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CRYPTOSPORIDIUM AND CRYPTOSPORIDIOSIS: A BRIEF REVIEW

A. M. A. M. Z. SIDDIKI & M. MASUDUZZAMAN

Department of Pathology and Parasitology; Chittagong Veterinary and Animal Sciences University; Khulshi, Chittagong; Bangladesh

Summary

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Cryptosporidium parvum is an important zoonotic protozoan that has been found in human and animal populations throughout the world. It has a predilection for epithelial cells in the digestive tracts of a wide variety of hosts (humans, livestock, companion animals, wildlife, birds, reptiles and fish). The zoonotic form of infection was also reported by many investigators and led to many cross transmission studies to understand the complex epidemiology of cryptosporidiosis. More importantly, the opportunistic pathogen can produce an infection that may be chronic and even life-threatening for undernourished infants and AIDS patients. The waterborne transmission through drinking water or swimming pool is quite common resulting in a number of outbreaks in many countries worldwide. While many physical and chemical methods are employed to remove the oocysts from drinking water, it is still difficult to completely remove it through any single technique. The parasite has a unique intracellular but extracytoplasmic location and several other unusual features like presence of relict mitochondria, unique life cycle features like production of two types of oocysts, ability to autoinfection and lack of host specificity. Until now numerous *in vivo* and *in vitro* drug trials have been conducted against this important opportunistic pathogen but none was found to be completely effective against cryptosporidiosis. Recent release of complete genome sequences for *C. parvum* and *C. hominis* has facilitated further studies of this pathogen. It is expected that the use of modern bioinformatic tools along with mass spectrometry can be very useful to understand the global proteome of this parasite. Efforts to design a satisfactory treatment of cryptosporidiosis have not been successful due to a lack of understanding of basic cellular and molecular biology of the parasite. Therefore, considerable research is underway to explore the biology of *C. parvum* which will eventually lead to identification of suitable drug targets. In this review we attempted to highlight different biological aspects of *Cryptosporidium* along with present status of research and future directions.

Key words: biology, *Cryptosporidium*, genome, parasite, protozoan

INTRODUCTION

C. parvum is a member of Apicomplexa that has been found in human and animal populations worldwide. People from both developed and developing countries are vulnerable to these important opportunistic protozoa. It has a predilection for epithelial cells in the digestive tracts of a wide variety of hosts which include humans, livestock, companion animals, wildlife, birds, reptiles and fish (O'Donoghue, 1995). The protozoan is responsible for moderate to severe opportunistic infection in both immunocompetent and immunocompromised individuals, the latter group being more susceptible with fatal consequences. The im-

munocompetent individuals usually experience self-limiting disease often manifested by acute profuse, watery diarrhoea accompanied by abdominal pain and other enteric symptoms like vomiting, low grade fever, general malaise, weakness, fatigue, loss of appetite, nausea, chills and sweats. In contrast, the disease may be chronic and even life-threatening for undernourished infants and AIDS patients (Manabe *et al.,* 1998). Other groups at risk include people who have chronic disease, malnutrition or other debilitating conditions, which lead to compromised immune system. Further, the problem becomes more critical and complex by the lack of curative therapy.

Although the first report of *Cryptosporidium* infection in mice was published by Tyzzer in 1907, it was not until the 1980s when it was reported as a cause of death in AIDS patients. The earliest cases of human cryptosporidiosis were diagnosed in animal handlers. An outbreak at a day care centre was first documented in 1983. In 1987, 13,000 people in Carrollton, Georgia became ill with cryptosporidiosis. This was the first report of its spread through a municipal water system that met all state and federal drinking water standards. It also sparked great public health concern after the large human waterborne outbreak in Milwaukee in 1993 when over 40,000 people were affected with nearly 100 deaths (MacKenzie *et al.,* 1995). The total cost of outbreak-associated illness was $96.3 million: $31.7 million in medical costs and $64.6 million in productivity losses. The average total costs for persons with mild, moderate, and severe illness were $116, $475, and $7,808, respectively (Corso *et al.,* 2003). These outbreaks focused attention on the risk of waterborne cryptosporidiosis and the possible need for stricter drinking water standards. Again, the impact of cryptosporidiosis on animals can be devastating, resulting in morbidity, poor growth, and even mortality, with associated economic losses. The important thing is that infection in domestic and wild ruminants provides the biggest sources of environmental contamination. Apparently healthy calves can also become subclinically infected and contribute to oocyst excretion in faeces (Tzipori, 1988). Since the Milwaukee outbreak, until 1998 there have been at least 23 reported waterborne outbreaks of cryptosporidiosis in the UK and North America (Fricker & Crabb, 1998). The *Cryptosporidium* oocysts may remain viable in water for over 140 days. They are very resistant to commonest disinfectants and therefore, difficult to be destroyed by conventional chlorination treatment (Ramirez *et al.,* 2004). The waterborne transmission through swimming pool or public water parks thus plays a prominent role when it is almost impossible to determine the origin of many individual cases of cryptosporidiosis.

