henriksen & Krogh 1980), West Germany (Heine & Boch 1981) and Australia (Jerrett & Snodgrass 1981). Although the most serious outbreaks were multi-aetiological, involving rotavirus, coronavirus and enterotoxigenic *Escherichia coli* infections as well as cryptosporidia, less severe outbreaks with low mortality have been reported in which Cryptosporidium was the only pathogen identified (Bergeland *et al.* 1979, Tzipori *et al.* 1980*c*, Anderson & Bulgin 1981). Although interaction between cryptosporidia and enteric bacteria must be considered a possibility in such outbreaks, bacteria-free isolates of Cryptosporidium can cause clinical disease in gnotobiotic piglets (Tzipori *et al.* 1982*c*), calves (S Tzipori, unpublished data) and lambs (Snodgrass *et al.* 1983).

It seems probable that cryptosporidial infections represent a serious complication of virus-induced enteritis, particularly in young calves. Recently Snodgrass *et al.* (1982), as part of a survey of calf diarrhoea in 25 Scottish herds, found a significant ($P < 0.02$) association between cryptosporidial infections and diarrhoea on two farms where at least 33.3% of scouring calves were excreting the organism. The overall diagnosis in the survey showed that cryptosporidiosis was the sole recognizable cause of diarrhoea on one farm; that cryptosporidia and rotaviruses were jointly concerned in three outbreaks; and that in one further outbreak these two agents were associated with a bovine coronavirus. Cryptosporidial infections may also play an important contributory role in the 'cachexia syndrome' found in veal calf units in the Netherlands (Hage-Noordam *et al.* 1982).

The first reports of sporadic cases of cryptosporidiosis in sheep were made by Barker & Carbonell (1974) and Berg *et al.* (1978). Later, Tzipori *et al.* (1981*a*) found that significant mortality occurred in a group of artificially-reared lambs, a high percentage of which were excreting Cryptosporidium oocysts, though one was infected with a rotavirus. The occurrence of diarrhoea with high mortality was also recorded in naturally-reared lambs, in which Cryptosporidium was virtually the only pathogen identified (Angus *et al.* 1982*a*).

Although piglets can be experimentally infected with bovine isolates of Cryptosporidium, with resultant diarrhoea and severe pathological changes in the intestine (Tzipori *et al.* 1981*d*, 1982*c*), reports of the natural disease in pig herds suggest that cryptosporidiosis has little clinical significance (Morin *et al.* 1976, Kennedy *et al.* 1977, Links 1982). Cryptosporidiosis has also been confirmed in red deer calves, in which there was high mortality, though several also were infected with an astrovirus (Tzipori *et al.* 1981*b*). There are reports of diarrhoea associated with cryptosporidial infections in goats (Mason *et al.* 1981, Tzipori *et al.* 1982*b*).

The economic significance of cryptosporidial infections in poultry is unknown, but infections have been reported in chickens (Tyzzer 1929, Fletcher *et al.* 1975, Dhillon *et al.* 1981), domestic geese (Proctor & Kemp 1974) and turkeys. In the latter species, diarrhoea and deaths occurred in poults in one outbreak (Slavin 1955), while a report by Hoerr *et al.* (1978) described outbreaks of tracheobronchitis in young birds, with cryptosporidial infections in the nasal passages, trachea and primary bronchi.

No treatment has produced any significant mitigating effect in outbreaks of cryptosporidiosis in livestock (Tzipori 1983), although Morin *et al.* (1976) claimed that sulphonamides were helpful in some outbreaks. Snodgrass *et al.* (1980) found that clinical improvement occurred with oral sulphadimidine in the early stages of one outbreak, but later in the same outbreak this treatment failed to control diarrhoea. Presumably oral sulphonamides are active only against concomitant bacterial infections. Attempts at chemoprophylaxis with a number of antiprotozoal and other drugs were unsuccessful (Moon *et al.* 1982).

Cryptosporidial infections in companion animals and exotic pets

Naturally-occurring cryptosporidial infections were found in adult cats and kittens by Iseki (1979). Five cats were shedding oocysts and the disease was transmitted to susceptible cats. The infections were subclinical, and no significant intestinal lesions were found. There are no reports of cryptosporidial infections in dogs; the only report in horses relates to infections in Arabian foals with an inherited immunodeficiency (Snyder *et al.* 1978).

