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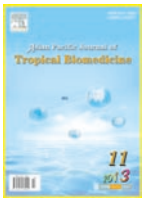

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Cryptosporidiosis as threatening health problem: A review

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PEER REVIEW

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Comments

This is a nice review article that has summarized information on cryptosporidiosis including classic microbiology, epidemiology, clinical manifestations, diagnosis and treatments based on articles published from 1907 to 2013. Information in this article will give physicians and young scientists a comprehensive overview on cryptosporidiosis and this article will be helpful in controlling and prevention of cryptosporidiosis.
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ABSTRACT

The protozoa under the genus *Cryptosporidium* is a zoonotic apicomplexan obligate intracellular parasite. Cryptosporidiosis, the term used to designate infection caused by *Cryptosporidium* sp., is considered as one of the most common food and waterborne diseases with worldwide spread, acting as a common cause of diarrhoea in animals and man. In immunocompetent individuals, *Cryptosporidium* typically induces self-limiting diarrhoea, which may resolve on its own after 2-3 d. However, cryptosporidiosis may turn life-threatening and subsequently lead to death in small children, the elderly and immunocompromised person, especially in AIDS patient. The diagnosis for *Cryptosporidium* infection is usually carried out through examination of stool for the presence of oocysts which measured 4-6 μm with spherical appearance. Morphometric identification is often difficult because of the diminutive size and obscure internal structure of the protozoa. Often, the identification of *Cryptosporidium* is realised through the combination of methods incorporating data from morphometrics, molecular techniques, and host specificity. However, limitations to some of these techniques still exist whether because of cost, duration, expertise, or reliability. Drugs combination is implemented in treatment of cryptosporidiosis. The efficiency of paromomycin, an aminocyclitol antibiotic isolated from *Streptomyces*, can be effective when combined use with protease inhibitors or recombinant IL-12. Since there is no drug that achieves the complete removal of *Cryptosporidium* from the host, supportive therapy was preferred in both human and domestic animals.

KEYWORDS

Cryptosporidium, Prevalence, Taxonomy, Outbreak, Diagnosis

1. Introduction

Cryptosporidiosis is a zoonotic protozoal disease caused by coccidial species of the genus *Cryptosporidium* and which is reported in more than 40 countries in the world[1]. In stool surveys of patients with gastroenteritis, the reported prevalence of *Cryptosporidium* is 1%-4% in Europe and North America and 1%-37% in Africa, Asia, Australia, and South and Central America[2-6]. In most cases, *Cryptosporidium* infection results in gastrointestinal problems such as severe diarrhoea in both immunocompromised and immunocompetent people. Among the five common *Cryptosporidium* species in humans, *Cryptosporidium parvum* (*C. parvum*) and *Cryptosporidium hominis* (*C. hominis*) are responsible for more than 90% of human cases of cryptosporidiosis[7].

Initially, *Cryptosporidium* sp. was reported by Tyzzer as infective in mice[8], and then was pushed to the background until the first human case noted in 1976[9,10]. Afterwards, more concerns over cryptosporidiosis have arisen since it was determined to cause death in one AIDS patient[11]. Mainly, cryptosporidiosis is responsible for acute self-limiting diarrhoea in immunocompetent persons and life-threatening diarrhoea in immunocompromised persons, particularly in persons receiving immunosuppressive drugs and AIDS patients[12].

Among all identified species, *C. hominis* (previously known as the *C. parvum* genotype I) almost exclusively infects humans. *C. parvum* (previously known as the *C. parvum* genotype II) has a wide range of hosts including

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humans and animals and has been considered as a zoonotic species. Furthermore, *Cryptosporidium canis*, *Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium andersoni*, *Cryptosporidium muris*, and *Cryptosporidium suis* have also been isolated from immunocompetent humans[13-15].