HISTORICAL PERSPECTIVE

Cryptosporidium parvum was first identified in laboratory mice in 1907 (Tyzzer, 1907; 1910). Taxonomically *C. parvum* belongs to the phylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order Eucoccidiorida, suborder Eimeriorina, family Cryptosporidiidae (Levine, 1985). However, recent molecular studies have shown that members of the genus are actually more closely related to the gregarines than to eimerias or plasmodium (Carreno *et al.,* 1999)**.** After the first report in mice, in 1955 the organism was recognised as a potential cause of diarrhoea in turkeys (Slavin, 1955). Subsequently it was identified in other animal

species in which the infection was thought to be either opportunistic and harmless or associated with individual cases of diarrhoea (Tzipori & Griffiths, 1998). Cryptosporidiosis has long been a veterinary problem, mainly in young farm animals, such as calves. The first two cases of human cryptosporidiosis were reported in 1976 (Meisel *et al.* 1976; Nime *et al.* 1976). Until 1982 however, *Cryptosporidium* was rarely reported in humans. Later, it has been reported to infect humans of 95 different countries throughout the world (Morgan-Ryan *et al.,* 2002). The number of detected cases began to rise rapidly along with the AIDS epidemic and the development of methods to identify the parasite in stool samples. Until now at least 22 species of *Cryptosporidium* have been named based on host occurrence, parasite morphology, host predilection and site of infection. However, only 13 species are considered valid by most investigators (Ramirez *et al.,* 2004). Among them *C. parvum* is the most commonly reported species with a host range of 152 species of mammals (Fayer *et al.,* 2000). Again, an increasing number of genetically distinct intraspecific variants or genotypes of *C. parvum* have been described, many of which appear to be host-specific and could represent distinct species. *C. parvum* has some unique features like unusual location within the host cell, sequestered between the cell cytoplasm and cell membrane, lack of host specificity, and innate antimicrobial resistance which distinguish it from other enteric protozoa. Unlike other Apicomplexa, *C. parvum* produce two types of oocysts (thin and thick walled) and the thin walled oocyst is responsible for autoinfection within the host. Thus, the repeated first generation schizogony contributes considerably to its pathogenesis.

THE UNIQUE LIFE CYCLE

The life cycle of *C. parvum* has been outlined in a number of reviews (Fayer & Ungar, 1986; Current & Garcia, 1991; O'Donoghue, 1995). In experimental animals, the prepatent period (PPP), i. e. the interval between infection and the first appearance of oocysts in the faeces, is generally 4 days (3 days in heavy infections). However, human infection with low oocyst ingestion can extend the PPP up to 6 days depending on the dose size and frequency. Patency, which is the length of time oocysts are shed in the faeces, generally lasts 6−18 days (4−10 days of diarrhoea) in immunocompetent individuals but may be prolonged in immunosuppressed patients. Some individuals shed oocysts but appear asymptomatic which is the subclinical form of cryptosporidiosis.

Cryptosporidium is monoxenous, that is, its life cycle is completed within one host. The parasite moves from host to host via the faecal-oral route (Fayer & Ungar, 1986). The life cycle begins with the ingestion of oocysts which excyst in the intestine, releasing sporozoites. Thereafter, two cycles of schizogony are followed by gametogony with the production of male and female gametocytes (Tzipori & Griffiths, 1998). The asexual cycle of *C. parvum* life cycle starts with the ingestion of thick walled oocysts from the external source or autoinfection with the thin walled oocysts of earlier infection. The sporozoite is the infective stage and after its invasion into the epithelial cells, schizogony takes place with production of two different types of merozoites. After ingestion, the oocysts excyst in the gastro-intestinal tract releasing the infective sporozoites. Various factors including reducing conditions, carbon dioxide, temperature, pancreatic enzymes and bile

salts are thought to be associated with triggering the excystation (Fayer & Leek, 1984; Reduker & Speer, 1985; Sundermann *et al.,* 1987; Robertson *et al.,* 1993). The release of sporozoites takes place through a slit-like opening created at one end of the oocyst by dissolution of a special suture in the oocyst wall (Reduker & Speer, 1985; Reduker *et al.,* 1985). The sporozoites then are ready to attach with epithelial cells where they become enclosed within parasitophorous vacuoles and develop trophozoites.

