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Review article

Cryptosporidium species a "new" human pathogen

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SUMMARY Publications describing aspects of the coccidian protozoan parasite *Cryptosporidium*, increased greatly during 1983 and 1984 as a result of not only increasing veterinary interest but also in the role of the parasite in the newly recognised acquired immune deficiency syndrome (AIDS). The reports reflected widespread collaboration, not only between clinicians, microbiologists, and histopathologists, but also between veterinary and human health care workers. *Cryptosporidium* was first described in mice in 1907 and subsequently in various other species; it was not described in man until 1976. Several likely putative species have been described, but there is probably little host specificity. Experimental and clinical studies have greatly increased the knowledge about the organism's biology. The parasite undergoes its complete life cycle within the intestine, although it may occasionally occur in other sites. The main symptom produced is a non-inflammatory diarrhoea, which, in patients with AIDS and children in third world countries, may be life threatening: even in immunocompetent subjects this symptom is usually protracted. Attempts to find effective chemotherapeutic agents have been unsuccessful. Epidemiologically the infection was thought to be zoonotic in origin, but there is increasing evidence of person to person transmission. Diagnosis has depended upon histological examination, but simple methods of detection have now been described: more invasive methods need no longer be used. The parasite, which is found more commonly in children, occurs in about 2% of faecal specimens examined and seems to be closely associated with production of symptoms. A serological response has been shown. Much remains to be learned about its epidemiology and pathogenic mechanisms, while the expected increase in incidence of AIDS makes an effective form of treatment essential.

The investigation of uncomplicated diarrhoeal disease comprises a large proportion of the workload of microbiological laboratories, and routine methods often fail to indicate a causative agent. The list of causative agents continues to increase. Recently the investigation of opportunistic infections affecting the gastrointestinal tract, particularly in immunocompromised subjects, has increasingly entailed diagnostic work by histopathologists and immunologists as well as microbiologists.

Interest has focused on the coccidian protozoan organism *Cryptosporidium* sp. This agent, already well known to veterinarians as a cause of diarrhoea in animals, was first recognised in man in 1976. The infection has subsequently been recognised in both immunocompromised subjects (with severe and often life threatening diarrhoea) and normal subjects (predominantly children and young adults in whom it produces a characteristic self limiting flu like gastroenteritis). Cases occur both sporadically and in outbreaks. The illness is often more protracted than that caused by other agents, and invasive investigations may be carried out if *Cryptosporidium* is not diagnosed.

The infection probably has several reservoirs and routes of transmission in both rural and urban communities. Early veterinary interest led to the assumption that infection was almost entirely zoonotic, but

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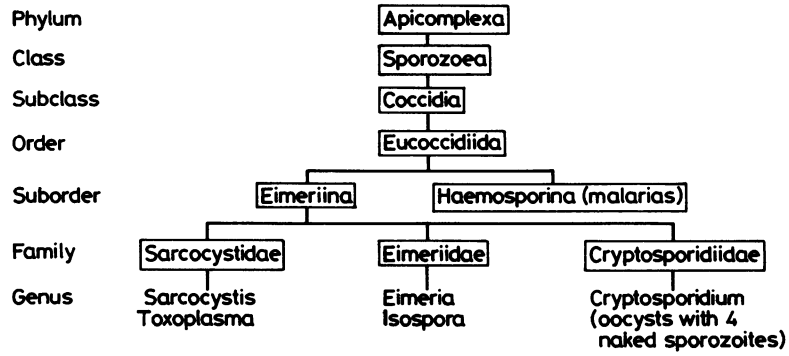

Fig. 1 *Taxonomy of Cryptosporidium: simplified scheme, showing relations with other medically important species.*

doubt has now been cast on this, and person to person spread is probably more common than was first thought. The natural history of the organism and some laboratory evidence suggests that environmental contamination is probably widespread and infection may additionally be acquired through food and water. Histologically, much is known about the infection because many of the early cases were detected in biopsy material, and infection has been shown to extend beyond the gastrointestinal tract in some cases.

Little, however, is known about the pathogenic mechanisms, although Koch's postulates have largely been fulfilled. The parasite is notably resistant to chemotherapeutic agents, although a wide range have been tested in veterinary studies and administered in the more serious cases in man. Recent work with experimental models, including induced infection in laboratory animals, cell culture, and fertile hens' eggs, has begun to elucidate the biological and pathogenic mechanisms of the organism. Microscopical and serological methods for diagnosis have been developed. It is now recognised that this organism is an important cause of gastrointestinal infections in both normal and immunocompromised subjects throughout the world. Routine laboratory investigation of such infections should now include *Cryptosporidium* among the list of suspected agents.

Historical aspects

The first description of *Cryptosporidium* is credited to Tyzzer in 1907, who found the parasite in the peptic glands of laboratory mice and considered it to be an extracellular species related to the coccidian protozoa.[1] He subsequently suggested in 1910, probably incorrectly, that it had already been described in 1894–5 by J Jackson-Clark, who identified it as a coccidian, *Eimeria falciformii*. The reports of Tyzzer were remarkable for the quality of their description and illustration.[2,3] Before the first report of infection in man cryptosporidia were described in a variety of host species and were each generally designated a species of their own. They were classified according to the host in which they were found. The validity of these identifications was first questioned by Vetterling in 1971.[4] Subsequently, Tzipori *et al* suggested that on the basis of wide host tolerance and lack of tissue specificity there may be only a single species in the genus *Cryptosporidium*.[5]

The report by Panciera *et al* in 1971 on the importance of *Cryptosporidium* in diarrhoea in calves[6] was a major stimulus to veterinary interest and subsequently to much experimental work: this was excellently reviewed by Tzipori,[7] Angus,[8] and Current *et al*.[9] The importance of veterinary work in relation to human medicine was amplified in a recent leading article.[10]

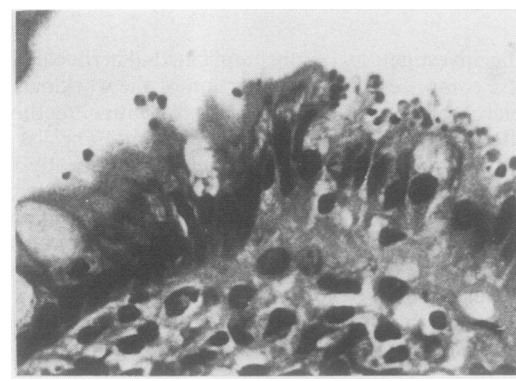

Fig. 2 *Light micrograph of human rectal biopsy, showing Cryptosporidium infection of epithelial surface. (Haematoxylin and eosin.)* × *650.*

