6 Viruses and protozoa

A.R. Eley

In up to 10% of all reported general community outbreaks of food poisoning in the UK, bacteria cannot be identified as the causative organisms. In many of these instances it is becoming increasingly evident that viruses are playing an important role; hospital outbreaks of viral gastro-enteritis are now known to be relatively common. One of the reasons for the lack of detailed knowledge about viruses that have been implicated in food poisoning is that traditionally they have only been considered after bacterial causes have been excluded; this often means that there is no food left for examination. Another problem has been the difficulty of observing or isolating viruses from foods: they may vary greatly in composition, with each requiring a different extraction procedure. Furthermore, techniques for isolating viruses are more complex and more time-consuming than those used for isolating bacteria. Unfortunately, electron-microscopy has only a limited level of sensitivity (approximately 10^5–10^6 viral particles/g of food), whereas with certain viruses we know that the infective dose may be as low as 10 to 100 particles/g. It is hoped that new methods for detecting viruses, including DNA probe technology, may have much greater sensitivity and provide new information on the natural history of food-borne viral gastro-enteritis.

Although the role of viruses in gastro-enteritis is under investigation, so far only a relatively small number of different organisms have been frequently associated with food poisoning. These are discussed below in order of importance:

1. small round structured virus (SRSV) group;
2. rotavirus;
3. astrovirus;
4. other viruses.

Although protozoa are not considered to be classical agents of food poisoning, recent evidence has indicated a food mode of transmission for some cases of cryptosporidiosis, as well as a small number of outbreaks of giardiasis. Amoebic dysentery caused by *Entamoeba histolytica* is usually a water-

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borne infection, but it has potential for food-borne transmission, especially in endemic areas of the world such as the Indian sub-continent and tropical Africa.

Improvements in methodology and increased awareness should allow us to look more closely for protozoa, so that we may appreciate their true role in the causation of gastro-enteritis.

Like viruses, these protozoa cannot replicate in food, but their cysts may remain infectious in foods for prolonged periods, constituting a potential hazard. Furthermore, only a small number of cysts are required to cause disease (i.e. they have a low infective dose).

6.1 SRSV GROUP

Unfortunately, the taxonomy and nomenclature of this group of viruses is confusing, misleading and subject to rapid change, and differs between the UK and the USA.

Perhaps the best known virus in this group is the Norwalk agent, which is an important cause of gastro-enteritis in older children and adults, usually during the winter months ('winter vomiting disease'). This virus was initially described in 1972 and derived its name from an outbreak of acute gastro-enteritis in a secondary school in Norwalk, Ohio. In the UK, however, this virus is known as SRSV. In addition to the Norwalk virus, there are a small number of related viruses, which may be described as structurally similar but antigenically different. These are called Norwalk-like-viruses (NLVs), or other SRSVs, and these include Hawaii virus, Snow Mountain virus, Montgomery County virus and Taunton virus; the names usually relate to the location of the initial outbreak.

At present the nucleic acid type, which is an important characteristic in viral classification, is not known for all SRSVs. However, recent reports suggest that the Norwalk and Snow Mountain viruses contain a single major structural protein similar to that found in human caliciviruses. This finding has prompted the suggestion that the Norwalk and related viruses may be possible candidates for consideration as members of the Caliciviridae. Briefly, the major features of the Caliciviridae are: non-enveloped, roughly spherical and approximately 35–39 nm in diameter, capsid has a single major polypeptide (60–71 000 molecular weight), one molecule of single-stranded RNA. These viruses are not sensitive to lipid solvents or mild detergents, but are inactivated at pH 3–5.

Viruses within the SRSV group are generally described as virions with a diameter of between 27 and 32 nm (Figure 6.1). This makes them slightly smaller than human caliciviruses and slightly larger than parvoviruses (18–26 nm). The latter distinction should be noted, as SRSVs have been and still are called 'parvovirus-like particles'. This name may now be even more