VIRULENCE

Among the 13 identified species, *C. parvum* is the major species responsible for disease in humans and domestic animals such as cattle, horses, sheep, goat and pigs (de Graaf *et al.,* 1999). Several virulence factors that appear to be important in *Cryptosporidium* infections have been described and comprehensively reviewed (Okhuysen *et al.,* 2002). It has long been speculated that clinical severity and/or attack rates for *C. parvum* infections might be related to genotype and/or specific virulence differences among isolates. Analysis of genotypic heterogeneity in single and multiple loci has identified 2 major genotypes: genotype 1 (human) which infects primarily humans, and genotype 2 (bovine), which infects both humans and other mammals (Sulaiman *et al.,* 1998; Smith & Ronald, 2001). The genotype 1 is later re-classified as *Cryptosporidium hominis* for several important reasons (Morgan-Ryan *et al.,* 2002). While the two genotypes are similar in their morphology, there are reports of considerable genetic differences (Morgan *et al.,* 1998; Xiao *et al.,* 1999; Sulaiman *et al.,* 2002). It is remarkable that although a number of *Cryptosporidium* isolates can replicate and produce oocysts in a variety of mammals, not all of them develop the same symptoms and sometimes remain asymptomatic. Understanding the factors that determine the infectivity of *C. parvum* and regulate host-specificity is thus very important in the study of the pathogenesis of cryptosporidiosis in man and animals, and in providing information to formulate effective therapeutic and control strategies.

VIABILITY OF *CRYPTOSPORIDIUM* OOCYSTS

The oocyst of the *Cryptosporidium sp.* is double layered and can persist in the environment very well. It can survive in water at temperature range of 4−22°C under ambient conditions. Several authors have studied the viability and infectivity of *Cryptosporidium* oocyst in different environmental conditions (Fayer *et al.,* 1996; Pokorny *et al.*, 2002; Jenkins *et al.*, 2003). A number of chemical and physical methods of oocyst inactivation has been reported where ozone, ultraviolet rays, chlorine, chlorine dioxide, monochloramine, ammonia etc has been tried with variable result (Carey *et al.*, 2004). Further investigation is underway using other physical and chemical methods for complete and effective destruction of *Cryptosporidium* oocysts from drinking water sources.

INCIDENCE OF DISEASE

Immunocompromised hosts

Cryptosporidiosis continues to be a serious problem in immunocompromised patients and particularly in undernourished infants and children. The lack of an effective treatment and the propensity of

the parasite to survive in and be transmitted through water sources are important public health hazard issues. *Cryptosporidium* has been reported in immunocompromised patients with impaired cell-mediated immunity due to primary states like common variable immunodeficiency, hypogammaglobulinaemia, severe combined immunodeficiency, X-linked hyper-IgM syndrome or gamma interferon deficiency. Other patients with secondary deficiencies like HIV/AIDS, organ transplantation and treated with immunosuppressive drugs, haematological malignancies and anti-cancer chemotherapy are also vulnerable to severe *Cryptosporidium* infection (Farthing, 2000). A number of epidemiological studies of cryptosporidiosis in HIV/ AIDS patients have been reported from throughout the world and reviewed by several authors (Angus, 1990; Casemore, 1990; Chacin-Bonilla, 1995; Griffiths, 1998). Prevalence of cryptosporidiosis among AIDS patients has been reported as 3−4% in USA and over 50% in Africa and Haiti. The severity and duration of illness depends on the host's immune status and most infections are chronic and debilitating, contributing to severe dehydration, weight loss and malnutrition, extended hospitalizations and mortality. Moreover, in AIDS patients the infection may spread throughout the bowel, extending into the bile ducts, gallbladder, or other mucosal surfaces exposed via vomiting and/or aspiration (Farthing, 2000).