Cryptosporidiosis has a higher incidence in developing countries, especially in children, institutionalized patients, malnourished, and immunocompromised individuals (AIDS) [16]. *Cryptosporidium* mostly infects children less than five years old and peaks for children less than two years old[17]. In industrialised countries, cryptosporidiosis also occurs in adults due to foodborne or waterborne outbreaks[7,18]. Currently, nitazoxanide (Alinia) is approved for treatment of cryptosporidiosis in children and immunocompetent adults in the USA. However, nitazoxanide is not effective without an appropriate immune response and is therefore ineffective against immunocompromised individuals[19].

2. Morphology and life cycle

Oocysts of *Cryptosporidium* sp. are small, measuring 4-6 μm in diameter and are spherical-to-ovoid in shape[20]. The life cycle of *Cryptosporidium* is completed in a single host. It involves both asexual and sexual stages. There are six major developmental stages as described by Tzipori and Ward[2], which are excystation, merogony, gametogony, fertilization and zygote development, formation of environmentally resistant oocyst wall, and sporogony (Figure 1)[21].

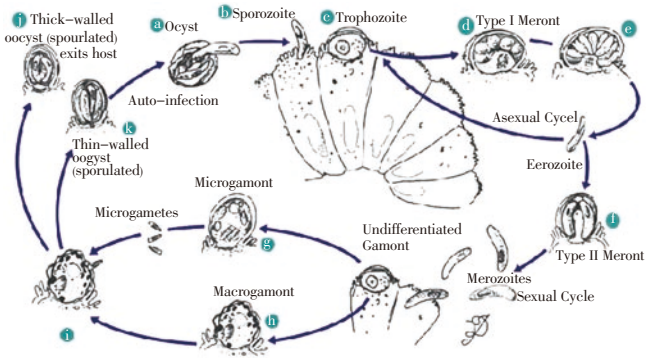

Figure 1. Life cycle of *Cryptosporidium* sp. consisting of asexual and sexual stages.

Infection starts with the ingestion of the oocysts by the host. Oocyst is the exogenous stage, containing four sporozoites within a tough two-layered wall excreted in the faeces[22]. Oocyst stage is environmentally stable, able to survive through routine wastewater treatment and is resistant to inactivation by commonly used drinking water disinfectants[23].

After oocysts are ingested via contaminated food or water, from fomites, or from direct contact with infected persons or animals, the oocyst undergoes excystation, releasing four sporozoites in the small intestine (ileum) which invades epithelial cells[24]. The sporozoites affix to the luminal surface of epithelial cells and differentiate asexually into trophozoites which to produce two different types of meront by merogony[11,24]. Type I meronts form eight merozoites, which enter neighbouring epithelial cells and either develop into type II meront or complete another cycle of type I meronts[25]. Type II meronts produce four merozoites which become microgamonts or macrogamonts[24,26].

Fertilization between gamonts results in formation of a zygote which develops into oocyst containing four sporozoites[24,27]. Two types of oocysts are produced, thick-walled oocysts which are excreted in the faeces and thin-walled oocysts which re-circulate in the intestinal tract causing autoinfection[11,24,26]. This phenomenon may explain the mechanism of persistent infection in AIDS patients in the absence of subsequent oocyst exposure[2].

3. Taxonomy

In 1907, Tyzzer was the first to discover the genus *Cryptosporidium* who described it as a coccidian-like organism in the stomach of mice, which he named *Cryptosporidium muris*[8,28]. Later, Tyzzer discovered a second isolate in the small intestine of mice, which he named *Cryptosporidium parvum*[29]. Hence, species nomenclature within the genus *Cryptosporidium* was initially based on host occurrence, which led to more than 60 species being described[24]. The classification based only on morphology has been difficult as *Cryptosporidium* species are morphologically identical.

Until recently, *Cryptosporidium* was classified as belonging to the family Cryptosporidiidae, suborder Eimeriorina and order Eucoccidiorida[30], which also includes in its ranks *Toxoplasma*, *Cyclospora*, *Isospora* and *Sarcocystis*[31]. To date, over 20 species and 60 genotypes of *Cryptosporidium* have been classified with invalidation of some old ones and introduction of new others[15,32]. Of these, *Cryptosporidium parvum* and *Cryptosporidium hominis* are the most common species causing the disease in human[33] (Table 1).

