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SPECIAL ARTICLE

Epidemiological aspects of human cryptosporidiosis

INTRODUCTION

The coccidian protozoan parasite *Cryptosporidium* has been described in many host species since its discovery in the early part of the century, but it remained obscure until the recognition by veterinary workers in the 1970s of its importance as a cause of scours in young livestock animals [1–4]. Subsequently, particularly as a result of collaborative studies involving both medical and veterinary workers, cryptosporidiosis was also recognized in man [1, 4–7]. Many of the early reports of the infection in humans were in immunocompromised subjects, either immunodeficient subjects, particularly those suffering from the then newly emerging disease, AIDS, and in the immunosuppressed. Such early cases were often diagnosed histologically, sometimes as a chance finding. Diagnosis by means of detection of oocysts in animal faecal smears had been demonstrated by veterinary workers in the 1970s [1, 3] and subsequently also in humans [5–8]. However, the presence of oocysts in faeces, and the significance of the infection, continued to be in doubt [9, 10]. Some cases, particularly among the immunocompetent, appeared to have an association with animals, particularly cattle, but even when such a source was not apparent the infection was generally referred to as an emerging opportunist zoonosis [11, 12]. At that time, little if anything was known of the epidemiology of the infection in the general population. The recognition of an apparently opportunist zoonosis occurring particularly in young calves and in AIDS patients who were primarily urban adult males seemed, however, to be epidemiologically inconsistent. A prospective study was therefore set up at the Rhyl Public Health Laboratory in early 1983 to look for the parasite in a mixed urban and rural population including representatives of all age groups [13]. Such preliminary prospective surveys, from north Wales and elsewhere, indicated the importance of the parasite as a cause of acute, sporadic gastroenteritis in otherwise healthy subjects, particularly children, in developed countries [14, 15], in under-developed countries [16, 17], and in travellers, most of whom were adults [18, 19].

Increasing awareness of the parasite, and that it could be identified by means of detection of oocysts in faeces using variations of conventional microbiological staining methods [20–24], led the way to widespread investigation and reporting. *Cryptosporidium* is now widely recognized as a cause of acute, self-limiting, infective gastroenteritis in otherwise normal human subjects and of potentially fatal infection in the immunocompromised. However, examination of presumptive positive samples referred to the Rhyl Public Health Laboratory from various parts of the world indicate that some over-reporting undoubtedly occurs as a result of failure to discriminate between oocysts and the various oocyst-like bodies

(fungal spores, etc.) which are not oocysts (see below). Conversely, dependence on microscopical detection of individual oocysts may result in some under-reporting. Few of the early reports included detailed descriptions of epidemiological field studies. The emergence of evidence of outbreaks indicated the need for control measures based on an understanding of the natural history of the infection using methods established for other gastrointestinal infections [25]. There was, therefore, clearly a need for more detailed epidemiological investigation of the infection, particularly in immunocompetent hosts [26]. Such studies were initiated in the UK, including north Wales.

In many laboratories worldwide, in both developed and developing countries, *Cryptosporidium* is now among the most commonly reported enteric pathogens. Varying patterns of incidence, by age, season, and geographical location, and of routes of transmission, have now emerged and have been reviewed [4, 27–34]. Cryptosporidiosis continues to represent a serious threat to AIDS sufferers and others who are immunocompromised. Although there are some differences in the epidemiology of the infection between immunocompetent and immunocompromised subjects, e.g. in age distribution and in the severity and duration of symptoms, in general the epidemiology differs little except where indicated below.

BIOLOGY AND CLASSIFICATION

A knowledge of the biology of the parasite is essential to an understanding of the epidemiology of human cryptosporidiosis. Much of the earlier literature on *Cryptosporidium* describing its biology and classification is confused and contains inaccuracies in addition to areas of uncertainty. *Cryptosporidium* is a coccidian protozoan which, however, differs from other coccidia in several important respects. *Cryptosporidium* is so named because it appears to lack the sporocyst structure found in other coccidia. It is an obligate parasite with a complex life-cycle, which it completes without the need for development in a secondary host; i.e. it is monoxenous [7, 28–31] (Figs 1, 2). Infection is initiated following ingestion of an environmentally resistant oocyst containing four, naked, motile sporozoites. Development is through several characteristic stages: excystment, merogeny (asexual), gamogony (sexual), zygote and oocyst formation, and sporulation. The fixed endogenous (tissue) stages have a pseudo-external location and are intracellular but extracytoplasmic. They are found primarily in the brush-border of the apical enterocytes of the small bowel but may develop on any epithelial surface and have been reported in a number of different organs and tissues [32]. The non-flagellate motile forms (zoites) are released into the lumen and are initially actively invasive. Once they have entered a cell they develop as a fixed (endogenous) stage within a parasitophorous vacuole thought to be formed from the host cell outer membrane. Recycling of asexual stages, and the presence of thin-walled oocysts which result in endogenous reinfection (autoinfection), permit very heavy infection to develop [31, 34]. The infection is probably limited by a combination of humoral and cellular immune mechanisms. The oocyst stage does not require a period of external ripening. The various developmental stages have been elegantly illustrated by Current and his co-workers [31, 35, 36].

Cryptosporidium is an ubiquitous parasite described in a large number of host species [28, 31]. Early reports tended to assign species status to isolates from each

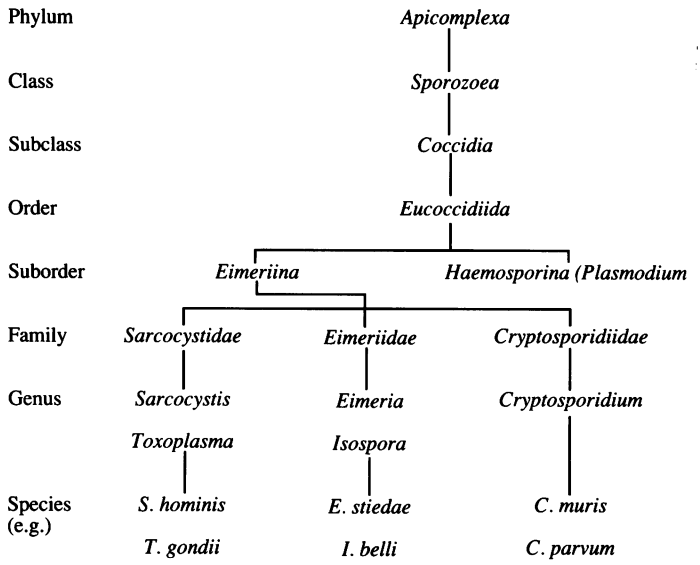

Fig. 1. Taxonomic position of *Cryptosporidium* simplified to show relationships with other medically important species. (Reproduced with permission from Casemore, Sands and Curry [7].)

newly reported host species in accordance with the practice for other coccidia which are generally both host- and tissue-specific. Some of these identifications were based on inadequate information and were incorrect. Subsequently, evidence was adduced to suggest the possibility of a single species genus [4, 37]. However, examination of the original findings of Tyzzer [38–40], disputed by some [4] but largely confirmed by others, reveal that *C. muris* and *C. parvum* are distinct species, both found in mice [7, 31, 34–36, 41–43]. *C. muris* appears to be restricted to the glands of the stomach. It has subsequently also been found to occur in cattle in which it is found in the abomasum and oocysts may be detected in the faeces [35, 44]. Anderson in the USA [44], has reported finding the infection to be present in many herds of cattle, in which infection appears to be asymptomatic, but it has not yet been reported in the UK (K. Angus, personal communication). *C. parvum* was so described because of its smaller size. Tyzzer predicted that it might be capable of infecting more than one host species. The majority of infections in man and most livestock animals are probably with *C. parvum* [7, 31, 34, 35]. There is insufficient data yet to confirm whether isolates can be sub-divided into host adapted strains or antigenic or other sub-types although there is some evidence to support this possibility (see below). Some antigenic variation appears to exist which may reflect the host species from which the isolate was derived [43, 45, 46]. Until more definitive information is available it is probably safer to refer to isolates from humans as *Cryptosporidium* species.