Immunocompetent hosts

Human cryptosporidiosis has been reviewed by several authors (O'Donoghue, 1995; Griffiths, 1998). In general, everyone is at risk of getting cryptosporidiosis (Keusch *et al.,* 1995) but people from developing countries are more susceptible. The prevalence of cryptosporidiosis has ranged from 0.1−27.1% in developed industrialized countries compared to 0.1−31.5% in less developed countries (O'Donoghue, 1995). The epidemiological investigation reveals that almost 1−4% of patients with diarrhoea in developed countries and up to 16% in less developed countries are associated with *Cryptosporidium* infection. It is thought that the higher prevalence of infection in less developed countries is due to poor sanitation, contaminated water supplies, overcrowding or greater contact with domestic animals (Ungar, 1990). Children are more susceptible to infection than adults and a number of reports mentioned higher incidence of cryptosporidiosis in malnourished compared to well-nourished children (Sarabia-Arce *et al.,* 1990; Garcia-Velarde, 1991; Duong *et al.,* 1991, 1995). Cryptosporidiosis is also reported as a risk factor of childhood death (Molbak *et al.,* 1993). Malnutrition affects cell-mediated immunity while acute cryptosporidiosis leads to malabsorption and anorexia making the condition further complicated.

Domestic and companion animals

Cryptosporidiosis in animals have been reviewed by different authors (Angus, 1983; Tzipori, 1983, 1988; Currant & Garcia, 1991; O`Donoghue, 1995; Olson *et al.,* 2003; Ramirez *et al.,* 2004). Infection of domestic and wild animals provides the biggest source of oocysts, which are responsible for environmental contamination. In USA, *Cryptosporidium* is reported to be present in more than 90% of all dairy farms and 50% or more of all dairy calves will shed detectable number of oocysts (Sischo *et al.,* 2000). Young animals are more susceptible to infection and disease while in adults it is asymptomatic in most cases. Calves are more susceptible shortly after birth and infection

has been reported in both dairy and beef calves (Garber *et al.,* 1994; Xiao & Herd, 1994a; Atwill *et al.,* 1999). The duration of infection is usually short, lasting about two weeks with peak oocysts shedding at second week of infection (Ongerth & Stibbs, 1989; Xiao & Herd, 1994a, 1994b; Kemp & Wright, 1995; O'Handley *et al.,* 1999; Uga *et al.,* 2000). Clinical signs are usually manifested in calves 7−30 days of age with mild to moderate, pale or yellowish diarrhoea, which is accompanied by mucus. The condition can last for two weeks and alongside dehydration, calves become lethargic and anorexic contributing to weight loss. They do not respond to antibiotic therapy and in more severe cases, dehydration and cardiovascular collapse lead to mortality (Olson *et al.,* 2003). However, the healthy calves can be subclinically infected which contributes continuous oocyst excretion in faeces (Tzipori, 1988).

The prevalence of cryptosporidiosis in sheep and goats is similar to that in cattle. The clinical signs and pattern of infection are also identical. *Cryptosporidium* is also an important cause of enteric infection in young lambs and goats. Severe outbreaks with high case fatality rates have been reported by several authors (Tzipori *et al.,* 1981; Angus *et al.,* 1982; Johnson *et al.,* 1999). Cryptosporidiosis in swine and horses is typically asymptomatic and reported worldwide. Prevalence of swine cryptosporidiosis has been recorded as 5% to 21.9% by several investigators from different geographic regions (Tacal *et al.,* 1987; Kaminjolo *et al.,* 1993; Quilez *et al.,* 1996; Olson *et al.,* 1997; Yu & Seo, 2004). A number of surveys indicate a prevalence rate in horses between 0% and 31% (Coleman *et al.,* 1989; Xiao & Herd, 1994b, 1994c; Olson *et al.,* 1997; Majewska *et al.,* 1999; Sturdee *et al.,* 2003). However, most epidemiological studies of cryptosporidiosis have been conducted on humans and cattle ignoring swine and equine populations.

Cryptosporidiosis has also been reported in dogs, cats and other pets and they are important source of infection for children, elderly and immunocompromised owners (Morgan *et al.,* 2000a, 2000b; Fayer *et al.,* 2001). The prevalence of canine cryptosporidiosis varies between 2% and 44% while puppies are more frequently infected (Chermette & Blonde, 1989; Uga *et al.,* 1989; el-Ahraf *et al.,* 1991). Cats with or without diarrhoea have been reported to shed *Cryptosporidium* oocysts and there is a high prevalence of *Cryptosporidium felis* in HIV-infected humans (Pieniazek *et al.,* 1999; Morgan *et al.,* 2000a). Once infected, cats continue to shed large number of oocysts for over 6 months, which provides a threat of potential zoonoses.