Table 1
Recognized species in the genus *Cryptosporidium*.

Species	Host	Reference
C. hominis	Human	[34]
C. parvum	Cattle, human	[35]
C. andersoni	Cattle	[36]
C. muris	Rodent	[37]
C. suis	Pig	[38,39]
C. felis	Cat	[40]
C. canis	Dog	[41]
C. wrairi	Guinea pig	[42]
C. baileyi	Poultry	[43]
C. meleagridis	Turkey, human, zoo birds	[13,40,44-45]
C. bovis	Cattle, sheep	[46]
C. galli	Finches, chicken	[47]
C. serpentis	Reptile	[48]
C. saurophilum	Lizard, snake	[14,49]
C. molnari	Fish	[50]
C. scophtalmi	Fish	[51]
C. xiaoi	Sheep, yak, goat	[52]
C. fragile	Frog	[53]
C. ryanae	Cattle	[54]
C. marcopodum	Kangaroo	[55]
C. fayeri	Kangaroo	[57]

4. Pathology and clinical manifestations

Cryptosporidium has been recognized as a cause of gastrointestinal illness in both immunocompetent and immunodeficient people[11,20]. Clinical manifestations of cryptosporidiosis typically include persistent watery diarrhoea, nausea, vomiting, abdominal cramps, and fever[58].

Clinical history and physical examination are quite valuable as preliminary screening for the aetiology of diarrhoea. Food and water consumption could be the source of infection if patients have history of travelling to endemic or epidemic places prior to illness. Acute diarrhoea may point more to rotavirus while persistent diarrhoea is usually caused by protozoa such as *Cryptosporidium* and *Giardia*. Dysentery or bloody diarrhoea may be due to *Salmonella*, *Shigella*, or trauma to the gastrointestinal tract such as ulcer and irritable bowel syndrome. Nevertheless, these classifications are not concrete and sometimes mixed infection is detected in patients, particularly in endemic areas with poor hygiene and sanitation practices.

Cryptosporidium infects tissue on the superficial surface of the intestinal epithelium in the ileum and causes destruction to the epithelial layer[59]. Extensive studies in a piglet model of cryptosporidiosis demonstrate the loss of vacuolated villus tip epithelium accompanied with reduction in glucose-coupled sodium co-transport[60]. *Cryptosporidium* disrupts the intestinal barrier function and increases its permeability, causing impaired absorption and increased secretion of fluid and electrolytes, and nutrients, which leads to malnutrition and watery diarrhoea[61]. Persistent infection in the absence of subsequent exposure to oocysts is quite common, especially among the immunosuppressed patients. Thin-walled oocysts excyst in the intestinal tract without ever leaving the host and have the ability to cause autoinfection[2]. Moreover, attachment of motile sporozoites to the gastric epithelium induced the formation of parasitophorous vacuole and this unique structure provides protection for *Cryptosporidium* from the harsh environment of the host gastrointestinal tract.

In developing countries, where children are more susceptible to infection, cryptosporidiosis in early childhood may be associated with subsequent impaired physical and cognitive development, even in the absence of diarrhoea[62,63]. Cryptosporidiosis is one of the most serious opportunistic infections in AIDS patients. An AIDS patient with CD4 T-cell counts of <150/mL can develop persistent infection, often with profound and life-threatening diarrhoea[12]. The parasite can also spread from the intestines to the hepatobiliary and the pancreatic ducts, causing cholangiohepatitis, cholecystitis, choledochitis or pancreatitis[2,64].

5. Transmission and outbreaks

Cryptosporidium can be transmitted directly via person to person, animal to human, animal to animal, or indirectly by water, food and possibly via air[16]. Animals were considered to be a reservoir of *Cryptosporidium*, with potential for the contamination of household water sources and most human cryptosporiodiosis is associated with *C. parvum* and *C. hominis*[65,66]. In addition, children infected with *C. hominis* shed higher levels of oocysts because they have underdeveloped immune system and oocysts can proliferate easier, possibly contributing to the increased prevalence and spread of *C. hominis* within these communities[13,67].