Reports of natural and experimental infections in mice, rabbits and guinea pigs, species commonly kept by young children as pets, indicate that these are subclinical. Amongst possible exotic pets, cryptosporidiosis has been reported in rhesus monkeys (Kovatch & White 1972, Cockrell *et al.* 1974), parrots (Doster *et al.* 1979), peacocks (Mason & Hartley 1980) and certain species of snake (Brownstein *et al.* 1977). The capacity of all these species for contaminating their environment is unknown.

Cryptosporidiosis in man

The first recorded case of human cryptosporidiosis features in a report by Nime *et al.* (1976), the patient being a 3-year-old girl from Nashville, Tennessee, who was admitted to hospital with symptoms of vomiting, watery diarrhoea and crampy abdominal pains. The child's immunological status was not investigated and with supportive therapy she recovered uneventfully. Diagnosis of the infection was effected by rectal biopsy. More recent publications (Slavin 1980, Bryceson 1980, Tzipori *et al.* 1980*b*, Weinstein *et al.* 1981, Sloper *et al.* 1982, Anderson *et al.* 1982, Reese *et al.* 1982) give well-documented accounts of 7 further cases in the United States and 4 in the UK. Of these patients, whose ages ranged from 3 to 58 years, 8 either were seriously immunodeficient or were receiving immunosuppressive treatment. The outcome was fatal in 6 of these immunologically-compromised patients, though one patient, a 30-year-old man who apparently contracted the infection while under treatment for bullous pemphigus, recovered spontaneously following the withdrawal of immunosuppressive drugs (Meisel *et al.* 1976). Not unexpectedly, the clinical pattern in a number of these cases was complicated by other infections, including *Giardia lamblia*, cytomegalovirus, adenovirus or toxoplasma infections. Diagnosis in this group of patients was established by light and electron microscopical examination of intestinal mucosal biopsy samples.

The remaining 3 reports of individual infections refer to acute but transient infections in young adults, none of whom had any history of immunodeficiency. Their symptoms were similar: all had nausea or vomiting, protracted watery diarrhoea, cramping abdominal pains and anorexia, with spontaneous recovery in one to two weeks. In each case, diagnosis was made by demonstration of Cryptosporidium oocysts in stool samples in the absence of other significant enteropathogens. Oocysts were recovered by flotation techniques in two instances (Anderson *et al.* 1982, Reese *et al.* 1982) and in two instances further confirmation was made by transmission to laboratory animals (Tzipori *et al.* 1983*b*); Reese *et al.* 1982). In a preliminary report which included the case described by Reese *et al.* (1982), Current *et al.* (1981) demonstrated Cryptosporidium oocysts in stool samples from 12 previously healthy human patients with similar or less severe symptoms. None of these individuals had Salmonella infections, but the investigation did not exclude other possible bacterial or viral pathogens (Centers for Disease Control 1982).

Further evidence for the occurrence of transient cryptosporidiosis in man is provided by a 4-month survey of hospital patients with enteritis: 25 of 362 patients (6.9%) were excreting Cryptosporidium oocysts in their stools (Tzipori *et al.* 1983*b*); in only 4 of these patients were other possible enteropathogens identified. Oocyst shedding was also confirmed in two 18-month-old infants with diarrhoea in a city hospital (S Tzipori & C R Madeley, personal communication); in a 2-year-old child from a children's home during a diarrhoea epidemic; and in a mother and her child (S Tzipori, personal communication).

As in other species, no effective drug or other treatment capable of controlling Cryptosporidium infections in man has yet been found; consequently the disease is refractory in patients with immunodeficiencies or who are receiving prolonged immunosuppressive therapy. In circumstances favourable to the discontinuance of immunosuppressive drugs, natural resistance may develop, resulting in spontaneous recovery (Meisel *et al.* 1976). In healthy, immunocompetent individuals, infections appear to be transient and recovery takes place spontaneously with rest and supportive therapy.