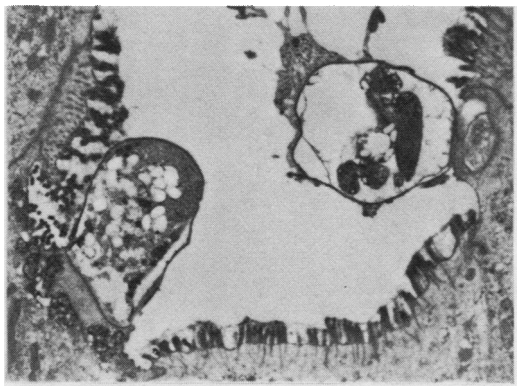

Fig. 3 *Electron micrograph of mouse colonic crypt showing two individual organisms of Cryptosporidium derived from a human case. One organism contains numerous polysaccharide granules indicating that it is a sexual stage. Apparently empty organism is a schizont containing merozoites. Tissue was initially fixed in formalin and embedded in wax and subsequently refixed and embedded for electron microscopic examination.* × *6250.*

Taxonomy and life history

Fig. 1 summarises the taxonomy of *Cryptosporidium* as a member of the coccidia.[11 12] Sporozoan protozoa are usually specific to a particular host and tissue. *Cryptosporidium* is an unusual sporozoan for several reasons. The endogenous stages seem superficially to be extracellular parasites attached primarily to the gastric, intestinal, and rectal mucosa (Figs. 2 and 3) of various animal species, including man, mammals, birds, and reptiles, and the epithelial surface of the respiratory tract can also be infected.[13–15] Electron microscopy, however, has shown that these parasites are not intracellular but extracytoplasmic and that they cause the deformation of the luminal host cell plasma membrane, producing a false appearance of superficial attachment. Several morphologically different developmental stages have been identified.

Reports on *Cryptosporidium* are confused because many species have been described.[16] Conventionally, it would be assumed that each "species" was different and would not infect other host animals. Studies on cross infection by several groups of workers both substantiate[4 16] and refute[5 17] this concept. Most recent publications on *Cryptosporidium* have suggested that this parasite is not restricted to either a particular host species or a specific epithelial surface. Current and Long infected chicken embryos with *Cryptosporidium* isolated from humans and calves.[17] These isolates were morphologically and developmentally indistinguishable. The authors therefore stated that these observations supported the proposals that *Cryptosporidium* has little or no host

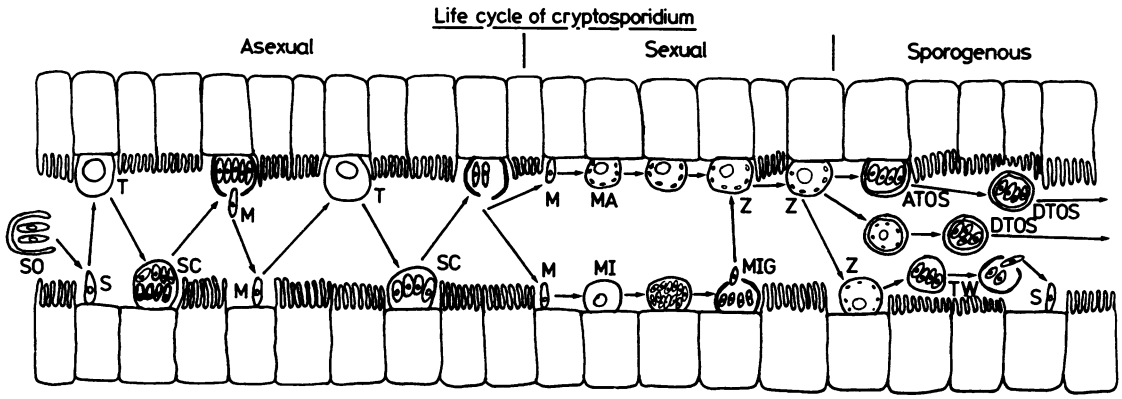

Fig. 4 *Diagrammatic representation of life cycle of Cryptosporidium. Infection starts with ingestion of an oocyst containing four sporozoites (SO). Digestive enzymes probably release sporozoites (S) from oocyst. Sporozoite enters an epithelial cell, takes up a pseudoexternal position, and matures into a trophozoite (T). Trophozoite feeds and undergoes asexual multiple budding process (schizogony) to produce a schizont (SC), which releases merozoites (M). Merozoites invade other epithelial cells and develop into trophozoites (T). Once again these undergo schizogony and release merozoites. Merozoites of this second cycle of asexual reproduction infect further epithelial cells but mature into either microgamonts (MI) or macrogamonts (MA) of the sexual cycle. Microgamont produces microgametes (MIG) which, on release, fertilise the macrogamont and produce a zygote (Z). Zygote may follow several sporogenous developmental routes: it may transform into an oocyst by secretion of a thick wall and development of sporozoites, while attached to the host cell (ATOS), before becoming detached (DTOS), and passing out of the gut; zygote may secrete a thick wall and oocyst produced may become detached (DTO) before development of sporozoites occurs (DTOS); or zygote may develop into a thin walled oocyst (TW) containing sporozoites (S). Thin walled oocysts (TW) may liberate sporozoites, thus spreading infection within the host. Microvilli have been omitted from infected cells for clarity.*

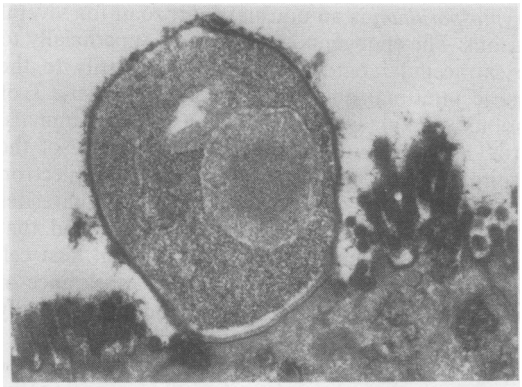

Fig. 5 *Young trophozoite of Cryptosporidium attached to mouse colonic mucosa. Note large nucleus with prominent nucleolus.* × *25000.*

specificity and that calves and other domestic animals may be a potential source of infection in man. Such speculation is supported by studies on the once confusing genus *Toxoplasma*. *Toxoplasma gondii* is now thought to be a parasite with a wide host tolerance and, as a consequence, many previous names of species have been invalidated.[18] If *Cryptosporidium* is a single species genus then *Cryptosporidium muris* would be the type species, as this was the first description of this parasite by Tyzzer in 1907.[1] It is our view,