Infectious oocysts could be excreted with the stool for up to 5 weeks even after diarrhoeal illness ends, which means that the absence of diarrhoea does not necessarily mean that the infection had subsided[68]. Numerous nosocomial outbreaks of cryptosporidiosis have occurred among healthcare workers as well as patients in bone marrow transplant units, paediatric hospitals, and wards with HIV-infected patients[69,70]. Furthermore, elderly hospitalized patients may also be at risk for *Cryptosporidium* infection[71].

Farm animals have long been implicated as sources of human infection. *C. parvum* has been the most prevalent species in farm animals particularly in calves. In sheep, the prevalence ranges from 10% to 68%[72,73] and between 11.0% and 35.2% in goats[74,75]. In the UK, human infection with *C. parvum* dramatically declined after applying several intervention measures that lessened human contact with livestock, which has been previously accounted as a risk factor of cryptosporidiosis[76]. Livestock has also been inferred as the source of waterborne outbreaks in Canada[16] and England[67]. Furthermore, *C. parvum* was the aetiological pathogen in 84% of sporadic cases detected in Scotland, supporting livestock faecal pollution of water sources as the leading cause of sporadic cryptosporidiosis[77]. Associations between animal contact with transmission of cryptosporidiosis in humans have been documented. Outbreaks have been reported in veterinary students and animal researchers working with infected calves; agricultural camps and fairs; and case control studies of human cryptosporidiosis[77-79].

Implicating domestic pets as potential source of infection for human starts gaining deliberation when *Cryptosporidium meleagridis*, a parasite originally described in turkeys, has been detected in human in the UK[67], Thailand[80] and Peru[65]. Dogs and cats seem to be most commonly infected with the predominantly host-adapted *Cryptosporidium canis* and *Cryptosporidium felis*[81] and may act as zoonotic reservoirs for human cryptosporidiosis[82].

During the last decade, *Cryptosporidium* has emerged as an important enteric pathogen and over 150 potentially waterborne pathogens. *Cryptosporidium* is the most notorious in developed countries, responsible for large waterborne disease outbreaks[83,84]. The significance of *Cryptosporidium* to water authorities resulted in introduction of specific regulations and guidelines to deal with this parasite. Many waterborne outbreaks of cryptosporidiosis have been reported in different countries[85,86]. *Cryptosporidium* sp. has been responsible for waterborne outbreaks associated with water intended for drinking in the United States[58,87]. These outbreaks have occurred in water systems that used

well and spring water treated solely by chlorination and in surface water systems that have been filtered. The first reported waterborne outbreak of cryptosporidiosis was in the summer of 1984 in San Antonio, Texas[88]. The largest documented waterborne disease outbreak was in 1993, which infected 403 000 people and more than 100 died with weakened immune systems in Milwaukee[58]. Subsequent analysis revealed that the Milwaukee outbreak was due to *C. hominis*[89]. Although cattle have been repeatedly implicated as sources of waterborne cryptosporidiosis outbreaks, genotyping the contaminating isolates in Milwaukee outbreak has implicated human effluent as the source of contamination[89]. Swimming pool-associated outbreaks of cryptosporidiosis have also been widely reported[83,90].

Foodborne outbreaks of cryptosporidiosis have been accounted worldwide. The main sources of food contamination comes from consumption of improperly cleaned raw fruits, vegetables and shellfish; faulty processing of meat, beverages and other foodstuffs; unhygienic food handlers; and contamination via vectors such as insects and birds (Table 2)[91,92].

Table 2
Food-borne outbreaks of cryptosporidiosis.