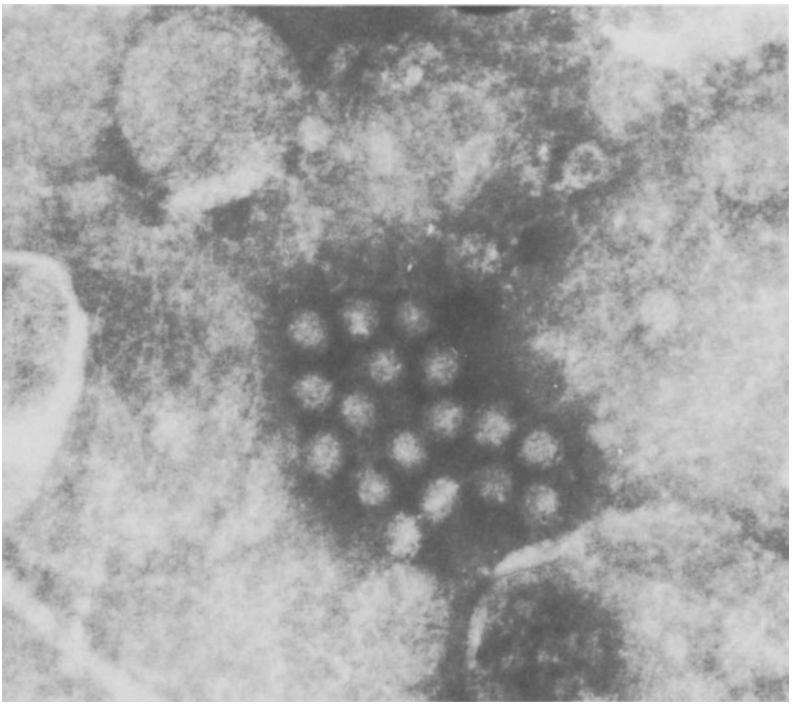

Figure 6.1 SRSV in faecal extract (× 200 000)

confusing, as we know that parvoviruses contain single-stranded DNA, in contrast to the RNA content of caliciviruses.

One of the major problems with SRSVs has been the difficulty of *in vitro* cultivation, which means that few of the viruses have been compared or characterized in detail; this has resulted in slow taxonomic progress.

6.1.1 Pathogenesis

At present disease mechanisms are poorly understood, although it is known that only a small infective dose is required ($10–10^2$ virions/g of food). It has also been established that these viruses are resistant to gastric acid, unlike some human caliciviruses, which would be inactivated in the stomach (see above).

6.1.2 Clinical features and prognosis

Symptoms of SRSV infection are typically nausea, vomiting, abdominal pain, malaise and a low-grade fever. Diarrhoea may also be present, though often

in a mild form. The disease has an incubation period of between 24 and 72 hours (Table 6.1).

Table 6.1 Clinical features of the illnesses produced by the major viral and protozoal causes of food poisoning

	SRSV	*Rotavirus*	Crypt. parvum	G. lamblia
Incubation time (hours)	24–72	24–168	4–14 days	1–3 weeks
Duration of illness (hours)	24–48	48–72	2–14 days	1–4 weeks
Vomiting	+	+	+	−
Nausea	+	−	+	+
Diarrhoea	±	+	+	+
Abdominal pain	+	−	+	+
Fever	+	±	±	−

− : rarely seen; ± : sometimes seen; + : often seen.

The disease is usually self-limiting, of 24 to 48 hours' duration, with no long-term sequelae.

6.1.3 Incidence and epidemiology

Due to the limitations on the detection and identification of these viruses, it is impossible to quote figures on the incidence of this type of food poisoning. However, it is generally believed that this group of viruses may be the most common non-bacterial cause in the UK and the USA. It is also thought to play a significant role in Australia and Japan. The epidemiology of infection is further complicated by the fact that some patients may be asymptomatic excreters for a period of time. Symptomless food-handlers may be an important source of food contamination.

Epidemics often occur in families, communities, schools, camps, institutions and cruise ships and spread rapidly, as these viruses have a low infective dose.

6.1.4 Ecology and foodstuffs

SRSV food poisoning has been associated with certain foods, especially shellfish. This is because aquatic bivalve molluscs (clams, mussels, oysters) filter and concentrate microscopic organisms from faecal-contaminated water into their own bodies. When eaten raw or improperly cooked, these infected shellfish can then cause disease. However, these viruses are not capable of multiplying in such foods.

6.1.5 Control

Since our knowledge of these organisms is limited, the only practical means of control are adequate cooking of foods, the prevention of cross-contamination and other good food hygiene practices.