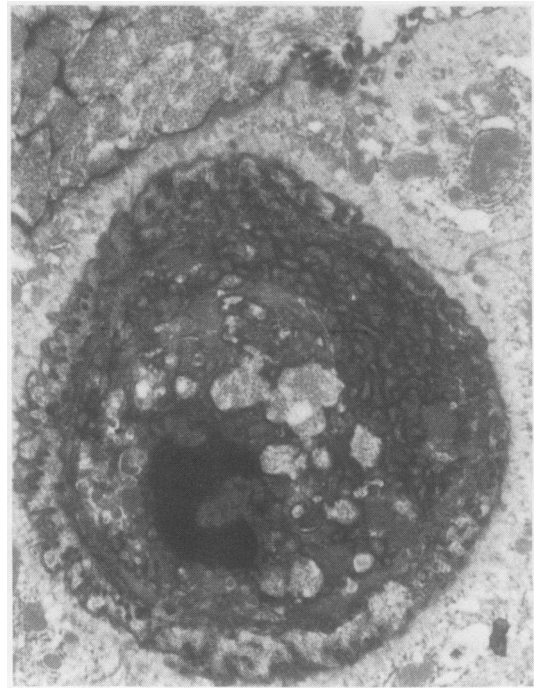

Fig. 6 *Transverse section through attachment zone showing membranous folds present in this area.* × *10000.*

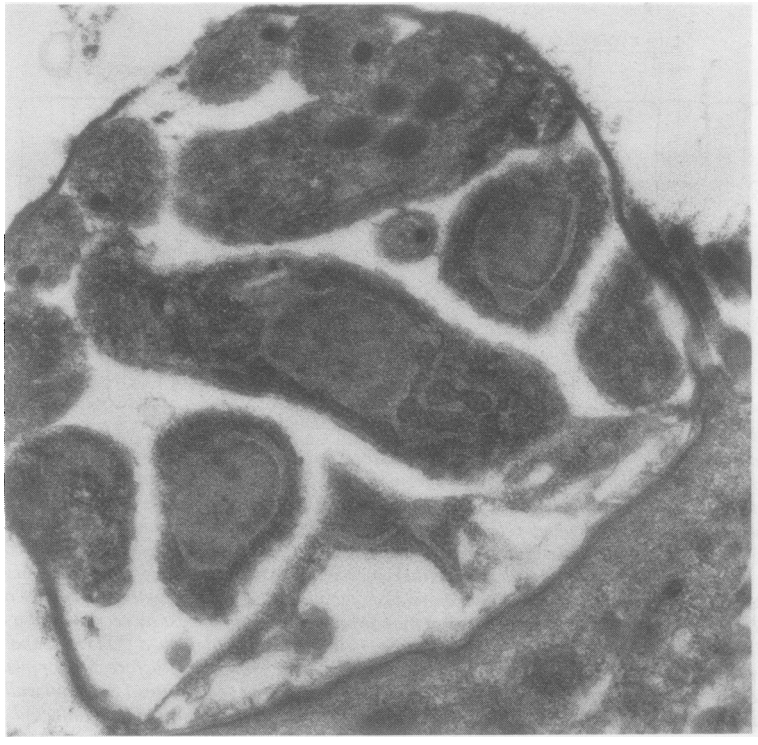

Fig. 7 *Schizont containing merozoites.* × *40000.*

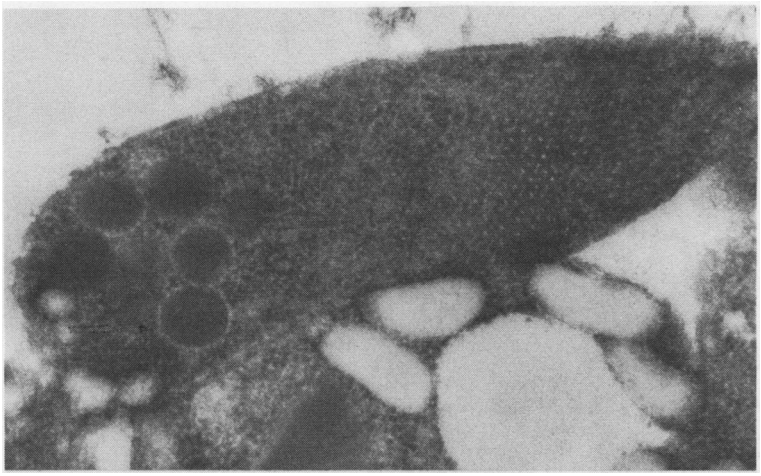

Fig. 8 *Merozoite inside schizont of Cryptosporidium. Note dense granules at posterior end of organism.* × *62000.*

however, and that of some veterinary workers (KW Angus, personal communication), that *C muris* may differ from *C parvum* and that many of the more recently identified organisms relate more closely to *C parvum*. At present there is insufficient evidence for a definitive statement. Workers should therefore continue to use the name *Cryptosporidium* species until such time as definitive evidence becomes available.

The life cycle of *Cryptosporidium* (Fig. 4) has not been completely elucidated, and, furthermore, documented evidence is somewhat confusing because of speculations about some facets of the cycle, notably those outside the host and within the intestinal contents. The life cycle has been reconstructed, using both light microscopy and electron microscopy, by several authors.[4 7 11 19 20 23] These reconstructions are, however, open to several different interpretations.

The initial infective stage is the sporozoite (1·5 μm × 0·75 μm) liberated from an oocyst by partial digestion after passage through the stomach.[2 21] The sporozoite must reach and penetrate a suitable host

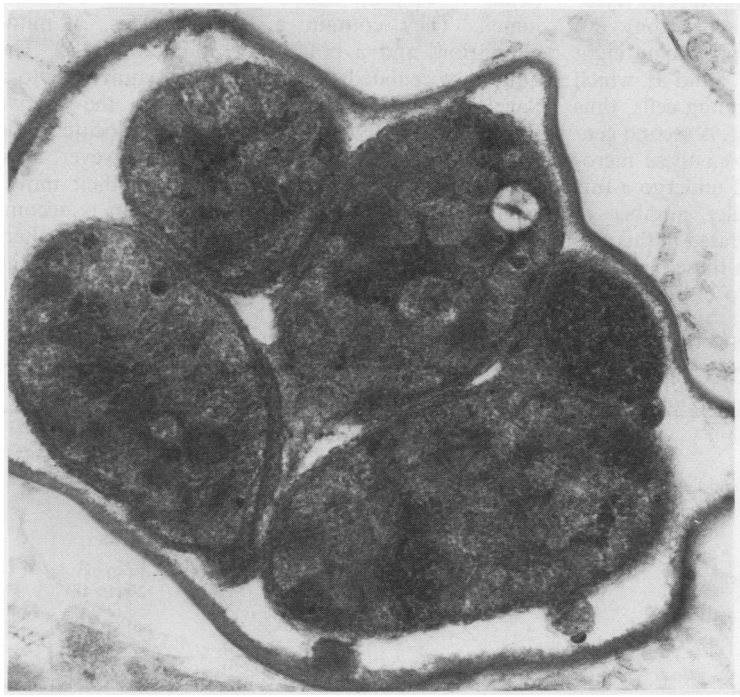

Fig. 9 *Thin section of human faecal specimen positive for Cryptosporidium. Oocyst contains four sporozoites and residual cytoplasmic mass.* × *40000.*