Wild animals

Cryptosporidium infection has been reported in a wide range of wild animals including birds, reptiles and fish (Sturdee *et al.,* 1999; Olson *et al.,* 2003). It has been reported that up to one-fifth of oocysts in agricultural drainage is contributed by the wildlife in UK (Hooda *et al.,* 2000). The most common species *C. parvum* has been reported in 155 species of hosts, mostly wild animals and birds. The zoonotic transmission either by direct exposure to contaminated animal faeces or during waterborne outbreaks may become contributory factors for human infection. On the other hand, exposure of domestic and wild mammals to human sewage may be a source of animal infection (Olson *et al.,* 2003). Wild birds are suspected to be the source of infection in farm animals and may also be responsible

for the contamination of surface water. Although wild animals contribute greatly for environmental contamination of *Cryptosporidium* oocysts, not all species are infective for human and domestic animals. Further molecular genotyping of environmental samples is highly essential to determine the impact of cryptosporidiosis in wild animals. This also will increase our understanding on the waterborne transmission of cryptosporidiosis to other domesticated animals and human.

DIAGNOSIS OF CRYPTOSPORIDIOSIS

A number of tests have been developed for the diagnosis of *Cryptosporidium*. These involve direct detection by microscopy of faecal materials after using specialized staining techniques (Garcia *et al.,* 1983). The modified acid-fast (Ziehl-Neelsen) stain and auramine stain are widely used but have the limitation of relatively low sensitivity with faeces (Weber *et al.,* 1991). However, the examination under UV light with a rhodamine filter can increase the sensitivity up to 100 times (Nielsen & Ward, 1999). Use of polyclonal or monoclonal antibodies for detection by immunolabelling was also developed but has proved less sensitive like conventional staining (Garcia & Shimizu, 1997). Recently developed PCR amplification technique has been found very specific and highly sensitive to target different genes encoding oocyst wall protein, the small-subunit rRNA, β-tubulin, thrombospondin-related adhesive proteins of *Cryptosporidium* 1 and 2, internally transcribed spacer 1, polythreonine repeat, dihydrofolate reductase, unknown DNA sequences, mRNA of heat shock proteins (Sulaiman *et al.,* 1999). The efficacy of this technique for detection of *Cryptosporidium* in environmental and clinical samples was also reported in several studies. Certainly, the use of PCR technique with sequence analysis helps the genetic characterization to identify *Cryptosporidium* at species level which can contributes significantly for epidemiological investigations and assessment of sources and risk factors for zoonoses. The detection of oocysts in environmental water samples is a very important issue for the water industry. Additional research is needed for the development of easy, cost-effective, highly sensitive and rapid diagnostic tools for *Cryptosporidium* in clinical and environmental samples.

TREATMENT AND CONTROL OF CRYPTOSPORIDIOSIS

Chemotherapeutic agents

Chemotherapy of human and animal coccidiosis including cryptosporidiosis has been reviewed by Haberkorn (1996), Coombs & Muller (2002), Coombs (1999) and Gargala (2008). Until now, there is no effective treatment for cryptosporidiosis. Supportive therapy involving oral or intravenous rehydration is the important option to alleviate the clinical signs. The efficacy of over 200 therapeutic agents have been studied *in vitro* and *in vivo* by different investigators and reviewed extensively (Fayer & Ungar, 1986; Fayer *et al.,* 1990; Flanigan & Soave, 1993; O'Donoghue, 1995; Tzipori, 1998; Mead, 2002). Only recently, the US Food and Drug Administration (FDA) approved the first ever drug nitazoxanide (Alinia®) to treat diarrhoea of children caused by *C. parvum* and *Giardia lamblia* (Anonymous, 2002). Although nitazoxanide was showed to reduce the duration of diarrhoea and oocyst excretion, its safety

and efficacy in adult and immunosuppressed hosts are still unknown (Bicart-See *et al.,* 2000; Gilles & Hoffman, 2002; Ramirez *et al.,* 2004). The use of highly active antiretroviral therapy (HAART) in persons with AIDS has reduced the prevalence of infection with *C. parvum* and the length and severity of its clinical course. HAART has shown to decrease by 90% the incidence of cryptosporidiosis in the USA. Again, there have been a number of studies evaluating combination therapy (Kimata *et al.,* 1991; Fayer & Ellis, 1993; Giacometti *et al.,* 1996, 1999, 2001; You *et al.,* 1998). A good response was reported after prolonged use of a wide variety of combinations and dosages of protease inhibitors and/or nucleoside analogs that, along with paromomycin, spiramycin, or azithromycin, were examined in HIV-positive patients with chronic cryptosporidiosis (Maggi *et al.,* 2000). Development of effective treatment has been limited by lack of a simple *in vitro* cultivation system to study biochemical and metabolic requirements and a good small animal model of screening the efficacy of drug candidates.