Cryptosporidia are known to occur in wild rodents which may act as a natural reservoir for livestock animals [41–43]. Although *in vivo* laboratory studies require the use of suckling mice, infection and excretion of oocysts appears to occur in adult wild mice. There are at least two avian species which, fortunately in public health terms, appear to be poorly transmissible, if at all, to man and other mammals [31, 47–49]. Cryptosporidia have occasionally been reported in fish and

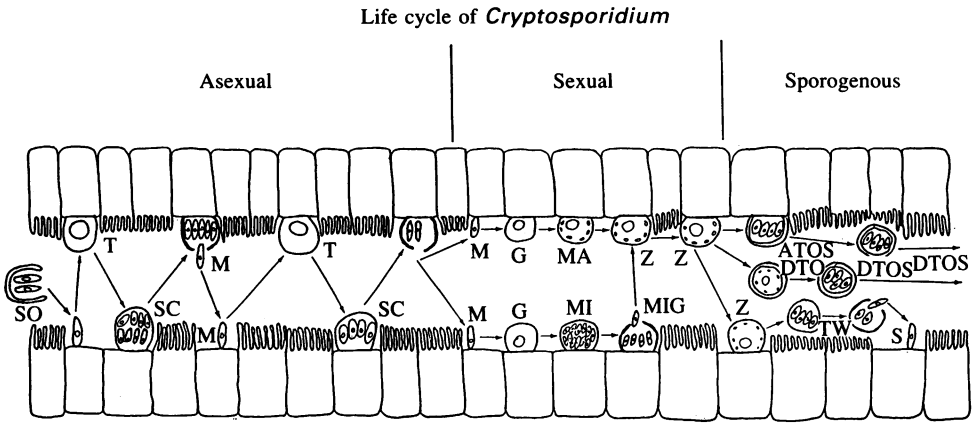

Fig. 2. Diagramatic representation of the life cycle of *Cryptosporidium* sp. Infection starts with ingestion of an oocyst containing four naked, motile, sporozoites (SO). Sporozoites (S) are released, attach to an epithelial cell and take up a pseudo-external position. They develop into a trophozoite (T) or uninucleate meront which, as it matures, undergoes schizogony (asexual multiple budding) to produce a schizont (SC). There are probably two asexual generations which undergo three and then two nuclear divisions, producing eight or four motile merozoites respectively (M). Merozoites of the first generation invade other cells to form new meronts. The second generation produce gamonts (G) of the sexual cycle. The latter may mature as either microgamonts (MI) or macrogametes (MA). Microgamonts produce 16 motile microgametes (MIG) which, on release, fertilize the macrogamete to produce a zygote (Z). The zygote may follow several sporogenous developmental routes: it may transform into a thick-walled oocyst in-situ (ATOS) which then sporulates before becoming detached from the host cell (DTOS), or be shed and subsequently sporulate (DTO); the zygote may develop into a thin-walled oocyst (TW) which releases its sporozoites while in-situ, thus spreading infection within the host. Microvilli have been omitted from infected cells for clarity. (Reproduced with permission from Casemore, Sands and Curry [7].)

reptiles but are poorly characterized and in some cases erroneously identified as such [31, 34, 49, 50].

The biological and other features which determine the medical and epidemiological importance of the parasite are summarized in Table 1.

DISTRIBUTION OF HUMAN CRYPTOSPORIDIOSIS

Age and sex distribution

A variety of factors may bias laboratory study findings, particularly towards children. Specimens submitted to the laboratory are usually from selected populations and even when derived from general population groups are subject to a variety of ill-determined biases. Despite such biases, the use of laboratory based data is of value although care is needed in interpretation [25, 51, 52].

Early reports of cryptosporidiosis in humans were often of infection in adults, reflecting the high proportion of immunocompromised subjects among such cases [7]. Attention was drawn in 1983 to sporadic infection in the community, especially in otherwise normal children [13–15]. Several subsequent studies have confirmed a peak incidence in children aged 1–5 years in most areas. A secondary

Table 1. *Biological/epidemiological characteristics of* Cryptosporidium parvum *associated with transmissibility to man*

Lack of species specificity (host-species cross-transmissibility) (also lacks tissue specificity)
Ubiquitous (Man, food and companion animals – ?not avian species)
Monoxenous (Full development in single host species)
Oocyst excreted fully sporulated (no external 'ripening' required)
Oocysts excreted in large numbers (?enhanced by autoinfective cycle)
Probable low infective dose-size (? < = 10–100 oocysts)
Oocysts environmentally resistant
Oocysts resistant to common disinfectants
Faecal–oral mode of infection (Plus? others – aerosol, vomit, etc.)
Person-to-person transmission (especially young children)

peak in laboratory proven incidence may also be seen in adults aged 20–40 years and which may result from family contact with children, or occupational exposure [53, 54]. Detailed examination of laboratory data from a national PHLS surveillance study [55] (Tables 2, 3) shows that approximately 60% of positive findings are from children and 30% from adults aged less than 45 years. Hence, the role of *Cryptosporidium* in diarrhoeal disease in young adults should not be under-estimated. Cryptosporidiosis in susceptible adults may be particularly unpleasant and may occasionally lead to severe disease and even death in some cases [32] (D. Casemore, unpublished observations). In our experience clinical infection is uncommon over the age of 40 although it occurs from time to time. Asymptomatic infection may be found in adults who are close family contacts of cases. There is no evidence of increased incidence in the elderly. Infection in some Scandinavian countries appears to occur mainly in adults, most of whom acquire their infection abroad, especially Russia, or as a result of occupational exposure [18, 54, 56–58]. The reason for the preponderance of adult cases in Scandinavian countries and the relative infrequency of positive findings in children does not yet appear to have been adequately explained. Travellers would be predominantly adult: the low incidence in children may imply that autochthonous infection in Scandinavia is uncommon except in rural subjects in whom the infection is, apparently, often asymptomatic [58].