SRV group

Another group of viruses with a similar terminology are the small round (featureless) viruses (SRVs). These were first described in 1978 and are generally smaller in size (20–30 nm in diameter) than SRSVs. As with the SRSVs, their nucleic acid type is unknown, they have a low infective dose, and disease is often associated with consumption of raw or improperly cooked clams or oysters. However, their true significance in causing disease is still not fully established.

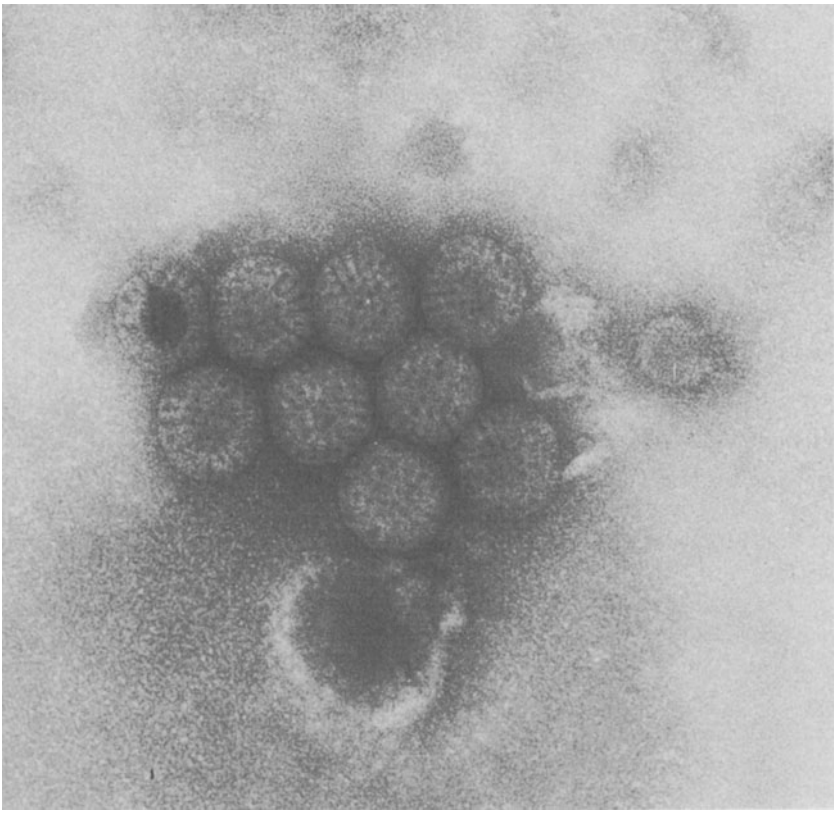

Figure 6.2 Rotavirus in faecal extract (× 200 000)

6.2 ROTAVIRUSES

This group of viruses was first discovered in 1973; the first types to be isolated are now known as group A rotaviruses. These are the commonest cause of gastro-enteritis in infants worldwide, and in developing countries they account for high levels of infant mortality. Usually, rotaviruses are responsible for a winter disease, with a peak in autumn and early winter.

Rotaviruses are described as reovirus-like particles of approximately 70 nm in diameter, which have a wheel-like shape, a double-shelled capsid structure, and contain double-stranded RNA (Figure 6.2).

There is still a certain amount of controversy about whether rotavirus infections can be food-borne, which has not been helped by the fact that these viruses grow poorly or not at all in cell-culture systems. Food is almost certainly not the principal mode of transmission, which is more likely to be faecal-oral, a suggestion supported by the fact that rotaviruses can survive on human hands for a number of hours. However, there is some evidence that food contamination may be responsible for single isolates and a number of outbreaks.

6.2.1 Pathogenesis

Little is known about their pathogenesis apart from the fact that these viruses can infect the mucosa of the jejenum and ileum, causing desquamation and villous atrophy. This could then lead to carbohydrate malabsorption and finally diarrhoea. It should be noted that only a very low infective dose is necessary to cause disease, while the virus can be excreted in large numbers in faeces, up to 10^{10} virions/g. Maximum viral shedding in stools tends to occur from two to five days after the onset of diarrhoea.

We do not fully understand yet why many adults with infection are asymptomatic, or why others may display a wide variety of symptoms. A number of factors may be involved, including a pre-existing immunity, differences in immune response, infections with other pathogens, differences in inoculum size, and host factors such as stress or drug ingestion. A further complication is that these viruses may often be found in large numbers in the stools of healthy neonates, but are then associated with symptomatic disease in children older than six months.