In vitro trials

More than 200 antibacterials or other therapeutic agents were tested *in vitro* for their activity against cryptosporidiosis (O'Donoghue, 1995; Tzipori, 1998; Mead, 2002). In a single study, the efficacy of 101 antimicrobials and other agents were evaluated *in vitro* against cryptosporidiosis (Woods *et al.,* 1996). In another study, anticryptosporidial activity of 71 compounds and their possible cytotoxic effects were assessed using a semi-quantitative screening method (Armson *et al.,* 1999). The *in vitro* system for drug screening has been described using different cell lines and procedures. The most commonly used cell lines are Madin-Derby canine kidney cells (Tzipori & Griffits, 1998), the human lung adenocarcinoma epithelial cell line A-549 (Giacometti *et al.,* 1996), epithelial human colon carcinoma cell line T84 (Flanigan *et al.,* 1991), Caco-2 cells (McDonald *et al.,* 1990) and human colon tumour (HCT-8) cell line (Woods *et al.,* 1996; Armson *et al.,* 1999). As there are many variations regarding cell line types, time of onset of drug treatment in relation to infection with oocysts or sporozoites, days of incubation, methods of fixation, quantitation, analysis, presentation of data etc., they often make it troublesome to compare the results obtained from different investigators (Tzipori, 1998). A highly reproducible *in vitro* test result is thus recommended while a standard *in vitro* culture of *C. parvum* is highly in need.

In vivo trials

Although many compounds have demonstrated activity using *in vitro* assays, only few of them showed therapeutic efficacy in animal models. A number of clinical trials have been reported while many drugs have been tested in experimentally infected animals (O'Donoghue, 1995; Mead, 2002). During late 1980s, the macrolide antibiotic spiramycin was found effective but its toxicity was unacceptable (Portnoy *et al.,* 1984; Saez-Llorens, 1989; Wittenberg *et al.,* 1989). This was one of the first *in vivo* drug trials for cryptosporidiosis. After that numerous drug efficacy studies involving more than 50 therapeutic agents have been performed in animal models or in clinical trials. Among them nitazoxanide and its derivatives are tested extensively in a number of human patients and animal models and were found somewhat effective against cryptosporidiosis (Rossignol *et al.*, 2006). Moreover, combination therapy involving

more than one or two drugs was also investigated with some significant outcome. Although the result was variable for different agents, none was found to eliminate the disease completely or was officially approved for the treatment of cryptosporidiosis (Mead, 2002).

Conventional anticoccidials

Despite the intensive efforts, treatment of cryptosporidiosis with available anticoccidials is still not satisfactory (Haberkorn, 1996; Coombs & Muller, 2002). The macrolides, spiramycin and azithromycin have been found ineffective and less tolerable in both immunocompetent and immunodeficient individuals (Saez-Llorens, 1989; Fayer & Ellis, 1993; Galvagno *et al.* 1993; Vargas *et al.* 1993; O'Donoghue 1995). Diclazuril was tested in humans with unsatisfactory results (Connolly *et al.,* 1990; Soave, 1990). In AIDS patents, letrazuril induced clinical improvements in up to 50% patients and some inhibitory activity on oocyst excretion (Harris *et al.* 1994). In an *in vitro* trial, monensin and halofuginone were found to reduce the parasite multiplication by more than 90% while tested *in vitro* (McDonald *et al.,* 1990). Unfortunately none of the widely used anticoccidials was suitable to treat clinically infected patients while some were suggested for prophylactic use. Still, the search for a new anticoccidial to treat cryptosporidiosis as well as other drug-resistant coccidia continues and the success of the modern drug-discoverer is highly dependent on the unknown, peculiar metabolic features of this important opportunistic parasite. While recent reports suggest *Cryptosporidium* to be rather a member of gregarines than a coccidian (Carreno *et al.,* 1999; Hijjawi *et al.,* 2002), it may offer an explanation to why all anticoccidials are ineffective against *C. parvum*.