In the large 2-year PHLS UK survey [55] (Table 2) and locally in north Wales over more than 5 years [43, 53], infection was most common in the 1–5 years age group. These surveys included patients of all ages and examination of denominator data indicates that the peak is not the result of age sampling bias. Infection was less common in children aged under 1 year and occurred infrequently under 6 months of age. This pattern is similar to that reported by some others, in the UK [59], the USA [60], in rural Costa Rica [61], Liberia [62], Rwanda [17], Guinea-Bissau [63] and in Haiti [64]. However, in some other surveys in the UK [15, 65, 66], in Eire [67], and in some Third World countries [68–70], infection has been found to occur commonly in children aged less than a year. The reasons for these differences are not yet clear but may relate to levels of maternal immunity and of exposure [43, 45]. In rural settings in north Wales, clinical cryptosporidiosis in

Table 2. *Cryptosporidium-positive stools – age distribution symptomatic patients in PHLS (CDSC- UK Survey* 1985–87*

Age (years)	GP samples (*n*)	Positive (%)	Hospital (*n*)	Positive (%)
< 1	2057	31 (1·5)	3537	24 (0·7)
1–5	4520	183 (4·0)	1972	59 (3·0)
5–15	2160	88 (4·0)	1306	28 (2·1)
15–25	3491	70 (2·0)	1778	19 (1·0)
25–35	4079	75 (1·8)	1011	13 (1·3)
35–45	3049	35 (1·1)	795	7 (0·9)
45–55	2904	14 (0·5)	707	2 (0·3)
55–65	2060	7 (0·3)	1634	1 (0·1)
> 65	2987	12 (0·4)	5971	14 (0·2)
NK	2721	23 (0·8)	1861	3 (0·2)
Total	30043	538 (1·8)	20572	170 (0·8)

* Adapted from Palmer and Biffin [55].

adults, and in infants aged under 6 months, is particularly uncommon and tends to be mild. Infection in rural infants seems to occur most frequently when they are becoming mobile, are teething, and are changing to a shared diet, including in some cases raw cows' milk. At this point, maternally acquired immunity will also be waning. This pattern of incidence thus probably reflects both the frequency of exposure and levels of immunity, including passively acquired immunity. Corbett-Feeney [67] in Eire reported finding a protective effect in infants from breast feeding. This had previously been noted in some developing countries (see below). Though not studied critically, this would also appear to be the case in rural subjects in north Wales (D. Casemore, unpublished observation). There is, however, conflicting medical and veterinary evidence for the protective role of breast feeding and of colostral antibody in resistance to infection with *Cryptosporidium* [34, 43, 45]. Perinatal infection has been described and may lead to fetal distress or a failure to thrive [71, 72]. Extra-intestinal infection has been demonstrated but there is no evidence of transplacental transmission [32].

The distribution of cases by sex appears to be generally unremarkable. However, experience with campylobacter infections shows that detailed attention to rates of infection may reveal differences in both age- and sex-specific distribution to those shown by analysis of simple incidence figures [52]. Such detailed analysis has yet to be reported for *Cryptosporidium* infection.

Frequency of occurrence

Most early reports of human cryptosporidiosis were of single sporadic cases, small clusters of cases, or of short clinical series. Population surveys began to be reported in 1983, from Australia [14], Finland [18] and the UK [13], and subsequently from numerous other centres [7, 27–33]. Surveys were often of selected populations, usually based on specimens routinely submitted to the laboratory, and sometimes studied over short periods of time. Few studies were adequately controlled. Denominator figures were often not recorded other than as the total sample size. Other recognized potential pathogens were not always excluded. Accurate identification cannot be assumed in all studies (D. Casemore, unpublished data).

Table 3. *Age-Group distribution of Cryptosporidiosis cases (PHLS National Survey)*

Proportions of positives (excluding NKs)

Age group	Numbers examined (% of total *n*)	Positive (% + ve) (% of all + ves)
0–14	18792 (33)	769 (4·1) (62·4)
15–44	23993 (42·4)	398 (1·7) (32·3)
45–> 65	13770 (24·3)	65 (0·47) (5·3)

Adapted from Palmer and Biffin [55].

Laboratory-based surveys are subject to a variety of biases, as discussed above. From some of these studies therefore, it is difficult to gain an accurate picture of incidence rates, or of prevalence, or even to demonstrate unequivocally an aetiological role. They do, however, indicate the ubiquity of the parasite.

A few surveys have shown low prevalence in certain areas (< 1 %) and the need for routine laboratory screening has been questioned [73–79]. Generalized recommendations discouraging screening, when based on localized and sometimes short-term, low-yield studies, should be viewed with caution. Even if such low figures are an accurate reflection of local prevalence, the simplicity and low cost of such screening, together with the clinical, epidemiological, and other benefits obtained, suggest that the search for *Cryptosporidium* should now be undertaken by most laboratories, at least on selected specimens assessed over a sufficient period of time to allow for temporal variation [80–82].

Relative frequency of occurrence

In several studies, *Cryptosporidium* was found to occur as the third or fourth most commonly identified pathogen and at certain times was the most commonly detected of the agents looked for [17, 23, 30, 55, 59, 65, 67, 69, 80, 83, 84]. Such findings will tend to reflect the age of subjects sampled and seasonal variation in incidence of the different agents involved. Thus, in north Wales for example, although campylobacter is the most commonly identified enteric pathogen overall, and rotavirus in infants, during certain periods of the year *Cryptosporidium* is the most common finding in children. In the PHLS survey [55], *Cryptosporidium* occurred twice as often as salmonella and several times more commonly than shigella, in the 1–5 years age group over a 2-year study period.

Geographic distribution

Several reviews have tabulated the frequency of detection in a variety of survey populations in many countries worldwide [7, 27–30, 33]. Reported rates varied from less than 1 % to more than 30 %. Some of this variation may be attributed to genuine geographic variation, some to demographic or temporal factors. In some instances methodological factors may be important given the variation in sensitivity and specificity of the various methods used [24]. Composite tables of reported laboratory confirmed incidence generally yield average figures in developed countries of approximately 1–2 % positivity overall and about 4 % in children. As might be expected, reported rates are generally higher in Third World countries, especially in children.

Table 4. *Outbreaks of cryptosporidiosis in livestock animals reported to veterinary data collection centre (VIDA)*

Cattle	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1984	14	34	43	45	15	7	11	10	8	21	21	24	253
1985	41	48	94	67	50	17	10	12	14	21	34	37	445
1986	53	44	41	54	32	13	22	20	26	25	44	64	438
1987	72	79	94	120	53	49	22	16	58	57	83	102	805
Total	180	205	272	286	150	86	65	58	106	124	182	227	1941
Sheep													
1984	0	1	5	16	3	0	0	0	0	0	0	1	26
1985	1	3	4	29	9	3	0	0	0	1	0	1	51
1986	3	3	1	34	14	1	0	0	1	0	0	0	57
1987	4	5	18	37	22	1	1	0	0	0	0	0	88
TOTAL	8	12	28	116	48	5	1	0	1	1	0	2	222

Data courtesy of Mr A. B. Davies (DVO, Bangor).

Temporal distribution and seasonality

Seasonal or temporal trends have been noted by a number of authors but these vary from country to country including; summer in Australia [14], rainy season in Central America [61] and India [85], spring [84] or late summer in north America [60, 86], and late summer in Germany [87]. Our own studies [43, 53, 88] and some others in the UK [55, 59, 65, 66] (CDS/CDSC, unpublished reports) and in Eire [67], indicate a peak of incidence in the spring and sometimes also in late autumn or early winter. These trends may indirectly reflect rainfall, farming events such as lambing and calving (Table 4), and practices such as slurry muck-spreading [53, 88]. Paradoxically, more detailed study of data from the PHLS study [55], failed to demonstrate clear seasonality nationally (S. R. Palmer, personal communication). Experience over the past 5 years suggests that outbreaks, or temporal clusters of apparently sporadic cases, occur in different parts of the UK, often at about the same time each year, but not recurring in the same locality year by year. Whether this relates to local herd immunity levels affecting either primary incidence or secondary person-to-person spread, or to other factors, is not yet known. Detailed sero-epidemiological studies might help in elucidating this [43, 45].