6.2.2 Clinical features and prognosis

This type of food poisoning is characterized by vomiting (sometimes projectile) and diarrhoea; there may be an associated upper respiratory tract infection. If fever is also present then this may indicate a more severe illness. The

incubation period may vary from one to seven days, but is usually between two and four days.

A complete recovery usually occurs within two to three days, although fatalities may very occasionally be reported. Deaths tend to occur among young children and geriatric patients; both groups tend to suffer more severe symptoms, including dehydration.

6.2.3 Incidence and epidemiology

Group A rotaviruses, so-called because of the presence of a group A specific antigen, are the most common isolates from Western Europe and the USA. Recently, other serologically distinct groups have been discovered, which have a rather different geographical distribution. The group B viruses have been responsible primarily for larger-scale epidemics amongst infants and adults in China. In 1989 there was a report from Japan of the first large outbreak due to group C rotaviruses: out of 3102 people, 675 became ill. Previously, group C viruses had only been implicated in a few sporadic cases. Furthermore, in the last few years there have been reports of viruses found in patients with gastro-enteritis which have rotavirus morphology but lack the common group specific antigen; these have been called atypical rotaviruses or pararotaviruses.

In institutional outbreaks in hospitals, nursing homes, day-care centres and schools, epidemics are characterized by a large-scale sudden onset of presenting symptoms (see above), usually of short duration.

For epidemiological purposes, rotaviruses have been divided into groups based on major distinct antigen specificities. Subgroups have been based on the major inner capsid protein VP6 which has a molecular weight of 45 000, and serogroups divided on the basis of the major outer capsid glycoprotein VP7 which has a molecular weight of 35 000. In addition, analysis by polyacrylamide gel electrophoresis, which allows separation in an electrical field according to molecular size, has shown that the genome consists of 11 segments of double-stranded RNA which vary from strain to strain, resulting in a characteristic pattern or electropherotype.

6.2.4 Ecology and foodstuffs

Infection is thought to occur by ingestion of faecal-contaminated food or water, although no specific foodstuffs are known to be particularly hazardous at present. What is known is that rotaviruses are fairly stable in groundwater and sewage, and are resistant to many commonly-used chemical disinfectants.

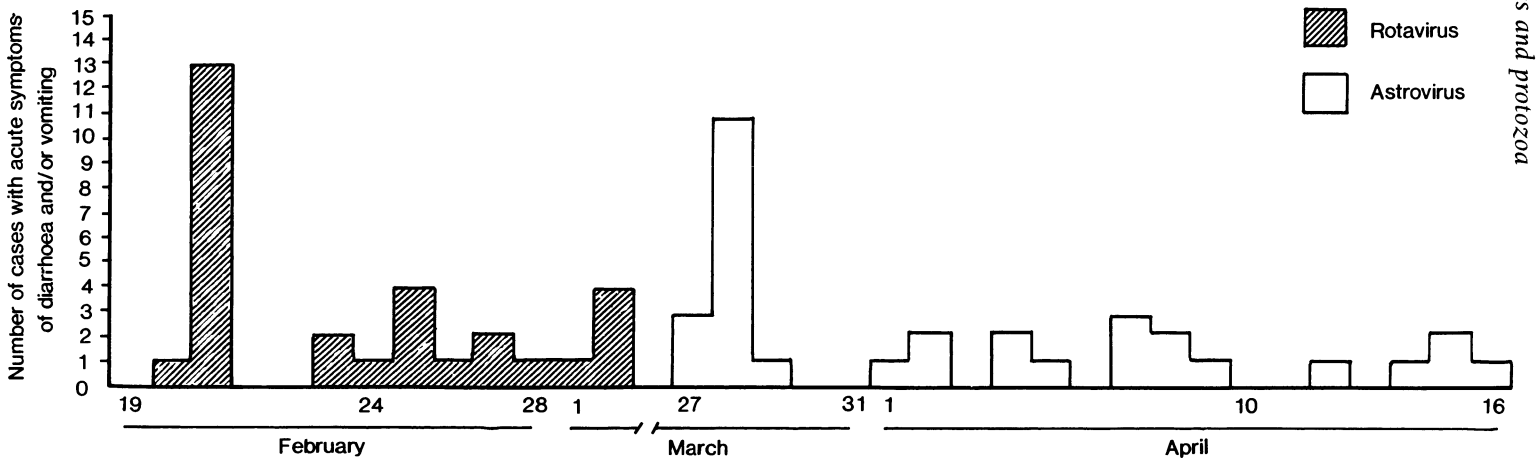

Figure 6.3 Cases of gastroenteritis on a geriatric ward, caused by rotavirus followed by astrovirus. After Lewis, D.C. *et al.* (1989), *Journal of Hospital Infection*, **14**, 9–14