Immunotherapy

With the paucity of existing therapeutic agents and their resistance, immunotherapy has been thought to be a significant alternative for cryptosporidiosis and has been reviewed by several authors (O'Donoghue, 1995; Crabb, 1998; Theodos, 1998; Ramirez *et al.,* 2004). It has been found that both innate and parasite-specific cell mediated immune responses are involved in immunity to cryptosporidiosis while most of the components of these responses have not yet been identified. Until now, many of the cytokine immune modulators, interferon-γ, interleukin-12 and other modulators like dehydroepiandrosterone, diethyldithiocarbamate, dialyzable leukocyte extract etc. have been experimentally found unsatisfactory against cryptosporidiosis (Theodos, 1998). Passive immunotherapy using bovine hyperimmune serum and hyperimmune bovine colostrum containing antibodies against *C. parvum* surface proteins as well as antisporozoite monoclonal antibodies have also been tested to treat cryptosporidiosis (Arrowood *et al.,* 1989; Doyle *et al.,* 1993; Okhuysen *et al.,* 1998; Hunt *et al.,* 2002; Riggs *et al.,* 2002). However, these preparations of antibodies have shown only a limited degree of efficacy both in animals and humans. Possibility for a vaccine to treat cryptosporidiosis is also under consideration (de Graaf *et al.,* 1999; Jenkins, 2001).

Novel therapeutic targets

Modern approaches for drug design rely upon identifying possible drug targets. The understanding of distinct features of the parasite metabolism and their metabolic enzymes can be exploited for the design of specific antiparasitic agents. The elucidation of the function and mole-

cular trafficking through the feeder organelle and through the parasitophorus vacuole membrane is also essential to determine the pharmacodynamics of any drug candidate. A number of hypotheses have been reported by several authors to highlight different aspects regarding drug discovery (Tzipori, 1998; Coombs & Muller, 2002; Armson *et al.,* 2003).

One of the recent concerns for the molecular biologists is whether *C. parvum* possesses membrane bound organelles. Preliminary evidences suggest the absence of plastid structures and the presence of relic mitochondrion with some associated functions (Tetley *et al.,* 1998; Riordan *et al.,* 1999, 2003; Rotte *et al.,* 2001; LaGier *et al.,* 2003; Stejskal *et al.,* 2003; Roberts *et al.,* 2004; Slapeta & Keithly, 2004). The similarity of this organelle of *C. parvum* can be compared with hydrogenosomes of trichomonads (Dyall & Johnson, 2000) and the mitosome of *Entamoeba histolytica* (Tovar *et al.,* 1999). While the function of this organelle or its existence as a relic mitochondrion is not clear, its possible involvement in metabolic events can be exploited in search of potential therapeutic targets.

PREVENTION OF CRYPTOSPORIDIOSIS

Prevention is the most effective approach to control cryptosporidiosis. Contamination of water sources is the major source of human infection and thus prevention of environmental spread of oocysts is crucial to check this infection. Water companies should follow the regulatory status and environmental laws regarding safety standards for water for public consumption. Cattle farms should be constructed away from streams and rivers to avoid possible water contamination. Prophylactic measures should be taken to reduce the transmission between animals, as they are the main source of zoonotic infection. This involves effective herd management without overcrowding or reducing stocking density, treatment of infected cattle separately, keeping young animals from the adults, minimizing personnel-calves contact etc. (Ramirez *et al.,* 2004). The destruction of oocysts with 5% ammonia solutions with heat is recommended for cleaning houses (Campbell *et al.,* 1992). Immunosuppressed persons should take special care in avoiding contact with any pets or diarrhoea patients (Juranek, 1995).

PRESENT STATUS & FUTURE NEEDS

In vitro culture of C. parvum

Despite various efforts to develop a suitable *in vitro* culture technique for *C. parvum*, there is limited success for continuous propagation and simultaneous production of oocyst and other developing life cycle stages. Since the first report of *C. parvum* growth in cell culture (Current & Haynes, 1984), a number of papers have been published on its *in vitro* culture by several investigators (Arrowood, 2002). Different investigators used a number of 17 different cell lines and the HCT-8 cell line were reported most suitable when cultivated under 5% CO_2 at 37 ºC in RPMI 1640 medium (Upton *et al.,* 1994; Hijjawi *et al.,* 2001). Unfortunately, the culture of *C. parvum* in cell culture was not effective but labour intensive. Moreover, the culture is influenced by many factors such as host cell type and age, pH, culturing conditions like temperature and CO_2, media supplements etc. which limit the success. In 2001, Hijjawi *et al.* were the first to report a complete

development and long-term maintenance of *C. parvum* human and cattle genotypes in HCT-8 cell culture but it has not been widely practised for several reasons. Recently, complete *in vitro* development of all life cycle stages of *C. parvum* (cattle genotype) has been reported in host cell free culture (Hijjawi *et al.,* 2004) using a modified RPMI-1640 maintenance medium devoid of any cell and this was the first report of *C. parvum* growing extracellularly. However, further study is essential to find out a suitable *in vitro* culture technique, which will be of great value for drug evaluation studies as well as other complex biological investigations.