RESERVOIRS AND ROUTES OF TRANSMISSION

Zoonotic and non-zoonotic infections

The natural history of cryptosporidiosis is complex and involves both zoonotic and 'urban' or non-zoonotic reservoirs and modes of transmission.

Zoonotic transmission. Current and his co-workers drew attention to the association of human infection with exposure to infected calves [5]. In recent reviews, more than 40 host species from which *Cryptosporidium* species had been isolated have been listed [28, 31, 49, 89]. Cross-transmission has been demonstrated experimentally or naturally between a variety of host species [28, 31, 49, 89]. There is, therefore, a potentially large zoonotic reservoir of infection. Most

clinical infections in humans and livestock mammals, as stated above, are probably with a single species, *C. parvum*, which has spherical or slightly ovoid oocysts measuring some 4·5-5·0 μm [7, 31, 35, 36, 43]. Cryptosporidial oocysts morphologically identical to *C. muris*, and distinct from *C. parvum*, have been identified in cattle in the USA [35, 44] but the epidemiological importance of this is not yet clear. The oocysts are more ellipsoidal and measure approximately 6 × 8 μm. The parasite appears, as with the original isolate discovered in mice by Tyzzer, to be restricted to the glands of the stomach (abomasum) and is not associated with symptoms other than poor weight gain in some cases [44] (K. W. Angus, personal communication). *C. muris* infection has not yet been reported in man. Large ($\geqslant 8$ μm) spherical acid-fast objects sometimes found in human faeces, particularly in subjects from Asia and Africa, and thought by some to be cryptosporidia are probably fungal spores (D. Casemore, unpublished observation). The importance of measuring presumptive oocysts cannot be overemphasized.

Livestock. Calves and lambs are commonly infected with *Cryptosporidium* in the UK. Pigs are susceptible to infection but this does not appear to be common. Infection in foals may be more common than is currently recognized. Deer and goats are increasingly commonly kept as stock animals and may be severely affected by cryptosporidiosis [2, 89]. Many human infections with *Cryptosporidium* are derived from livestock, particularly cattle, either directly, or indirectly. There is also evidence, at least in the UK, to implicate sheep in human infections [88, 90, 91]. The spring peak in incidence in humans in the UK closely parallels that reported by the Central Veterinary Laboratories for lambs (Table 4). In an outbreak in north Wales an association was noted in two related cases with exposure to infected bottle-fed orphan lambs [88]. A case-control study was subsequently carried out during 1988 which confirmed a significant association between human cryptosporidiosis in north Wales during March and April and contact with sheep or lambs, particularly between children and bottle-fed lambs ($P = 0{\cdot}00006$) [90]. Similar association has again been noted in the spring of 1989 (D. Casemore, unpublished data). In some cases studied, the recently increasing trend towards lambing in deep litter sheds close to the farm house had lead to increased exposure of family members and visitors to infection. Exposure sometimes occurred as a result of educational visits to working farms during the lambing season, or away from farms when lambs were taken to urban schools and nurseries for educational purposes. In several instances there was evidence of secondary spread within households or play-groups. Educational visits to farms and livestock markets may also lead to contact with other livestock such as calves which may also lead to cryptosporidiosis and to exposure to a variety of other infectious agents. There is, therefore, clearly a need for such zoonotic exposure to be controlled and for the importance of hygienic measures to be emphasised to those responsible. Guidelines to help limit hazards to children from this type of exposure have been circulated in the UK [92]. Zoonotic exposure of urban subjects also occurs when camping on farmland but, in such circumstances, exposure to more than one confounding risk factor (livestock animals, their excreta, raw milk and water, etc.) is common, often combined with lower standards of hygiene (D. Casemore, unpublished observations).

Although symptomatic infection in animals seems to be restricted generally to the very young, adult animals, especially deer, horses, pigs and sheep may excrete low numbers of oocysts [2, 89] (K. W. Angus, personal communication). Such excretors may provide a reservoir for the young of their own species and for humans. A number of cases of human cryptosporidiosis have been investigated in north Wales for whom the only identified risk factor was recent attendance at riding stables or recent delivery of horse manure for garden fertilizer use in which small numbers of oocysts were identified (D. P. Casemore and P. Robinson, unpublished data).

Companion animals. Companion animals have been shown to be infected [28, 31, 34, 54, 89] and have been implicated in human disease from time to time. They include cats [93, 94] and possibly dogs [86] (D. Casemore, unpublished observations). However, despite the frequency with which pets are present in households of infected patients, they do not seem to have been often implicated as a source of infection. With the possible exception of cats, companion animals probably do not represent a zoonotic reservoir for human cryptosporidiosis [2, 34]. The finding of small numbers of oocysts in the stools of household pets need not imply that the animal is the source of human infection. In a recent episode investigated in north Wales, the family pet labrador dog was found to be excreting small number of oocysts. The dog had shown signs of mild gastrointestinal upset at the same time as the first affected family member but was not sampled until some time later. A similar isolated incident had previously been encountered with a cat. It is difficult to interpret the significance of such findings as it was not possible to exclude the possibility that the pet and the humans had been exposed to a common source of infection. Wild rodents in the home may potentially transmit infection to pets directly or by contamination of food.

Occupational zoonotic exposure. Human infection has been documented following occupational exposure to naturally and experimentally infected animals, including during the inoculation of infected material, especially by veterinary students [54, 58, 85, 95–98]. A negative association has been noted in some instances between bovine and human cases, particularly with rural adults who are occupationally exposed [12, 86]. Occupational exposure probably leads to repeated mild or asymptomatic infection and high levels of immunity. Repeated or recurrent infection has been noted occasionally in sporadic cases [99] (D. Casemore and P. Robinson, unpublished observations). In two cases in north Wales, the second episode was clinically milder than the first. In such cases, it is not yet possible to distinguish between continued low-level or intermittent excretion, recrudescent infection or reinfection.

Indirect zoonotic transmission. Occupationally exposed individuals may inadvertantly carry infected material home with them to indirectly infect family members, especially toddlers. Such indirect transmission may have been the route for severe perinatal infection reported by Dale and co-workers [72]. In north Wales, indirect transmission was thought to have occurred on a number of occasions with toddler children of mechanical-digger operators living in urban areas, whose clothing and footwear had become contaminated with animal excreta while working on farms [43]. Rahman and his co-workers in Bangladesh emphasized the importance of person-to-person transmission among household contacts of calf handlers [85].

Food. The pattern of food-borne infection generally has been changing in recent years and an increasing variety of pathogens recognized [100]. Direct incrimination of food in the transmission of *Cryptosporidium* is hampered by the lack of the equivalent of bacteriological enrichment culture for recovery of small numbers of oocyts and for confirmation of viability. *In vitro* cultivation in tissue cultures and in fertile hens eggs is possible but does not result in amplification [31, 34].