Country	Source	No. of cases	Reference
Mexico	Cow's milk	22	[93]
USA	Chicken salad	15	[94]
UK	Cow's milk	50	[95]
USA	Apple cider	154	[96]
USA	Green onions	54	[97]
USA	Apple cider	31	[97]
Spain	Clams/mussels/oysters	–	[98]
Spain	Mussels/cockles	–	[35]
USA	Fruit/vegetables	148	[99]
Australia	Cow's milk	8	[100]
USA	–	24	[101]
USA	–	88	[101]
USA	Mussels	–	[102]
USA	Apple cider	23	[97]
Japan	Raw meat and raw liver dish	4	[103]
Denmark	Salad buffet	99	[104]
Finland	Salad	72	[105]
Chile	Snails	–	[106]
Sweden	Fresh herbs	–	[39]

–: Unknown.

Genotyping analysis of outbreaks in USA from apple cider identified *C. parvum* which revealed that the outbreak may have been due to dropped apples on the ground contaminated with cattle faeces[96]. A recent investigation of food handler contamination of a salad in Denmark identified *C. hominis*[104]. Another foodborne outbreak in Japan was caused by eating raw beef and liver contaminated with *C. parvum*[103].

The nature of *Cryptosporidium* sp. oocyst makes it quite resistant to the changes in the environment. It is extremely resistant to chemical disinfection and not effected by chlorine, chloramines, and chlorine dioxide permissible in drinking water treatment[107]. Ozone is quite potent as chemical disinfectant for *Cryptosporidium* oocyst, but also more hazardous[108]. Overexposure of ozone can give rise to formation of high concentrations of possibly genotoxic by-products. Oocysts can survive for months in surface water and soil and still remain viable for infection[109,110].

6. Diagnosis

A wet mount using saline and/or iodine is the basis of all microscopic technique. Routine diagnosis of cryptosporidiosis in most countries has been based on microscopic detection of oocysts after staining of faecal smears. Concentration methods using the principals of flotation and sedimentation have been widely used, with solutions such as sucrose, salt, zinc sulphate and formol-ether[111,112]. The staining methods of most commonly used are the modified Ziehl-Neelson acid-fast stain and modified Kinyoun's acid-fast stain[113,114]. Differential staining methods such as safranin-methylene blue are also used[115]. Negative staining techniques with nigrosin[116], light green, merbromide[117] and malachite green[118] stains yeasts and bacteria but not oocysts. These techniques, despite being easy to use with low cost, unfortunately can not distinguish between species of *Cryptosporidium*[56](Figure 2).

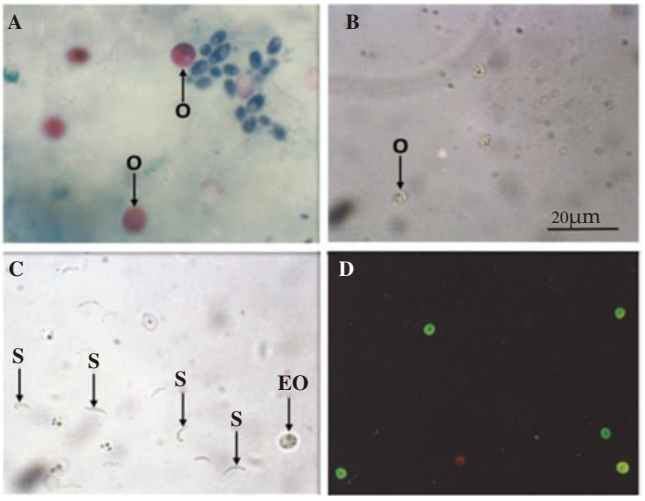

Figure 2. Microscopic observation of *Cryptosporidium* sp.
(A) Modified acid-fast staining 100×, (B) Wet mount 40×, (C) Wet mount 40×, (D) Fluorescent monoclonal antibody staining 20×. O=oocyst, S=sporozoites, EO=empty oocyst. (Source: Latif B, unpublished data).

Immunological based antigen detection methods that use antibodies to detect intact oocysts have been developed. Monoclonal antibody test is preferable to polyclonal antibody since it showed no cross-reactivity with other species[119]. Immunofluorescence-based kits, using a fluorescein isothiocyanate-conjugated anti-*Cryptosporidium* monoclonal antibody that recognises exposed surface epitopes of oocysts are more specific and also sensitive for detecting *Cryptosporidium* oocysts. Indirect fluorescence antibody tests show superior sensitivity compared to modified acid-fast stain[120]. However, it is expensive and needs specific equipment like fluorescence microscope. Antigen detection using enzyme linked immunosorbent assays and passive agglutination has also

been developed[121]. However, antibody cross-reaction and the costs associated with such techniques have limited their use[14]. Although enzyme linked immunosorbent assays showed sensitivities ranging from 66% to 100%, false positive results have been reported[114].