6.2.5 Control

For general measures see section 2.6. It also needs to be stressed that because this disease can be transmitted in a number of different ways and the infective dose is low, strict isolation and barrier nursing are recommended to prevent hospital outbreaks.

6.3 ASTROVIRUSES AND OTHER VIRUSES

These viruses were first described in 1978. They are 28–30 nm in diameter and have a characteristic five or six-pointed star-shaped surface pattern. Their nucleic acid type is single-stranded RNA. At least five serotypes have been recognized in the UK.

It is generally believed that these viruses are less pathogenic in adults than those belonging to the SRSV group. In two recorded outbreaks it was interesting to note that astrovirus infection occurred a few weeks after an outbreak of a different viral gastro-enteritis. In one instance, of those patients previously infected by rotavirus, 50% were infected by astrovirus in the second outbreak. This suggests that infection with one gastro-enteritis virus may predispose to infection by another (Figure 6.3).

Astroviruses have been detected in normal and diarrhoeal faeces from animals and humans, although they are difficult to cultivate *in vitro*. Partial characterization, however, has been made possible by the extremely large numbers of virions shed in the faeces.

Few outbreaks of gastro-enteritis in adults involving astrovirus infection have been reported, although they are more common among school children and in paediatric wards. In one recent outbreak it was suggested that food contamination provided a plausible explanation for the pattern of infection, although no proof was found of virions in the food.

A number of other viruses have been associated with gastro-enteritis (Table 6.2), and at least one, enteric adenovirus, may be excreted in large numbers in faeces. However, these other viruses have been difficult to cultivate *in vitro* and as yet no food-associated outbreaks have been reported. Further studies and newer technological advances should allow us to understand them more fully.

Table 6.2 Other viruses associated with gastro-enteritis

Virus	*Year described*	*Virion diameter (nm)*	Nucleic acid type
Enteric coronavirus	1975	100–150	ss RNA
Human calicivirus	1976	27–32	ss RNA
Enteric adenovirus	1979	70–80	ds DNA

ss: single-stranded; ds: double-stranded.

6.4 *CRYPTOSPORIDIUM PARVUM*

Cryptosporidium is a genus of protozoa which are pathogenic for man and other animals.

6.4.1 Pathogenesis

Infection follows the ingestion of a small number (probably < 10) of oocysts (cysts forming sporozoites), typically 4–6 μm in size. These banana-shaped motile sporozoites are released in the small intestine, where they adhere to enterocytes of the villi and develop into trophozoites beneath the cell membrane. Fertilization of macrogametes may follow, which results in the production of oocysts. Two types of oocyst can be formed: thin-walled oocysts which release sporozoites into the host's intestine, causing re-infection ('auto-infection') of the host, and acid-fast thick-walled oocysts which constitute approximately 80% of the total, and are released in the faeces (Figure 6.4).

The precise mechanism of pathogenesis is unknown, although the diarrhoea produced is of a secretory nature, with damage to the villi and some resulting malabsorption. However, invasion beyond the host cell-membrane does not usually occur.