Based on epidemiological evidence, consumption of certain foods, especially sausages, offal, and raw milk appear to be risk factors [53, 55, 59, 83, 88, 101]. An unexpected finding of the study in north Wales [43, 53, 88] was the frequency with which sausages were admitted to have been consumed raw, either prior to cooking during meal preparation or even by using the contents as a pate. This practice is now known to occur elsewhere in the UK. The risks of bacterial milk-borne infection are well established [102]. The retail sale of raw milk continues to provide a vehicle or source of infection with a variety of agents, especially in rural areas. The epidemiological evidence adduced for the association of raw milk consumption with transmission of *Cryptosporidium* emphasizes further the need for pasteurization of all milk supplies. Clinical cryptosporidiosis associated with raw milk consumption in north Wales appears to occur most often in those who only occasionally consume it or have recently commenced doing so. Presumably those who regularly consume raw milk will maintain sufficient levels of immunity to prevent symptomatic infection. Whether infection from milk arises as a result of faecal contamination or from ascending infection of the teat duct is not yet known.

Environmental sources

Co-infection with *Giardia* has been noted in some cases, particularly in travellers (see below), suggesting the possibility of contaminated water or fruit and vegetables, as a source of infection as well as by person-to-person transmission [56, 60, 74, 103–105]. Water-borne giardiasis has been reported from the UK [106]. Environmental sources of such infection have been identified in the USA and may occur more commonly than is currently realised [107–109]. This may result from either human or animal pollution of the environment in the case of both these parasites.

Several studies have noted temporal or seasonal peaks in incidence of cryptosporidiosis (see above) which in some cases coincided with periods of maximal rainfall. The widespread practice, even in advanced countries, of disposal of both animal and human excreta to land, e.g. by muck and slurry spreading on pasture, may lead to infection directly, by aerosol spread, or indirectly by contamination of water courses and reservoir feeder streams. Surface waters polluted naturally or by these practices may lead to contamination of water supplies or of food crops during irrigation. *Giardia lamblia* may share a common mode of transmission via water or food polluted by sewage as noted above. Methods have been developed for the investigation of water (see below). Using such methods contaminated water has been identified including surface waters, and in a few instances potable supplies, in the UK [43, 50, 88, 110–112] and in North America [105, 107–109, 113–117]. A number of cases or outbreaks have been investigated in which water was thought to have been the route of transmission. The epidemiological investigation of an outbreak in Texas in 1984,

described by D'Antonio and others [118], suggested contamination of the communal water supply with human sewage. Other cases in New Mexico, USA, also investigated epidemiologically, have been attributed to the water route [119, 120] involving swimming in, or consumption of, surface water.

One confirmed water-borne outbreak in Carroltown, Georgia, USA, resulted in an estimated 13000 cases [121, 122]. This followed a partial failure of the water treatment system, particularly of flocculation, of river water abstracted for the municipal supply at a time of high demand although the water met all EPA quality standards. Although the river flowed through cattle pasture, evidence of infection was lacking in cattle on that land but there was evidence of pollution with sewage. Breakthrough may have resulted from unusually heavy challenge from a bolus of heavily polluted water at a time when the flocculant mixing mechanism was operating at less than optimal efficiency (J. Rose, personal communication). The finding of apparently satisfactory standards for the water may have resulted from sampling at an inappropriate point – at the point of distribution. For example, for turbidity levels, it is essential to check individual treatment plant sections (filter outputs) prior to mixing. In this episode, failure to adequately back-wash some filters almost certainly led to part of the supply having raised turbidity levels (> 1 nephelometric turbidity unit, NTU) although the final mixed supply had a satisfactory NTU value.

Several possible waterborne outbreaks have been investigated in England and Wales but most have not been confirmed [43, 50, 123, 124] (D. Casemore, unpublished data). Most of these investigations, however, preceded the availability of the methods described below. Some outbreaks, at first thought to be waterborne, have shown epidemic curves suggestive of urban transmission when examined in detailed [124]. Nonetheless, the initial cluster of cases may well have been of water-borne infection followed by person-to-person transmission. In a few sporadic cases investigated in north Wales, the only risk factor identified has been a breakdown in the local water supply during the preceding 1–2 weeks [53]. In an outbreak in the Sheffield area [50, 112] cases showed a statistical association with consumption of potable water. Oocysts were demonstrated in surface waters in the catchment area and in cattle grazing on catchment land. An outbreak of cryptosporidiosis in Scotland [110, 111] was confirmed both epidemiologically and microbiologically as water-borne. Contaminated surface water shown to contain oocysts gained access to the treated potable supply via a previously undetected land drain and resulted in at least 27 confirmed cases, of whom 12 were admitted to hospital. Other outbreaks and clusters of cases have occurred more recently in different parts of the UK some of which may have been water-borne although proof is lacking in most cases.

Early in 1989, a very large outbreak of cryptosporidiosis occurred in the Oxford and Swindon areas of England supplied by Thames Water Authority [125, 126]. This has been attributed to consumption of contaminated potable water supplies derived from a polluted reservoir. Samples were taken and examined using the methods developed in the USA, originally for *Giardia* and subsequently adapted by Rose and her co-workers [113, 114, 116]. Surface water, water-treatment filter backwash samples, and potable water were all shown to contain oocysts (J. Colbourne, personal communication). The total number of cases attributable to

the water is difficult to estimate but 516 confirmed cases were identified in the affected area during the course of the outbreak between late December and late April (R. Mayon White, personal communication). The size of the outbreak and the public recognition of risk from treated water caused considerable media and political interest and concern. A number of issues were raised by the outbreak which have been addressed by a Government committee of enquiry and others [125–128]. These include, for example: the practical difficulties of the sampling methodology; the significance of changes in water-treatment practice including the change of flocculants used resulting from concern about the use of aluminium compounds; the problems arising from imposition of a water 'boiling order' including the practical difficulties this imposes upon hospitals and food industry users, and the relative risks of acquiring infection and of scalding injuries.

The safe disposal of sewage and the provision of safe water supplies have been pursued for more than a century [25, 129–131]. For largely historical reasons, standards for evaluating the wholesomeness of potable supplies are based primarily on bacteriological parameters. The demonstration of transmission of *Cryptosporidium*, and *Giardia*, via potable water demonstrates that the safety of such supplies cannot be assumed. Neither can the standard bacteriological parameters be used to guarantee freedom from the risk of infection. The fiscal and political implications of this situation are considerable. Currently, only adequate physical methods of removal are likely to be effective. Although breakthrough of such treatment systems resulting from a heavy challenge may account for some episodes, some areas of the country receive water which is chemically treated but unfiltered. It is probable that, where filtration and/or flocculation are missing or defective, contamination of water supplies will occur which, with the known resistance of oocysts to chemical disinfection [1, 3, 132–135], makes it likely that further outbreaks will be confirmed. The results of more recent disinfection studies are described below. Contamination of water supplies may in some cases be low-level and intermittent making monitoring difficult. Such contamination may account for some unexplained sporadic cases or small clusters of cases. The precise identity and the viability of oocysts found in water or other environmental samples cannot yet be reliably determined. Their significance is therefore likely to be uncertain although their presence should be taken as presumptive evidence for remedial action.