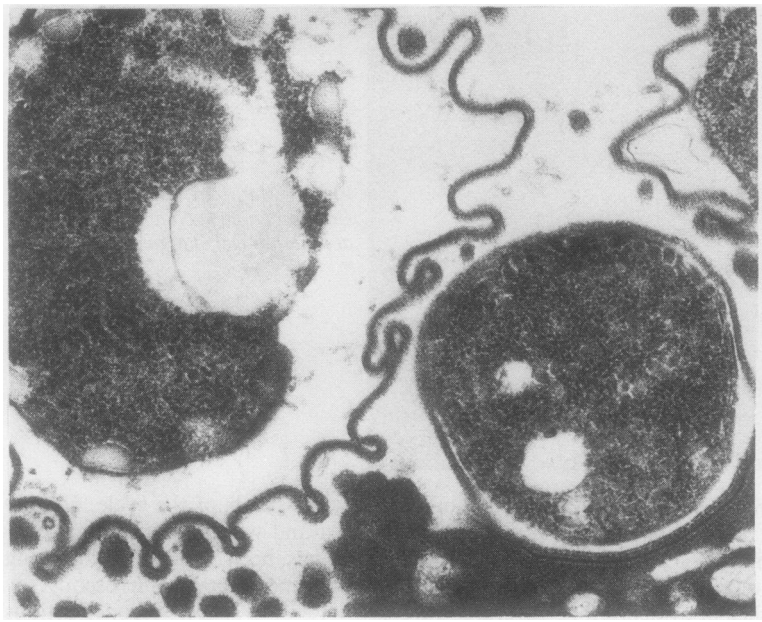

Fig. 10 *Human rectal biopsy showing several cryptosporidial endogenous stages. Attached form (right) is a trophozoite and detached form a thin walled oocyst which has yet to divide internally to form sporozoites.* × *40000.*

cell, when a parasitophorous vacuole is formed. The trophozoite (Fig. 5) then develops in a pseudo-external location and derives nutrition from either the host cell (Fig. 6) or the contents of the gut, or both. The mature trophozoite (1·5–6·0 μm in diameter) contains the normal complement of eucaryotic cytoplasmic organelles and undergoes schizogony (an asexual multiple budding process), producing eight merozoites (2–5 μm × 0·4 μm) (Figs. 7 and 8), which are liberated and invade the surrounding cells, thus spreading the infection within the host. A second generation of trophozoites is derived from these merozoites. These again feed, mature, and undergo a further phase of schizogony. A smaller number of merozoites may be produced and liberated in this second phase of schizogony. Once again these penetrate more cells but now differentiate into either macrogametocytes or microgametocytes. This differentiation is the initial stage of the sexual cycle of *Cryptosporidium* and has been elegantly shown by Goebel and Braendler in the small intestine of mice.[22]

The microgamont produces up to 16 microgametes (2 μm × 0·7 μm), which escape into the intestinal lumen. They contain a large nucleus, a mitochondrion, and a polar ring of microtubules. The authors speculated that this may be equivalent to a flagellum and, presumably, produces the motive force, enabling the microgamete to translocate to the macrogamete. No flagellar filaments, however, have been described that might account for their movement, and it is thought that locomotion is accomplished by a form of flexing or gliding.[17,21] An alternative suggestion is that the ring represents the "conoid" structure found in other coccidians, which

Table 1 *Incidence of Cryptosporidium world wide 1983–4*

Source	*Population*	*No examined*		*No positive %*	
Australia	Hospital patients		884		36 (4·1)
	Children	697		33 (4·7)	
	Adults	187		3 (1·6)	
Bangladesh	Animal attendants and families		320		14 (4·4)
Boston	All patients		1290		33 (2·6)
Costa Rica	Children		278		12 (4·3)
	Rural	95		4 (4·2)	
	Urban	183		8 (4·4)	
Finland	All patients (travellers)		1422		14 (0·98)
Rwanda	Children		193		20 (10·4)
	Adults		100		3 (3·0)
United Kingdom	All		6580		140 (2·1)
	Children	1363		59 (4·3)	
	Adults	1739		35 (2·0)	

Table 2 *Incidence of Cryptosporidium in 1983–4*

Source	*Population*	*No examined*	*No positive %*
Brighton	All patients	800	10 (1·25)
Blackburn	All patients	2174	24 (1·1)
	<15	NS	21
	>15	NS	3
Bristol	All patients	867	43 (5·0)
	<15	394	27 (6·8)
	>15	406	16 (3·9)
	NS	67	
Liverpool	Children in hospital	NS	NS (1·3)
North Yorkshire (rural)	All patients	166	12 (7·2)
North Wales (semirural)	All patients	2573	51 (1·98)
	<15	969	32 (3·3)
	16–40	524	17 (3·2)
	>40	809	2 (0·2)
	NS	211	
Total		6580	140 (2·13)

NS = not stated.

is thought to aid penetration. The initial stages of attachment of the microgametes and macrogametes have been described previously.[17,21,22]

The fusion of these sexual stages produces the zygote. An oocyst (4·5–6·0 μm in diameter) with a thickened wall is derived from this zygote.[23] Four sporozoites are formed inside the oocyst. The sporozoites may be produced while the oocyst is still attached to the intestinal epithelial cells, but our recent observations suggest that the sporozoites may form within the oocyst after detachment from the intestinal lining during passage in the faeces (Fig. 9). In addition, Current and Long suggested that there are both thin walled and thick walled oocysts.[17,23] As the thin walled oocysts (Fig. 10) are infective the infection is spread autogenously throughout the intestinal epithelium. Some confusion may arise because of the similarities between the oocysts of *Cryptosporidium* and the sporocysts of *Sarcocystis*. Iseki regarded the finding of endogenous stages—that is, those attached to the epithelial surface—as an essential tenet for identifying *Cryptosporidium*.[19] If biopsy material is available this may provide a definitive diagnosis. Non-invasive methods of diagnosis, however, are preferable.