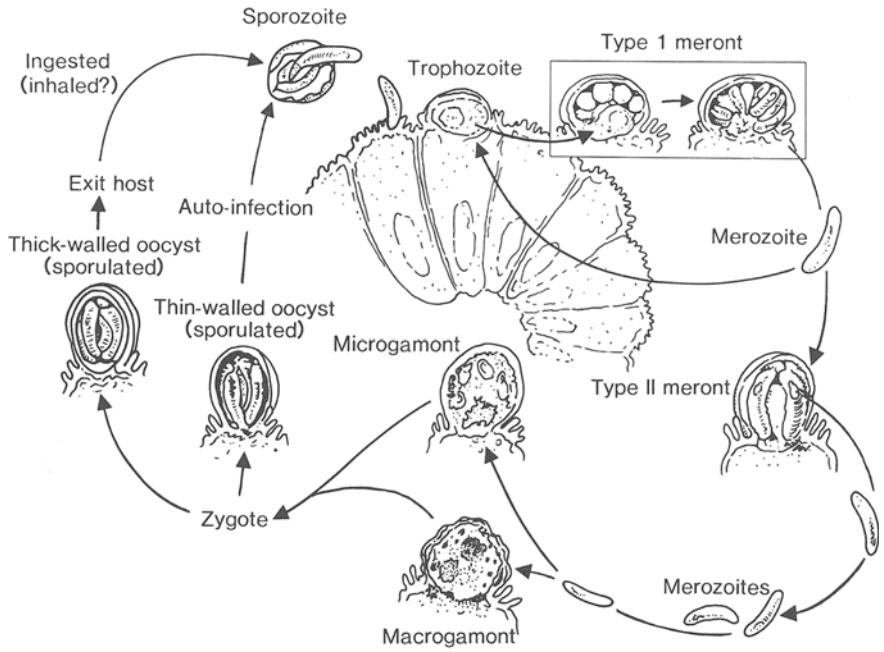

Figure 6.4 Life cycle of *Cryptosporidium parvum*. Source: Casemore, D.P. (1990), *The Lancet*, **336**, 1427–32

6.4.2 Clinical features

Symptoms of cryptosporidiosis in patients without immunodeficiency are diarrhoea of 2 to 14 days', duration, sometimes accompanied by an 'influenza-like' illness and fever. Additional features are nausea, vomiting and abdominal pain, usually after an incubation period of between 4 and 14 days.

In immunocompromised patients, especially those with AIDS, much more severe symptoms of diarrhoea, malabsorption and weight loss have been seen. In Africa the condition known as Slim's disease refers to AIDS patients who suffer the devastating consequences of cryptosporidiosis.

6.4.3 Prognosis

In children, where the symptoms are less severe, the illness is usually self-limiting. However, in immunocompromised patients the illness may be much more severe, with some fatalities.

Rehydration is important in severe cases such as malnourished children and AIDS patients. Chemotherapy is controversial, however, as there have been no definitive studies of proven efficacy, and because of this, cryptosporidiosis is extremely difficult to treat. Certain antibiotics have been shown to shorten the course of infection although relapses may occur. Furthermore, anti-peristaltic agents are usually successful in easing the symptoms of diarrhoea.

6.4.4 Incidence and epidemiology

The first human case of cryptosporidiosis caused by *Crypt. parvum* was described in 1976. This organism is now recognized as an important cause of diarrhoeal disease (including traveller's diarrhoea) worldwide. Initially, infections were associated with immunocompromised patients, especially those with AIDS, although nowadays most infections are seen in young children; infections are generally less prevalent in older children and adults.

Worldwide, there appears to be a much greater incidence of cryptosporidiosis in children in third-world countries than in Western Europe. Since these children are more likely to be malnourished, the disease is of greater severity and can lead to life-threatening dehydration. At the same time there appears to be a seasonal distribution of infections, with a predominance in late spring/early summer.

As food-borne transmission of cryptosporidiosis has only recently been suggested, there are no reported figures for the incidence of this type of food poisoning.

6.4.5 Ecology and foodstuffs

Until recently, cryptosporidiosis was thought to be a zoonotic disease, with cattle and other farm animals being implicated, but it is now well established that the disease may also be transmitted from person to person. Food transmission is thought to be linked with untreated milk and processed meats, as well as contaminated drinking water as a food component.

6.4.6 Control

Fortunately, pasteurization of milk renders the oocysts non-infective. In addition, oocysts are destroyed by heating and freezing, although they are resistant to many disinfectants, including chlorine.

6.5 *GIARDIA LAMBLIA*

Giardia lamblia is a well-recognized cause of diarrhoeal illness, and is the commonest gastro-enteritis parasite of man in the Western World. This flagellate protozoan (synonyms: *G. intestinalis*; *Lamblia intestinalis*) has both trophozoite and cystic stages and usually occurs in immunocompetent patients.