An outbreak of more than 70 cases of cryptosporidiosis associated with a swimming pool has been reported in the UK [136]. Defects in pool filtration and of sewage disposal resulted in pollution of the pool water. The only controlling factors currently for *Cryptosporidium*, if faecal contamination of a pool occurs from someone excreting oocysts, are dilution and efficient filtration. However, it is probable that in well-managed pools this infection will not normally be a problem. In the event of an outbreak being suspected, the pool should be closed and flocculants used, with backwashing to waste, to increase filtration efficiency. Increasing the temperature of the water to $\geqslant 45$ °C may also be used to kill oocysts. The activity of other disinfection systems is currently under investigation (see below).

Raw surface waters and private untreated supplies may be consumed by susceptible individuals, especially those engaged in outdoor pursuits such as

camping. This clearly carries a risk of acquiring enteric infection including cryptosporidiosis. Chemical treatment tablets may have little effect on the cysts of *Giardia* and *Cryptosporidium* but both are susceptible to heat treatment (see below).

Investigating environmental routes and reservoirs. The obligate parasitic nature and the ubiquity of *Cryptosporidium*, the variable but possible long incubation period, and the potential for subsequent person-to-person transmission, all make epidemiological investigation difficult. As with possible food-borne infection, the lack of an equivalent of bacterial enrichment culture is a constraint on investigation of environmental sources. Systems for the recovery and detection of oocysts from water have been developed, including Moore's swabs [43, 88, 124] and membrane [50, 112] or cartridge filtration [107–111, 113–117, 122, 137], usually combined with centrifugation and or flotation. Cartridge filtration was extensively used and evaluated in the UK during the outbreaks in Scotland [110, 111, 137] and in Oxford and Swindon (J. Colbourne, personal communication). A tentative standard method has been produced in the UK, under the auspices of the Department of the Environment (DoE) Standing Committee of Analysts, based on the same methodology. The method can be made quantitative but gives no indication of either viability of oocysts or of their pathogenicity or virulence for man. The methods for recovery and identification of oocyts are time-consuming and labour-intensive and are unsuitable for routine public-health monitoring purposes on any scale.

Caution is needed in identifying objects found in water samples as oocysts of *Cryptosporidium*. Conventional tinctorial methods are of limited value because a variety of fungal spores and other objects resembling oocysts may be found in environmental samples, particularly by modified Ziehl–Neelsen and saffranin staining. These may be present in addition to genuine oocysts. Accurate size measurement is helpful, oocysts of *C. parvum* having a modal size, in stained preparations, of 4·5-5·0 μm [43]. The identity and significance of isolates from fish from reservoirs is uncertain as these have not yet been characterized. Detection and definitive identification of oocysts in clinical or environmental samples may be achieved by means of immunologically-based test such as immunofluorescence (IFAT) using either polyclonal [88, 115, 124, 138] or by monoclonal antibody [107–111, 113, 114, 116, 120, 139–142], by ELISA [112] or by latex agglutination [54, 143]. However, caution is needed as some antibodies, particularly monoclonals, may be too restrictive in the antigens which they will recognize. Others, particularly polyclonals, may detect group antigens present in cryptosporidia, from both mammalian and avian or other host species, which are of doubtful medical significance or cross-react with other objects such as yeasts. There appears to be incomplete concordance in the findings with the two monoclones currently available. Oocysts exposed to water treatment, especially to chlorine, may be both morphologically and antigenically altered [135] (D. Casemore, unpublished observation; J. Colbourne, personal communication). The monoclonal antibody described by McLauchlin and co-workers [141] has reacted with all human isolates so far tested in the author's laboratory, but reacts also, though less intensely, with with *C. muris* and *C. baileyi*. It appears to work well with environmental samples, oocysts often showing a clearly defined suture or surface fold. Cross-reactivity has

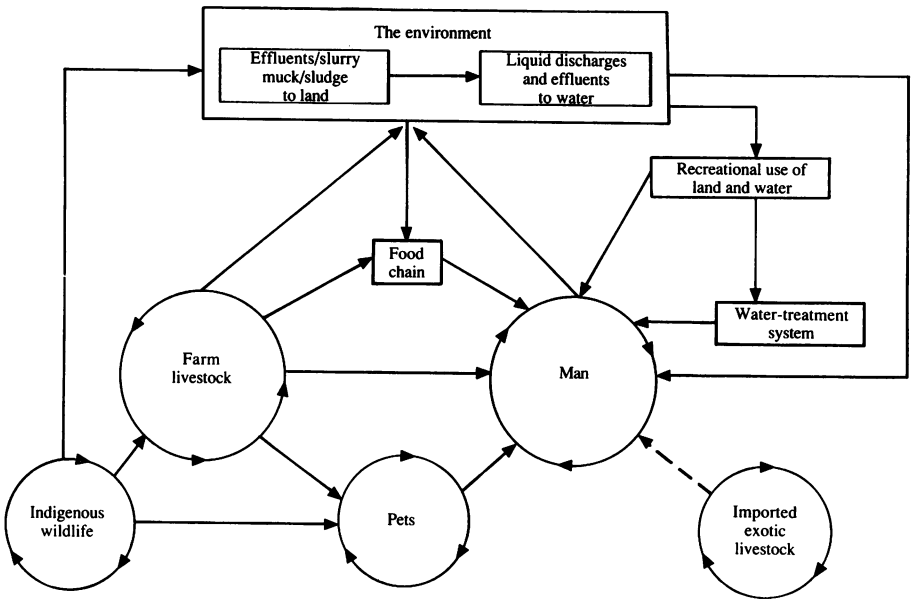

Fig. 3. Reservoirs and routes of transmission of *Cryptosporidium*.

not been noted with a variety of fungal species tested including yeasts, and some *Mucor* spp. which produce acid-fast spores of approximately 5 μm (D. Casemore, unpublished observations). The monoclone used by American workers reacts specifically with *C. parvum* but not *C. baileyi* [140] or *C. muris*.

There is a need to develop more sensitive but specific methods of detection which do not depend upon the labour-intensive and essentially subjective microscopical IFAT, particularly for screening out negative samples. These may be based upon ELISA, or gene-probe or polymerized chain reaction technology. There is also a need to be able to assess viability [135] and to identify and distinguish antigenic or iso-enzyme types. Little is currently known about the infectious dose-size (see below) and hence of the epidemiological significance of small numbers of oocysts.

The various potential reservoirs and routes of transmission are outlined in Fig. 3. Epidemiological investigation is often confounded by the presence of more than one of the risk factors indicated [88].