Pathology

The first recorded case of human cryptosporidiosis was reported in 1976 in a 3 year old girl from Nashville who had symptoms of vomiting, watery diarrhoea, and abdominal pains.[24] She lived on a farm, had been in excellent health and had developed normally. At rectal biopsy she was found to be infected with *Cryptosporidium*, and she recovered uneventfully with supportive treatment. Follow up investigations on the domestic animals proved negative, and thus the mode of transmission remained unidentified. A review of three cases between 1976 and 1979 focused attention on opportunistic cryptosporidial infections in immunocompromised patients.[25–27] Each patient had presented with fever and chronic watery diarrhoea, ranging from 10 days' to 3 years' duration. In one patient the symptom of dehydration was so severe that during 10 days 60 litres of parenteral fluid were administered, in addition to substantial oral intake.[25] In every case histological examination of jejunal biopsy specimens showed the presence of cryptosporidia either as the sole suspected pathogen[25,27] or in association with *Giardia lamblia*.[26] Moderate to severe abnormalities

Table 3 *Cryptosporidium sp reported to Public Health Laboratory Service Communicable Disease Surveillance Centre June 1983 to June 1984 (Table adapted from figures provided by Communicable Disease Surveillance Centre, Colindale)*

Age (years)	*Sex*			*Diarrhoea*	*Diarrhoea and vomiting*	*Symptoms not stated*
	M	*F*	*Unknown*			
<1	9	10	1	12	5	3
1–4	50	38	4	56	24	12
5–9	15	20	2	20	10	7
10–14	8	2	1	10		1
Child		1	10	2	3	6
15–64	32	31	9	44	12	16
≥65	1	1			2	
Not known	2	4	23	6	3	20
Total	117	107	50	150	59	65

Table 4 *Symptoms described in 23 published cases of cryptosporidiosis in immunologically normal subjects*

Symptom	*No of cases*
Diarrhoea	19
Cramps	12
Fever	9
Malaise	8
Nausea	8
Vomiting	4
Anorexia	3
Constipation	3
Asymptomatic	2
Headache	2

of villous architecture were reported with mild chronic inflammation of the lamina propria and slightly increased numbers of plasma cells, polymorphonuclear leucocytes, and lymphocytes. Two of these patients had no known unusual exposure to animals or house pets,[26 27] although the third patient[25] lived on a farm in a rural community. This patient, who had a history of ulcerative colitis and severe bullous pemphigoids, recovered uneventfully two weeks after immunosuppressive treatment was stopped.

Between 1980 and 1983 more than 80 cases of cryptosporidial gastroenteritis in man were reported either as a self limiting gastroenteritis in normal patients[24] or as the severe symptoms produced in immunocompromised patients, especially those with the acquired immunodeficiency syndrome (AIDS).[28]

THE DISEASE IN IMMUNOCOMPETENT SUBJECTS

A total of 159 cases have been reported as separate case studies,[24 29-36] outbreaks [9] (unpublished observations, DP Casemore *et al*), and surveys.[29 37-42] Selected reports[24 29-37 40] identified an age range from 2 months to 60 years with peak incidences during early childhood (less than 1 to 10 years) and between 21 and 30 years. Attention was first drawn by Casemore and Jackson to the incidence of sporadic cryptosporidiosis in children.[37] Other observations agree with this, suggesting that the incidence of cryptosporidiosis may peak in childhood (Tables 1, 2, and 3). Slightly more men than women have been infected.

The outstanding clinical features of cryptosporidiosis are: a flu like illness with watery diarrhoea; cramps; fever; malaise; and nausea (Table 4). Diarrhoea is often characterised by two to 10 watery stools a day, often beginning on the first or second day of illness.

Our experiences generally agree with the findings in Table 4, although anorexia and vomiting were noticed more often and, in a few cases, vomiting was the predominant or initial symptom. The diarrhoea, which was usually foul smelling, was accompanied by considerable weight loss (10% of body weight) and prostration in some cases. The abdominal pain tended to occur in the upper right quadrant. The incubation period usually lasted between five days and two weeks after initial contact with the organism,[29 30 32 36] inducing symptoms that could last for five to 14 days.[9 24 31-34] To date, no figures have been reported on the size of the infective dose, but it is thought to be low.[30 42] Verification of the size of the infective dose may indicate that the organism is efficient at causing the symptoms of disease and may continue to be excreted by patients for up to two weeks after their diarrhoea has resolved.[30 42] This continued infectivity makes such patients a potential hazard to contacts, including hospital staff.

THE DISEASE IN IMMUNODEFICIENT SUBJECTS

In contrast to the short term flu like gastrointestinal illness in immunocompetent patients, *Cryptosporidium* may cause severe protracted diarrhoea in immunodeficient patients. Most of the patients in the 71 published records of immunocompromised patients had AIDS[9 13 43-55] but others had hypogammaglobulinaemia[9 26 55 56] or were receiving immunosuppressive treatment.[25 27 57 58] The age distribution in these reports was influenced by the large number of patients with AIDS, resulting in a peak incidence between 31 and 40 years. This was reflected in a report on 21 patients with AIDS and *Cryptosporidium* in whom the age range was 23–62 years (mean 35·7 years).[51] Interestingly, of seven patients who did not have AIDS, five were aged under 20 years and three under 10 years.

Transmission of cryptosporidium from animals to man was not confirmed in any of the 71 cases investigated, although two immunosuppressed patients[25 57] and one patient with AIDS[45] had contact with farm animals. Contact with domestic pets was reported in three cases,[44 45 58] but follow up investigations were not made. In contrast to healthy

Table 5 *Symptoms described in 43 immunocompromised patients*

Symptoms	*No of cases among those with AIDS (n = 36) others (n = 7)*
Diarrhoea	35/7
Fever	25/3
Weight loss	23/1
Abdominal pain	12/3
Vomiting	9/2
Nausea	6/0
Anorexia	5/1
Headache	3/0
Malaise	2/0
Constipation	1/0

Table 6 *Organisms associated with Cryptosporidium in 57 immunocompromised patients*

Organism	*No of cases among those with AIDS (n = 50) others (n = 7)*
Candida	25/0
Pneumocystis carinii	19/0
Cytomegalovirus	15/0
Mycoplasma avium-intracellulare	7/0
Giardia	4/1
Entamoeba histolytica	4/0
Shigella	3/0
Herpes simplex virus	3/0
Neisseria gonorrhoeae	2/0
Histoplasma	1/0
Staphylococcus aureus	1/0
Adenovirus	1/0
Toxoplasma	1/0
Salmomella	1/0
Pseudomonas	1/0
Isospora belli	1/0

subjects who had cryptosporidial infections, immunodeficient patients inhabited urban rather than rural environments. Consequently, person to person transmission may be an important factor in the spread of cryptosporidiosis, particularly in homosexuals.[59] Among these subjects it may form part of the so called "gay bowel syndrome" of gastrointestinal infection acquired through homosexual practices.

Table 5 summarises the symptoms of 43 immunocompromised patients. All but one patient had severe protracted watery diarrhoea, either intermittently or continuously, for between seven days and six years. The mean duration of diarrhoea for patients with AIDS was 20·6 weeks (range one to 78 weeks), with a fluid loss of between 1 and 12 litres a day. Four of the seven patients who did not have AIDS complained of diarrhoea for between three and seven weeks, and three patients suffered episodes of diarrhoea lasting for three to six years. Fever, substantial weight loss (up to 50% of initial weight), and abdominal pain were often reported.