Genome sequence of C. parvum

The genome sequence projects of *C. parvum* (Abrahamsen *et al.,* 2004) and *C. hominis* (Xu *et al.*, 2004) are now completed and published. They are now accessible through public server domain. Moreover, an expressed sequence tag (EST) project has been initiated for *C. parvum* (Anonymous, 1999). The launch of new integrated database for *Cryptosporidium* genome and ESTs (Anonymous, 2009) also facilitates comprehensive data analysis (Puiu *et al.,* 2004; Anonymous, 2008; 2009). Ultimately, these projects will provide a vast amount of information that will shed light on many of the complex biochemical processes significant for intracellular parasitism. In another attempt, Strong & Nelson (2000) constructed the sporozoite cDNA and genomic DNA sequencing libraries from the IOWA isolates of *C. parvum* and determined ~2000 sequence tags by single pass sequencing of random clones. Together, they reported 567 ESTs and 1507 genome survey sequence (GSS).

Post-genomic investigations

The genome sequence of *C. parvum* has provided a vast hunting ground with enormous possibilities (Widmer *et al.,* 2002; Abrahamsen *et al.,* 2004; Keeling, 2004). The *C. parvum* possesses the most accessible apicomplexan genome. The relatively small size (9×10^6 base pairs) and the presence of few introns greatly facilitate gene identification with the use of simple gene-prediction algorithms. The genome database publicly available through worldwideweb (Amonymous, 2008) offers modern search tools including BLAST, sequence retrieval tool, sequence similarity search, DNA or peptide motif queries, text based gene search facilities etc. The use of comparative genomics with that of *Toxoplasma gondii* can outline a detailed *C. parvum* metabolic map and facilitate further analysis (Striepen & Kissinger, 2004). A complete or partial genome wide comparison of the type I and type II genotype will lead to a better understanding of host specificity and virulence mechanisms in this species. These genomes as well as completed *Plasmodium falciparum* genome will shed light on the origin and evolutionary processes which led to the emergence of these pathogens (Widmer *et al.,* 2002). Thus comparative genomics plays an important role in systematic analysis of different biological questions. That is why a comparative genomics database (Eupathdb) has been launched and is used by different investigators (Striepen & Kissinger, 2004; Striepen *et al.,* 2004).

The availability of complete genome sequence of *C. parvum* has also facilitated the global proteomic analysis of this important parasite (Siddiki, 2006; Snelling *et al.,* 2007; Sanderson *et al.,* 2008). The proteomics can answer numerous biological questions, which is currently impossi-

ble by any other technique. As an example, *Cryptosporidium* lacks an apicoplast (Zhu *et al.,* 2000) and presence of a relic mitochondrion has been confirmed by various reports (Rotte *et al.,* 2001; LaGier *et al.,* 2003; Stejskal *et al.,* 2003; Riordan *et al.,* 1999, 2003; Abrahamsen *et al.,* 2004; Roberts *et al.,* 2004; Slapeta and Keithly, 2004). Recently, Putignani *et al.* (2004) reported a model of highly evolved and functional mitochondrion for *C. parvum* based on ultrastructural evidence, phylogenetic analysis and genome sequence data-mining. They also presented evidence based on the phylogenetic analysis of mitochondrial hsp60 and hsp70 orthologs, indicating their evolutionary relationship with ancestral apicomplexan mitochondrion. However, complementarily to those investigations, the careful identification of all mitochondrial proteins using the proteomics approach would be the only most-reliable option for confirmatory evidence of any mitochondrion-like structure. Thus mitochondrial proteome prediction with identification of metabolic enzyme protein will help us to find out key mitochondrial pathways which will shed light on its mysterious metabolic events.

Efforts to design a satisfactory treatment of cryptosporidiosis have not been successful due to a lack of understanding of basic cellular and molecular biology of the parasite. However, few biochemical studies have been directed towards understanding the unique biochemistry and molecular mechanisms involved during host-parasite interaction and pathogenesis. With recent completion of *C. parvum* genome sequencing projects, it is now time to explore this organism at proteome level. The post-genomic analysis of *Cryptosporidium* thus will help us to improve our understanding about its biology and pathogenesis. This will further provide information towards identifying novel therapeutic targets to combat this most intriguing parasite.

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Correspondence:

A.M.A.M. Zonaed Siddiki, PhD
Associate Professor
Department of Pathology and Parasitology,
Chittagong Veterinary and Animal
Sciences University
Khulshi-4202, Chittagong, Bangladesh
E-mail: zsiddiki@gmail.com