Urban or non-zoonotic transmission

Person-to-person transmission is now recognized to be common, thus confirming the hypothesis that cryptosporidiosis is not necessarily a zoonosis [12]. Infection is transmitted within families [53, 88, 144–148] in day-care centres [53, 144, 145, 149–152], and elsewhere in an urban environment [53, 59, 153–159]. Opportunities for the spread of enteric infections in some Western urban day-care centres may rival those in some Third-World countries [103]. Both sporadic and day-care centre outbreak cases are often associated with confirmed secondary cases among families or of a history of recent diarrhoea among contacts who had not been investigated. Asymptomatic family contacts of confirmed cases are sometimes

found to excrete oocysts in small numbers [53, 88]. The existence of an asymptomatic carrier state, possibly involving the bile duct, has been reported [158] (see below). Clinically mild cases and asymptomatic excretors may provide a hidden reservoir of infection in the community. Evidence of either point-source or person-to-person transmission may be seen in family outbreaks [53]. Most urban infection probably occurs by the faecal-oral route, directly or via fomites. However, *Cryptosporidium* has been found in sputum and in vomit [32, 88, 159] and these may provide additional vehicles of transmission.

Nosocomial infection. Hospital cross infection, from patients to staff, has been documented and is further evidence of person-to-person transmission, [155–157]. The reverse route, or patient-to-patient transmission, is clearly possible and is of considerable potential importance for immunocompromised patients. There is a risk of indirect transmission via endoscopes, etc. [134, 160]. Given the known resistance of oocysts to disinfectants (see below), it is surprising that nosocomially acquired infection has not been documented more frequently.

Sexual transmission. Cryptosporidium may contribute to the so-called 'gay bowel syndrome' of sexually transmitted enteric infection [6, 7, 161]. However, continuing surveillance of infected practising homosexuals has failed to demonstrate an increased incidence of secondary cases in their partners (R. Soave, personal communication).

Travellers' diarrhoea. The infection has emerged as an important cause of travellers' diarrhoea [18, 19, 53–57, 104, 162–164]. Such cases often have mixed infections, especially with *Giardia*, suggesting a common epidemiology involving contaminated water or food. Increased exposure to livestock may be important in some areas. Direct and indirect person-to-person transmission will also be important given the generally poor hygienic conditions which prevail in many under-developed countries [165]. There is evidence to implicate flies in transmission in the Third World (C. Sterling, personal communication).

Travellers' diarrhoea is usually associated with travel to less-developed countries. However, urban subjects in the UK have sometimes been shown to have acquired infection when holidaying in more rural areas of the same country or in Western Europe. This often appears to be associated with consumption of raw (untreated) milk or water, or uncooked sausage, and with exposure to livestock [53, 55] (CDSC, unpublished reports; D. Casemore, unpublished observations).

Indigenous (autochthonous) infection in the Third World

Cryptosporidium has been reported from many Third World countries where it may be associated with severe morbidity and sometimes mortality or with asymptomatic infection. The reported age-specific rates of infection differ in different areas of the world and the reason for this is not yet clear. Mata [61] found such differences in incidence to occur between urban and rural populations in Central America which he attributed to differences in breastfeeding practices. An apparent protective effect from breast feeding was also noted in Haiti [64] and in Guatamala [68]. Hojlyng and his co-workers [62] in Liberia, reported a positive association with bottle feeding and with overcrowding in urban slums; Malla and co-workers [166] in India made positive links with bottle-feeding in rural children. Increased opportunity for exposure, in bottle-fed infants, and in children who are

being weaned may be as important as the immunological properties of breast milk for the differing rates of infection [167]. The evidence for the role of humoral immunity in resistance to cryptosporidiosis is conflicting [43, 45]. Transplacentally acquired passive immunity may be important and may explain the apparent low incidence of infection in infants under 6 months of age reported by some workers. Mathan and co-workers [70] in Southern India reported a high rate of asymptomatic infection in Southern Indian children and questioned the significance of the parasite. This differs from most other reports and the validity of the authors interpretation has been questioned [168]. However, such a pattern may reflect hyperendemicity with high levels of exposure and of antibody. There may be close association with livestock from an early age. Rahman and co-workers [85] emphasized the importance of person-to-person transmission among families of calf handlers, presumably introduced into the household from their animals. Carstensen and his co-workers [63] point out that the age-specific prevalence of cryptosporidiosis in their study was the reverse of that for most parasitic infections and suggest that early infection, probably by person-to-person transmission is followed by immunity to clinical infection. On the other hand, immunity impaired by malnutrition may delay clearing and hence prolong excretion. Cryptosporidiosis in the malnourished has been linked to severe persistent or chronic diarrhoea [169–171] which, in turn, may further increase the malnourishment. The severe diarrhoea sometimes associated with measles may be exacerbated by cryptosporidiosis in some cases [32].

Ethnic differences. Some reports have indicated differences in incidence in particular ethnic groups within the same study area, particularly of lower rates among muslims, which may reflect dietary differences, differing exposure to livestock animals, or to toiletting practices [59, 62] (T. Rowbotham, personal communication).

Mixed enteric infections

Polymicrobial infections are commonly found in subjects in developing countries and are now more commonly being recognized in developed countries, especially in AIDS sufferers. Although, in immunocompetent subjects, *Cryptosporidium* is most commonly found as the sole recognized enteric pathogen, a variety of agents have been reported in mixed infections. In developed countries, concurrent infection with such agents as *Giardia* [53, 60, 104, 105], *Campylobacter* [53, 67, 88] and *Shigella sonnei* [153], may indicate a common epidemiology and dual transmission in some of those cases. Mixed infection with *Cryptosporidium* plus *Giardia* have been noted particularly with children in day-care centres and in travellers to Leningrad. However, such infections may not always be significantly associated [74, 152] and may, particularly in under-developed countries, represent overlapping sequential infection or coincidental carriage of one agent detected as a result of acute infection with the other.

Incubation and excretion periods

The prepatent (incubation) period in naturally and experimentally infected animals has been shown to be usually 2–5 days, and the patent (excretion) period about 8–14 days, range < 7 to > 28 days [1, 2, 53, 66, 172–176]. Attempts have

been made to define incubation and excretion periods in man, both of which factors are epidemiologically important. Because of the ubiquity of the parasite and the diversity of potential sources and routes of transmission (see Fig. 3), it is usually not possible accurately to define the incubation period [177]. However, accidental laboratory infection [97] or other detailed exposure histories [18, 66, 95, 97, 104, 146, 174] suggest an incubation period of about 3 days to a week but longer in some cases. Some estimates are based on assumptions which may not be valid and ignore the existence of confounding factors. Periods of as little as 1 day have been suggested [174] but earlier exposure, or prior or co-infection with other agents having shorter incubation periods such as viruses, were not excluded [177]. The effect of infecting dose size is discussed below.

During the acute diarrhoeal phase, oocyst numbers may exceed $10^6/cm^3$ of stool. However, numbers of oocysts excreted during the course of the infection may fluctuate markedly and formed or semi-formed stools may be found to contain many oocysts while some fluid stools may contain few. The stools of some patients become oocyst-negative rapidly after cessation of their diarrhoea while others continue to excrete for a prolonged period, sometimes associated with the continuation of other symptoms. Accurate determination of the excretion period is limited by the availability of sequential specimens and by the sensitivity of oocyst detection systems. Excretion in immunologically normal subjects, assessed by conventional tinctorial methods, usually seems to cease within 1–4 weeks of clinical resolution but may be longer in some cases [32, 43, 53, 99, 174–176]. Use of a more sensitive IFAT method suggests that excretion may continue for longer [140, 141, 178]. The existence has been reported of 'atypical' oocysts not detectable by conventional staining methods [179–180]. Large numbers of oocysts may be detected in some stool samples when stained by IFAT, of which the majority can not be detected by other methods, even as unstained 'ghosts' [141] (D. Casemore, unpublished observation). The significance of these findings in relation to infectivity and control needs further elucidation.