DIAGNOSIS IN IMMUNOCOMPROMISED PATIENTS

Cryptosporidium infection in immunocompromised patients was usually diagnosed by histological examination of biopsy material. Small bowel biopsy specimens were used most often, although in seven of 30 cases biopsy together with faecal examination was preferred. Necropsy studies showed that the proximal jejunum was the most heavily infected region of the gastrointestinal tract, but infection may extend from the pharynx to the rectum.[27,56,60] In addition, cryptosporidia were also observed in lung biopsy specimens[13-15]; they may have spread to the respiratory tract either haematogenously[52] or more probably as a result of aspiration.[13] Histologically, the organisms may be easily overlooked (G Slavin, personal communication). In one case the organisms were found only after examination of six jejunal biopsies.[56] Histological changes are described as generally mild, consisting of slight blunting and distortion of the villi, lengthening of the crypts, and a modest mononuclear cell infiltration of the lamina propria.[25,27,52,56]

Patients with AIDS have been coinfected with a bewildering array of micro-organisms in stark contrast to those without AIDS and immunocompetent patients (Table 6).[9,13,28,43,45-54,61] Correlation between *Cryptosporidium* and AIDS has led to the proposal that it should be included as an important factor in the differential diagnosis.[9] Of 57 patients

Table 7 *Results of treating 60 immunodeficient patients suffering from cryptosporidiosis*

Treatment (n)	*No improvement*	*Improvement*	*Recovery*
Amphotericin (4)	4	0	0
Amprolium (2)	1	1	0
Bovine transmission factor (3)	3	0	0
Chloroquine (1)	1	0	0
Clindamycin (2)	2	0	0
Co-trimoxazole (21)	21	0	0
Diloxanide (5)	4	0	1
Furazolidone (15)	13	1	1
γ Globulin (2)	2	0	0
Iodoquinol (2)	2	0	0
Ketoconazole (5)	5	0	0
Metronidazole (10)	9	1	0
Paromomycin (2)	2	0	0
Pentamidine (5)	4	1	0
Primaquine (1)	1	0	0
Pyrimethamine (8)	7	1	0
Quinacrine (7)	7	0	0
Salinamycin (1)	1	0	0
Spiramycin (16)	8	4	4
Tetracycline (6)	4	2	0
Total (n = 118)	101	11	6

Table 8 *Chemotherapeutic agents evaluated prophylactically in calves, mice, or pigs listed alphabetically*

Amprolium	Nicarbazin
Bleomycin	Oxytetracycline
Difluoromethylornithine	Pentamidine
Diloxanide furoate	Phenamidine
Dimetridazole	Quinacrine
Ethopabate	Salinomycin
Furaltadone	Sulfadiazine
Halofuginone	Sulfadimidine
Ipronidazole	Sulfamethazine
Lasalocid	Sulfaquinoxaline
Metronidazole	Trimethoprim
Monensin	

with AIDS and cryptosporidiosis, 42 died, and the parasite was rarely eradicated.

Although parenteral nutrition has been used to sustain patients with hypogammaglobulinaemia, this procedure has not been successful in managing other immunocompromised patients. The only successful intervention occurred in subjects receiving immunosuppressive treatment, when the underlying immune deficiency could be reversed by stopping treatment.[25 58]

Immunosuppression occurs naturally as a direct result of measles infection. Cryptosporidiosis may occur in patients with measles,[62] and local experience with more than one such case suggests that the severity of symptoms is increased, more akin to the illness seen in patients with AIDS, although with spontaneous resolution. The first such patient, a 2 year old child, had as many as 19 bowel movements in one day at the peak of the illness. The serological response was poor. (DPC unpublished data).

Effective treatment for cryptosporidiosis, especially in cases of AIDS, has yet to be identified despite the large number of chemotherapeutic agents that have been tried (Table 7). Preliminary reports, however, suggest that a few patients may have responded to treatment with spiramycin given orally (1g three or four times a day).[44 63 64] Lack of a suitable laboratory model for assessing the efficiency of drugs in chronic infection has been a major limitation. Drug evaluation studies have therefore been confined to the prevention of infection in calves, mice, and pigs. Investigators in Australia and in the United States have concentrated on drugs known to be effective against coccidian parasites in animals or in treating protozoan parasites in man.[7 65-67] None of the drugs has been effective (Table 8).

Pathogenic mechanisms

Infection is initiated by the organism forming a stable attachment to the surface of the intestinal mucosa. Unlike many bacterial enteropathogens, this process is probably not mediated by extracellular organelles (colonisation factors) but may proceed by cell to cell recognition at the surface membrane. Electron microscopic studies on the ilea of infected calves showed thin, irregular, ruthenium red staining filaments extending from the parasite glycocalyx to the host cell glycocalyx.[68] Such a union of electronegatively charged surfaces may be facilitated by the "bridging" action of divalent cations or, more probably, by sugar-sugar binding proteins (lectins).[69] Adherence of *Entamoeba histolytica* trophozoites has previously been reported to be mediated by such a mechanism.[70] Possibly, similar proteins may be essential for cryptosporidia to colonise the mucosal surface.

The attachment phase is followed by penetration into the epithelial cell. These initial host parasite relations probably do much to change the appearance of the villi. Heavy infection with *Cryptosporidium* produces depressions or craters within the mucosal surface, strikingly illustrated by Snodgrass *et al* by scanning electron microscopy.[71] Several investigators described gross changes in the architecture of villi affected by parasites.[25-27 52 56 72 73] Stunting and fusion of villi were most commonly reported, together with damage to, and degeneration of, enterocytes.[73] This, coupled with the location of cryptosporidia predominantly within the posterior small intestine, probably comprise the important enteropathogenic factors.

The relations between the main clinical symptoms of disease and the pathophysiological events described are likely to be multifactorial. A heavy proliferation of *Cryptosporidium*, particularly in the immunocompromised host, may lead to impaired digestion, malabsorption, and profuse watery diarrhoea. The posterior small intestine is known to be particularly efficient at net fluid absorption.[74] Large numbers of cryptosporidia adherent to villi are likely to disturb normal villous function. Such a mechanism has previously been suggested for the symptoms of giardiasis.[75] Interestingly, however, lesions of similar severity in the anterior small intestine caused by rotavirus result in only mild diarrhoea.[76] Consequently, the distribution of cryptosporidia within the intestine may be crucial in producing the symptoms of disease.