6.5.1 Pathogenesis

As with cryptosporidium, only a few cysts (< 10) need to be ingested to cause infection. The cyst is oval (Figure 6.5), 7–14 μm in length and when mature has four nuclei and a diagonal axoneme (at this size the cyst is approximately

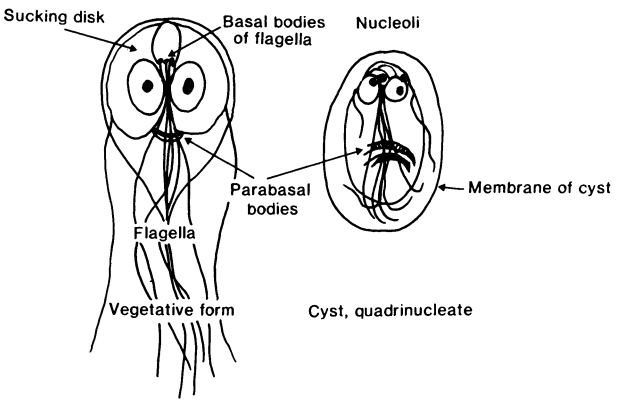

Figure 6.5 Vegetative and cyst forms of *Giardia lamblia*

300 times larger than SRSVs). Excystation and multiplication take place in the small intestine, particularly in the jejunum, where a four-nucleated trophozoite emerges and immediately divides by binary fission. Morphologically, the trophozoite is pear- or kite-shaped, measures 10–20 μm in length, has two oval nuclei and eight flagella, and has a prominant ventral sucking disc (Figure 6.5). The latter enables the parasite to adhere to the intestinal wall of the host, where it may act as a mechanical obstruction of the intestinal mucosa. In some patients the trophozoites become attached to the villi and cause mucosal damage. In contrast to cryptosporidia, giardia not only attach to the epithelium but also penetrate beyond it.

Although the mechanism by which *G. lamblia* induces diarrhoea is not clear, it has recently been reported that it produces a cholera toxin-like protein (which cross-reacts with antibodies to cholera toxin) and activates adenylate cyclase in a manner identical to cholera toxin.

6.5.2 Clinical features and prognosis

Symptoms of giardiasis include diarrhoea, which may develop into steatorrhea (fatty stools) if heavy infestation results in malabsorption of fat in the intestine. Nausea and abdominal pain are often present, although vomiting and the presence of a fever are rare. The incubation period is usually one to three weeks after the ingestion of contaminated food.

Infection is usually self-limiting after a period of a few weeks. In severe symptomatic infestations antimicrobial treatment may be recommended. Metronidazole is still probably the drug of choice, although the combination of metronidazole and mepacrine has been suggested when metronidazole alone fails to eradicate the infection.

6.5.3 Incidence and epidemiology

At least three food-borne outbreaks have been described, all of which have occurred in the USA. Home-canned salmon was implicated in the first outbreak, with noodle salad possibly implicated in the second. In the third outbreak no single food item was thought to be responsible, although there was an association between illness and eating sandwiches and uncooked food.

It is possible that similar outbreaks have occurred elsewhere and have gone undetected because of a number of factors, including problems in establishing a laboratory diagnosis, the long incubation period and a tendency not to consider giardia as a possible agent of food poisoning. Giardiasis is usually acquired by drinking inadequately treated contaminated water or by person-to-person spread by the faecal-oral route, and is transmitted by the ingestion of cysts.

In contrast to cryptosporidiosis, malnutrition does not predispose to a greater severity of infection. However, there is an increased prevalence of giardiasis in some immunocompromised patients, especially those with hypogammaglobulinaemia, where symptoms may be more severe or prolonged.

6.5.4 Ecology and foodstuffs

There appears to be no association between infection and consumption of particular foodstuffs, although very few outbreaks have been reported (see above). It is more likely that problems will arise whenever foods such as uncooked vegetables come into contact with water contaminated with faecal material, which is usually the source of infection.

6.5.5 Control

Normal cooking procedures will kill any infective oocysts present. However, raw food and cross-contamination are hazards, especially when there has been contact with faecal-contaminated water. Obviously, good personal hygiene practices will also prevent direct faecal contamination of foods.

Of particular importance is the fact that the cyst stage is resistant to the chlorine concentrations used in most water treatment plants. Therefore, adequate water treatment should include filtration combined with chemical treatment.