Infection in immunocompromised subjects is sometimes mild or resolves unexpectedly [181, 182] but the reason for this is not currently known. In some such cases anti-HIV treatment may permit sufficient immune response to return to induce control of the infection. This may parallel the situation reported in some leukaemic subjects [32]. Immunocompromised cases may harbour the parasite in the bile duct. Involvement of the bile duct has not yet been demonstrated in immunologically normal subjects [159]. One small study has adduced evidence for asymptomatic carriage by detection of oocysts in duodenal aspirates [158]. Endogenous stages were not found in biopsy sections but these may be easily overlooked [7]. The subjects did not have diarrhoea and oocysts were found in only a small proportion of their stool samples. Studies on more than 100 endoscopy fluids in north Wales have so far failed to confirm these findings (Casemore and Tynan, unpublished data). If the finding is confirmed, such cases may excrete oocysts at rates below the current threshold for detection but might, nonetheless, provide a reservoir of infection.

Infectivity and resistance

Little is known of the infectious dose size but it is thought to be small [28, 97, 183]. Infectious dose sizes in single figures have been determined for some other

protozoan cysts such as *Giardia* (D. Warhurst, personal communication). Experimental studies by Ernest and his co-workers [184] suggest that there may be a larger minimum infectious dose size and that the proportion of exposed subjects developing infection is a function of the dose size. Conversely, a low infecting dose size may simply extend the incubation period or reduce the likelihood of symptomatic expression. The possibility of a small infecting dose size, together with the known resistence of oocysts [1, 3, 4, 31, 132–135], emphasizes the potential for nosocomial and environmental transmission. Ten to 25% commercial bleach may be used for bacterial decontamination of oocyst samples prior to animal inoculation without significantly reducing infectivity [31] although a reduction in *in vitro* excystation may occur. In studies using an *in vitro* excystation model, plus newborn mouse inoculation for confirmation [133, 134], some compounds tested showed an effect in preliminary screening but, as might be expected, were ineffective when retested in the presence of protein. Some showed more effect if used at temperatures above ambient. Of the common disinfectants and antiseptics assayed, only 10 vol. hydrogen peroxide was found to be rapidly effective at ambient temperature and was unaffected by the presence of protein. An agricultural proprietary product, Oo-cide (Antec International Ltd) was also found to be effective but this depends upon the action of ammonia with a biocide in a highly alkaline solution and is unsuitable for medical or domestic use. Its use in the treatment of a polluted swimming pool (see above) led to considerable damage to the activated sludge system of the receiving sewage works. Oocysts are susceptible to freezing, to heating above 45 °C for 5–20 min, and to drying [133, 173, 185–187]. U.v. irradiation appeared to have some effect but less than that on bacteria or yeast cells similarly exposed (D. Blewett, S. E. Wright and D. Casemore, unpublished observations). Ozone at high concentrations (*c* 2·0 mg/l) has been reported to kill oocysts [188] but in other, preliminary, experiments exposure of oocysts at the time/temperature/concentration levels likely to be found in swimming-pool water treatment, excystation levels were not reduced (D. Casemore, unpublished observations). Laboratory scale studies by Upton and his co-workers show that pentaiodide resin may be capable of removing oocysts from water, probably by electrostatic forces [189] but the practical implications of this finding are uncertain. Thus, further studies are required to identify effective disinfectants suitable for domestic, hospital, and water-treatment purposes.

Seroepidemiology

Several attempts have been made to carry out serological studies using IFAT, ELISA, or laser densitometry [43, 45, 50, 88, 122, 156, 162, 190–196]. The significance of the findings in these preliminary reports is unclear because of variations in methodology. Some studies have employed infected tissues containing endogenous stages as antigen while others have employed purified oocysts. Studies on oocysts have indicated that, as might be anticipated, they are antigenically complex but include some common antigens [46, 140, 194, 195, 197–199]. The effect which differences in antigen source or test system might have is not clear but it has been suggested that titres may be significantly higher using sporozoites or endogenous stages as antigen [4, 45]. In some cases, titres were found to vary according to the host species from which the oocysts were derived

suggesting the possibility of different antigenic types [43, 45, 46]. In some studies, persistent IgA and or IgM, and poor IgG responses were reported. There is some evidence of pre-existing immunity in asymptomatic close family contacts of cases consistent with immunity, presumably associated with previous exposure [43, 45]. This seemed, in the north Wales study, to be associated mainly with the presence of IgG. As might be expected, seroprevalence rates tend to be much higher than the frequency of oocyst detection. In addition to giving some indication of prevalence in particular populations some of these studies have yielded information on the pattern of the immune response of value in the study of pathogenesis. Such studies may also provide alternative evidence of cases occurring in outbreaks [43, 45, 122]. Further studies are required to elucidate some of the questions raised.

Molecular epidemiology. Typing of epidemiologically important isolates of infective agents has usually depended on biotyping methods, based on both phenotypic and genotypic characteristics. The use of so-called 'molecular epidemiology' has proved of great value in the study of epidemiology, particularly of viruses [200]. Given the obligate parasitic nature of *Cryptosporidium*, the typing and sub-typing of isolates below species level must depend largely upon such molecular methods. Antigen and antibody analysis at the molecular level may yield epidemiologically useful information. Isoenzyme (zymodeme) profiling as used in the sub-typing of potentially pathogenic amoebae [201] may also be of value. The extraction and purification of nucleic acids and other molecular components has been achieved thus leading the way to the production of gene-probes and to other more sensitive means of detection and identification (D. Blewett and N. Gordon, personal communication).

CONCLUSION

Cryptosporidium sp. has emerged worldwide during the 1980s as an important cause of acute, often protracted but self-limited gastroenteritis in normal individuals while, in the immunocompromised, the infection may often prove chronic and life threatening. Infection in some cases extends beyond the gastrointestinal tract [32, 159]. The importance of close collaboration between veterinary and medical workers for the control of zoonoses has been emphasised [202]. Much has been learned from such collaboration about *Cryptosporidium* and human cryptosporidiosis. Molecular methods for the study of epidemiology are already under way, particularly by veterinary workers who have contributed so much to the understanding of human cryptosporidiosis. The infection is transmitted by both zoonotic and urban routes. It occurs sporadically and in both family and community outbreaks and is more common in children and young adults. There is a variable geographic and temporal distribution of incidence. Given awareness of the infection, the diagnosis may be achieved simply and relatively inexpensively, thus alerting others to the risk of transmission. In times of great fiscal constraint it is tempting to ignore the search for a pathogen which in some geographical areas, and at certain times, may have a low prevalence. In the hospital setting, such patients represent a potential source of nosocomial infection. The severity of the infection, particularly in the Third World and in the

ever increasing numbers of immunocompromised patients, makes awareness and control of the infection essential. In the UK, and elsewhere, cryptosporidiosis may be the most commonly detected enteric pathogen affecting AIDS patients [159, 203]. Control of human cryptosporidiosis can be achieved only by a thorough understanding of its natural history and epidemiology. Despite the advances made, much remains to be learned.

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