Diagnosis

Demonstration of oocysts in methanol-fixed faeces smears stained either by Giemsa's method (Pohlenz *et al.* 1978, Snodgrass *et al.* 1980) or by a modified Ziehl-Neelsen technique (Henriksen & Pohlenz 1981) is now used routinely in diagnosis of cryptosporidial infections in farm livestock. Field observations in calves have shown that oocyst shedding coincides with clinical illness and diarrhoea (Tzipori *et al.* 1980*c*, Anderson & Bulgin 1981), at which time oocysts are numerous in the faeces. In contrast, Tzipori *et al.* (1980*c*) noted that non-diarrhoeic animals in outbreaks pass very few or no oocysts. Similar observations have been made in lambs, red deer and goats. Oocysts can also be detected in faeces by flotation (Iseki 1979, Anderson 1981). Histological confirmation of infection, though valuable (Morin *et al.* 1976), is only practicable in recently-dead carcases or in moribund animals killed by intravenous barbiturates, due to autolytic sloughing of surface enterocytes. However, the organism may be detected in cadavers up to 6 hours after death by examining Giemsa-stained scrapings of ileal mucosa (Pohlenz *et al.* 1978). The significance of positive findings falls into perspective only when the presence or absence of other possible enteropathogens in the sample has been determined by specific techniques. Definitive confirmation of the presence of cryptosporidia in a faeces sample can be obtained by oral inoculation into suckling SPF mice of fresh material, faeces stored for up to several months at 4°C, or suspensions of oocysts obtained by flotation (Reese *et al.* 1982, Sherwood *et al.* 1982).

Diagnosis of acute but transient cases of cryptosporidiosis in man has been made by demonstration of oocysts in stool samples by stained smears or by flotation, and confirmed by transmission to mice (Tzipori *et al.* 1980*b*, Reese *et al.* 1982). In patients with immunodeficiencies, diagnosis of Cryptosporidium infection may be sought only when all other possibilities have been excluded, and the patient may be chronically debilitated and suffering from intercurrent infections which might increase the surgical risk of routine biopsy

procedures. Not all authorities accept that oocyst shedding occurs in these cases, and a further difficulty is that the oocyst of Cryptosporidium could be confused in Giemsa-stained smears with a stage of *Blastocystis hominis* (R G Bird, personal communication). Successful transmission to mice, with positive identification of endogenous stages by electron microscopy, would remove all doubts. However, recent evidence suggests that there may be strain differences in infectivity for laboratory animals of cryptosporidia found in human stools (Tzipori *et al.* 1983*a*), thus an unsuccessful transmission of a suspicious stool sample might require to be followed by a rectal, a colonic or even an ileal biopsy, before cryptosporidiosis could be ruled out. It has yet to be shown that the mouse transmission test is a necessary step in the diagnosis of chronic cryptosporidiosis in man.

Experimental cryptosporidiosis

The infectivity of intestinal contents from mice infected with an intestinal cryptosporidium designated *Cryptosporidium parvum* was demonstrated in mice by Tyzzer (1912). Later Vetterling *et al.* (1971) transmitted the disease in guinea pigs with intestinal scrapings from an infected guinea pig, and this technique was also used by Pohlenz *et al.* (1978) and Nagy *et al.* (1980) to transmit the disease in calves. The infectivity of fresh faeces containing oocysts was demonstrated by Tzipori *et al.* (1980*a*); diarrhoeic bovine faeces containing oocysts and possibly endogenous stages infected not only calves but six other species, confirming the lack of host-specificity of the organism. Inter-species transmission with infected faeces has subsequently been confirmed by other workers (Moon & Bemrick 1981, Reese *et al.* 1982).

Experiments with isolates from several different species induced clinical illness, diarrhoea and quite extensive and severe pathological changes in the posterior small intestines of lambs, piglets and calves (*see* review by Tzipori 1983). The lesions were widespread villous atrophy and fusion, with infiltrates of mononuclear cells and neutrophils into the lamina propria (Tzipori *et al.* 1981*d*, Angus *et al.* 1982*c*). It was initially considered that the gut lesions could result from interaction between the protozoan and enteric bacteria in the absence of known enteropathogens, even when intermediate passage through suckling SPF mice or rats was utilized as a 'biological filter' system (Angus *et al.* 1982*c*). However, recent studies using bacteria-free Cryptosporidium inocula in gnotobiotic pigs (Tzipori *et al.* 1982*c*), calves and lambs (Snodgrass *et al.* 1983) have confirmed the pathogenicity of Cryptosporidium for these species.