Watery diarrhoea is the major symptomatic expression of cryptosporidiosis and indicates an appreciable increase in lumenal water content. This may reflect

Table 9 *Incidence of Cryptosporidium by age 1983–4*

Source	*No examined*	*No positive (%)*
World wide	11067	272 (2·46)
Children	2531	124 (4·9)
Adults	2026	41 (2·0)
United Kingdom only	6580	140 (2·13)
Children	1363	59 (4·33)
Adults	1739	35 (2·01)

either a deficiency in the flow of water and nutrients from the lumen to plasma compartments (absorption) or a net accumulation of fluid in which water and electrolytes enter the lumen from the plasma (secretion), or both. Secretory diarrhoea is usually associated with bacteria capable of elaborating an enterotoxin. Such a mechanism, however, has not been discovered for *Cryptosporidium*. In our experience the inoculation of faeces heavily infected with cryptosporidia into Vero and other cells sensitive to toxins does not produce a cytotoxic effect. Jervis *et al* suggested that coccidial enteritis produced in guinea pigs may be caused by the response of the mucosa.[77] The discharge of toxic metabolites directly from the parasite to the infected enterocyte, coupled with the widespread reaction of the lamina propria, may provide a pathogenic mechanism. Cryptosporidial infection in guinea pigs, however, seemed to have little outward effect. Most probably a reduction in the mucosal surface and a decrease of many mucosal enzymes may be responsible for lowering the absorptive capacity of the small intestine, producing an osmotic diarrhoea.[78]

The intestinal mucosa acts as a semipermeable membrane, and absorption of water occurs as a passive response to physical forces, the most important of which is that derived from osmotic pressure. Pressure gradients are established by absorption of solutes, and water follows across the membrane to maintain osmotic equilibrium. Malabsorption of any water soluble nutrient will result in the retention of water within the lumen, triggering an osmotic diarrhoea. This is clearly the response in lactase deficiency. The disaccharide remains in the lumen with the attendant water until it reaches the colon. The lactose is then split by colonic bacteria, increasing the number of solutes, which in turn draws more water into the lumen. Many enteric infections are known to induce malabsorption of carbohydrates; including viral enteritis,[79] cholera,[80] shigellosis,[81] *Isospora belli*,[82] and giardiasis.[83 84] Tzipori reported that cryptosporidial infections had a pronounced effect on the activity of membrane bound lactase and suggested that there was a strong correlation between the degree of infection of the mucosa, the extent of mucosal changes, and the severity of the clinical illness.[7] Similarly, malabsorption of fat and low serum carotene concentrations were also detected in several patients with cryptosporidiosis.[56 85]

Malabsorption induced by infection may increase the intestinal water load, causing fermentative osmotic diarrhoea (LA Turnberg, personal communication). It has been estimated that if 5% of the average carbohydrate content of the American diet reached the colon a load of 100 mmol(mOsm) capable of supporting 300 cm^3 of water in the intestine would be produced. Bacterial fermentation of these sugars to fatty acids would stimulate an additional sixfold increase to 18 litres of water.[86 87] Several workers reported that acid stools, containing large quantities of reducing sugars, characteristic in such conditions.[88-91] Possibly, therefore, the offensive smelling stools which are often reported in cases of cryptosporidiosis may result from the bacterial fermentation of unabsorbed nutrients. It is our experience that most patients when questioned complained of rumbling sensations in the bowel and extremely offensive fluid stools.

Vomiting may exacerbate the loss of fluid produced by diarrhoea. In some patients this may be the predominant symptom with little or no diarrhoea. The mechanism for this emetic effect is unclear, but cryptosporidial endogenous forms have been detected in the stomach.[28 44 92] We found cryptosporidia in the vomit of one of our patients.

The severity of cryptosporidiosis in immunocompromised patients may indicate that functional cellular and humoral immunities are necessary to resolve infection. Although systemic antibodies would not be thought to play a part in this because of the superficial position of the organisms on the mucosa, circulating antibodies to *Cryptosporidium* have been detected,[12 93 94] and these were confirmed by our own findings. The presence of humoral antibodies against other enteric pathogens, occupying a similar site, clearly shows that the response to *Cryptosporidium* is not unique. It is known, for example, that both systemic and local antibodies are produced against *Giardia lamblia*,[95] *Escherichia coli*,[96] and *Vibrio cholerae*.[97] Campbell and Current concluded that infection with *Cryptosporidium* may not result in protective immunity but may reduce the severity of subsequent infections.[93] The actual role of circulating antibodies to *Cryptosporidium* is uncertain, however, and the presence of locally produced antibodies remains to be determined. Finally, the common occurrence of the parasite in newborn domestic animals suggests that infection and perhaps disease may occur in infants before the development of resistance[98] when passive immunity might be expected to be present. Studies on animals indicated that colostral antibodies fail to exert a protective effect.[12]

Epidemiological considerations

The ubiquitous nature of the parasite in man, wildlife, farm livestock, and pets and its apparent ability to cross host species barriers implies that the reservoir of infection to man is large. The oocysts are inherently stable in excreta and resistant to chemical agents[99 100]: oocysts have been found in the environ-

ment.[101] These factors would imply that various routes of transmission are possible. Person to person spread has also been reported.[7 30 36 64 101]

There are a few reports of cryptosporidiosis in normal subjects associated with other intestinal pathogens, especially *Campylobacter*[101] and *Giardia*.[38 39] The findings of Jokipii *et al* suggested that their cases largely represented a form of travellers' diarrhoea.[39] Our own findings included one subject who seemed to have acquired mixed *Cryptosporidium* and *Giardia* infection in Leningrad. In our experience, however, the most common organism, to occur with *Cryptosporidium* is *Campylobacter*. A few cases reported to the Communicable Disease Surveillance Centre (CDSC) have been in people who have returned from abroad, and in some of these cases there seems to have been an association with the consumption of raw milk. Two recent reports highlighted *Cryptosporidium* as a cause of travellers' diarrhoea.[102 103]

Water, raw milk, and foods have all been proposed as sources of infection, although this is difficult to substantiate directly in the absence of an enrichment culture system. Concentration methods such as filtration, Moore's swabs, and formol-ether centrifugation have been successfully used in environmental studies in one laboratory by (DPC)[101 120] but have so far failed to yield positive results with milk, piped waters, and food. The need for such environmental studies has already been proposed.[98]

A clear association between bovine and human cryptosporidial infections was established in 26 subjects who had direct contact with the faeces of infected calves[9 29 32 34]; one patient was a veterinary student in charge of calves used in transmission experiments with bovine cryptosporidium isolates.[32] Interestingly, the case reported by Nime *et al* may also have resulted from bovine transmission because the child was brought up on a cattle rearing farm.[24] Two other cases of possible transmission from animals, though not calves, were also reported: one patient was a 13 month old boy who was believed to have become infected from a pet cat which was later shown to be positive for *Cryptosporidium*[42]; and the other was a professional athlete who had worked in horse barns.[35] Koch *et al* also reported an association with domestic cats.[55] Possible sources of infection were not considered in 66 of an additional 130 patients.[9 31 33-38 41] Of the remaining 64 patients, 14 had acquired their infection while abroad,[39] 26 may have been associated with rural rather than urban communities and 22 may[101] (DP Casemore, unpublished observation) have been associated with urban rather than rural communities.[37] One case was an accidental infection in a research worker,[36] and one was reported as the first case of hospital cross infection.[30] More recent reports also highlighted urban infections without evidence of zoonotic transmission.[104-106] This evidence supports the proposal by Casemore and Jackson that cryptosporidiosis need not be viewed as a zoonosis.[107]

Overall, *Cryptosporidium* seems to occur in about 2% of specimens examined both abroad (Table 1) and in the United Kingdom (Table 2), although rates vary geographically and temporally. Some seasonal variation has been suggested although further data are required to verify this. The observation that infection among immunologically competent subjects occurs more commonly in children has been confirmed[37] (Table 9). Outbreaks in children attending day nurseries have been reported[105 106] (DP Casemore, unpublished data). The incidence of positive findings in relation to other recognised pathogens in man has not been widely reported, but studies in the United Kingdom[106] together with our own experience[120] suggest that *Cryptosporidium* may be found more often than many "common" pathogens.

Most positive findings reported are from symptomatic subjects. The first report to the CDSC was received in June 1983 from a case diagnosed at Rhyl Public Health Laboratory. It was found as a result of a prospective survey to determine the prevalence of *Cryptosporidium* in a semirural population. Results reported to the CDSC for 1983–4 (Table 3) include a proportion from subjects who were recorded as asymptomatic. This may reflect an absence of information rather than symptoms. It is our experience that such subjects, often contacts of cases, who are initially reported as asymptomatic are found on personal inquiry to have had mild symptoms. Occasionally, patients may have vomiting and abdominal pain without diarrhoea. Extended periods of excretion were also reported in those recovering from infection.[42] These mild cases and convalescent excreters may provide an important reservoir of infection in the community.

Laboratory methods

The methods now applied to detecting *Cryptosporidium* are all essentially conventional, or are modifications of conventional methods. Tyzzer tested various stains, including Romanowski stains, on histological sections and on smears of epithelial mucosa from the guts of experimental animals. Subsequent reports including electron microscopy first reported in 1966[108] established morphological details and an appreciation of the host parasite interaction. Oocysts in faeces were first detected and reported in 1978 by Pohlenz *et al*, who worked on calf material stained by Giemsa and opened the way for diagnosis by non-invasive means,[109] despite the view of Iseki.[19] This method was used by Tzipori on human faeces in

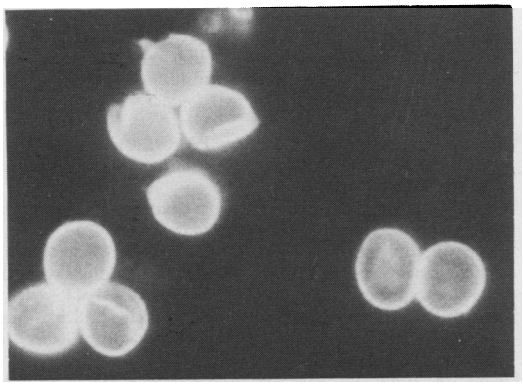

Fig. 11 *Immunofluorescence of purified cryptosporidial oocysts extracted from faeces.* × *2000.*

1980,[31] although a report in the same year suggested that oocysts were not present in stools.[73] The appearance of oocysts in preparations stained by the Romanowski method varies according to the particular stain and pH used. We found that consecutive staining with Jenner and Giesma stains and washing at pH 6·8 gave results that were most useful as an additional stain in equivocal cases.[120] The oocyst stained blue to azure with a crescentic, more deeply staining body and five or six eosinophilic granules: often a clear halo surrounded the cyst.

The use of cold Ziehl-Neelsen stain was introduced in 1981 by Henrikson and Pohlenz,[110] who again worked on calf material, and this was rapidly adopted by workers in human medicine.[111 112 116] With this method the internal structures, sporozoites, and residual body are stained red: empty oocysts and zygote cases remain unstained but are readily recognised with practice. The use of strong carbol-fuchsine as a negative stain has been described[113] but not generally adopted. Auramine-phenol has been recommended as an alternative to the Ziehl-Neelsen stain[114 115] and probably stains the outer cases as well as internal structures.[120] The auramine-phenol method seems, therefore, to be more sensitive than the Ziehl-Neelsen stain. Carbol-fuchsine negative staining combined with auramine-phenol was used in a simple rapid screening technique and has proved extremely useful in examining large numbers of specimens, including environmental material.[101 116 120] Safranin staining followed by counter staining with methylene blue is claimed to give more sensitive results.[117] Our own findings and those of others reported to us suggest that although the stain works admirably in some cases, failures occur owing to factors that are as yet unclear. Interestingly, although increased sensitivity is claimed, the incidence (1·3%) in a selected paediatric population was much lower than that reported in children elsewhere (4%) (Table 9).

In an effort to increase sensitivity concentration methods have been widely reported, especially by veterinary and American workers. The methods most commonly used are those of Sheather,[118] using concentrated sucrose and Ritchie's formol-ether method.[119] In our experience concentration is rarely required in acute cases, but it may have a role in the examination of specimens from contacts and late or follow up specimens. It is also of value for showing cryptosporidial contamination of environmental samples.[10] Contrary to some American findings,[111 112] detailed analysis here has shown that the formol-ether method, when suitably modified, is considerably more sensitive than Sheather's method. Sucrose also interferes with various staining methods and with the attachment of the material to the slide.[120]

Most light microscopy methods used for diagnosing cryptosporidiosis are not specific and can lead to misidentification. The desirability of a specific test is therefore obvious. The fluorescence antibody test or other similar methods should provide the necessary specificity. Although commercial reagents are not yet available, experimental evidence from veterinary workers (KW Angus, personal communication) and from our laboratory shows that this method does produce improved diagnostic specificity (Fig. 11).[120]

Availability of suitably sectioned biopsy material for examination in the electron microscope can produce a definitive diagnosis.[19] Other workers have used the negative staining method directly with faecal material.[121] The sensitivity of this method is necessarily very low and in the absence of specific morphological features it is of limited diagnostic value.

Proof of an immune response is required both clinically and immunologically. Studies on antibodies have been reported by Tzipori,[94] Current,[12] and Campbell and Current.[93] Similar studies in our laboratory showed that there was a response to infection, primarily rising titres of IgA and IgM. When serial specimens were examined the expected rise in IgG titres failed to occur the IgG titres remained low. Low titres of IgA and IgM were also present in some control subjects. This contrasts with the findings of Current[12] that, almost without exception, the response is IgG.

Conclusion

The role of *Cryptosporidium* in human disease is now established[122] Interest in human cryptosporidiosis has stemmed mainly from two sources: firstly, the cooperation and sharing of information between veterinary and human health care workers, and, secondly, from the concern related to opportunistic infections in immunocompromised patients,

especially those with AIDS. In view of the impending increase in the incidence of AIDS and the lack of chemotherapeutic substances effective against *Cryptosporidium* it is essential that work on this parasite should continue. The incidence in immunologically normal subjects makes it imperative to pursue epidemiological and environmental studies.

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