All isolates so far tested in mice have caused only subclinical infections with no significant pathological changes, although a number of mouse strains, including athymic (nu/nu) mice, have been utilized (Tzipori *et al.* 1980*a*, Sherwood *et al.* 1982). Infected mice shed oocysts from about day 5 post-inoculation (p.i.) to about day 12 p.i., and histological evidence of infection can be confirmed up to at least day 14 p.i. (Sherwood *et al.* 1982). Litters are infected uniformly, and the model has already proved useful for testing the effects of 16 potential therapeutic substances (Tzipori *et al.* 1982*a*) and the efficiency of disinfectants in sterilizing infected faeces (Campbell *et al.* 1982, Angus *et al.* 1982*b*). None of the drugs used controlled the infections in mice, and of the disinfectants used, only household ammonia and 10% formal-saline destroyed the infectivity of faecal stages.

Further experimental studies in animals should be useful in epidemiological studies, for further testing of possible chemotherapeutic agents, and for investigating the immunity to the organism.

Sources of infection and zoonotic implications

As Cryptosporidium can cross species barriers, an outbreak of disease in a group of animals of one species may act as the source of infection for a group of a different species on the same premises. For example, there is good circumstantial evidence that calves with diarrhoea and cryptosporidiosis transmitted the disease to artificially-reared lambs, probably by mechanical contamination from attendants' clothing or utensils (Tzipori *et al.* 1981*a*), and to red deer calves (Tzipori *et al.* 1981*b*). Wild rodents may also be a reservoir of infection; mice carry the

organism (Tyzzer 1907) and cryptosporidia have been demonstrated in the caecum of wild rats (*Rattus norvegicus*) by electron microscopy (K W Angus, unpublished). In human populations, recent observations on homosexual communities in the USA confirm infection by contact with infected individuals (R G Bird, personal communication).

A clear association between bovine cryptosporidiosis and human cryptosporidial infections has been established. In three separate outbreaks of bovine cryptosporidiosis, 12 handlers contracted the infection and 10 of these were acutely ill, though all recovered within two weeks (Current *et al.* 1981, Reese *et al.* 1982). The patient referred to by Anderson *et al.* (1982) was a veterinary student in charge of calves used in a transmission experiment with bovine Cryptosporidium isolates. In the case reported by Tzipori *et al.* (1980*b*) the patient worked at a veterinary institute, and could have had contact with lambs excreting a bovine strain of the organism. It may be of more than passing interest that the child referred to by Nime *et al.* (1976) was brought up on a cattle-rearing farm, while the subject of a report by Meisel *et al.* (1976) owned a small beef-rearing unit and had been working there shortly before his symptoms started. No associations between human cryptosporidial infections and outbreaks in lambs, pigs or other livestock have been reported.

Cryptosporidial infections can certainly occur in species which are commonly kept as pets, including cats, mice, guinea pigs, rabbits and monkeys, but reports indicate that these infections are subclinical, and oocyst shedding may be of such a low order that there is little or no risk to immunologically-competent individuals.

Conclusions

Like most coccidia, cryptosporidia are widespread in nature and will infect many species including man. Indeed, a random survey showed that sera from 10 mammalian species including man contained detectable antibodies to the organism (Tzipori & Campbell 1981). Transmission experiments show that Cryptosporidium can cause diarrhoea and severe pathological lesions in the intestines of calves, lambs and piglets. The organism has been associated with outbreaks of diarrhoea in calves, lambs, red deer and goats in the absence of other known enteropathogens, and may represent a serious complication of viral diarrhoeas in these species. Cryptosporidia have also been associated with outbreaks of diarrhoea or serious respiratory disease in some species of poultry. Attempts at treatment or prophylaxis of cryptosporidial infections with anticoccidial or other drugs have proved unsuccessful, and the infective stages in the faeces have been shown to be highly resistant to the action of disinfectants. No attempt has been made to assess the economic significance of cryptosporidiosis in livestock, but it is probably considerable.

A pathogenic coccidian capable of crossing species barriers, with a short life cycle which permits rapid build-up of infection in enclosed premises, constitutes a potential hazard for susceptible human individuals. The importance of cryptosporidial infection as a grave complication in immunologically-compromised patients cannot be over-emphasized.

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