The introduction of polymerase chain reaction technology provides specific diagnosis to the species level with high sensitivity. Polymerase chain reaction allows swift, repeatable and highly accurate examination, with the ability to analyse large number of samples[16]. A variety of gene loci are used in current DNA diagnostics and taxonomy of *Cryptosporidium*, including the small subunit rRNA (also known as 18S rRNA)[33,47,56,122], heat shock protein (HSP 70 gene)[123], *Cryptosporidium* outer wall protein gene[122], gene for thrombospondin-related adhesive protein of *Cryptosporidium*-1[124], and actin gene[125].

7. Treatment

In 2006, the only drug approved by the US Food and Drug Administration for treatment of cryptosporidiosis in children and immunocompetent adults is the anti-protozoal agent nitazoxanide (Alinia)[25]. However, nitazoxanide is not effective without an appropriate immune response and is therefore ineffective against immunocompromised individuals[19].

Recovery and survival rates has been dramatically improved with the use of highly active antiretroviral therapy, which cause increased CD4+ T-lymphocyte counts in immunocompromised individuals[25]. The introduction of protease inhibitors in highly active antiretroviral therapy also has additional benefits, as protease inhibitors appear to directly interfere with the life cycle of the parasite[126].

Drugs combination is implemented in treatment of cryptosporidiosis. The efficiency of paromomycin, an aminocyclitol antibiotic isolated from *Streptomyces*, can be effective when combined use with protease inhibitors[126] or recombinant IL-12[127].

Since there is no drug that achieves the complete removal of *Cryptosporidium* from the host, supportive therapy was preferred in both human and domestic animals. This consists of replacement of fluid and electrolytes, nutritional support, and anti-diarrhoeal drugs[128].

8. Prevention, control and conclusion

Cryptosporidium sp. could infect its host by three main routes. The first is through contamination of raw food ingredients from farms or abattoirs. The second is through contaminated water whether from unprocessed sources (rivers, wells) or from water treatment plants. The third is transfered from infected host such as unhygienic food handlers, pets, or pests like flies and cockroaches[129,130].

Taking preventive measures is the best way to manage *Cryptosporidium* sp. infection. Raw vegetables and food must be washed thoroughly before consumption. Clean tongs or utensils should be always used at a salad bar to prevent contamination from handlers' or patrons' hands. Water, especially untreated surface water, should be boiled before drinking to kill any oocysts in it. Water treatment plants have to be monitored regularly to prevent defective treatment and subsequent outbreak to houses receiving the water supply. Hands must be washed after contact with uncooked meat, soil, or animals before eating or touching around the mouth area. Pastures need to be fenced properly and the water bodies inside pastures (ponds or lakes) must never mix with the municipal water sources or reservoirs in order to prevent cross-contamination.

Outbreaks of cryptosporidiosis should be investigated quickly and the source isolated or quarantined to prevent further infection. Babies, toddlers, and diarrheic individuals must limit their contact with recreational water or use only pools made specifically for them. Train pets to defecate outside the house and bath them regularly, especially if there is any children teething and crawling around in the house. These measures may seem trivial at first glance, but they are in reality very important in minimising the risk of getting cryptosporidiosis, particularly in those with underdeveloped or immunocompromised immune system like children, the sick, and the elderly.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Cryptosporidiosis is a parasitic disease caused by coccidial species of the genus *Cryptosporidium*. Cryptosporidiosis has become recognized as one of the most common causes of waterborne disease in humans in every region of the world including developed and undeveloped countries.

Research frontiers

This review article has overviewed the pathogenesis, distribution, diagnosis and treatment of cryptosporidiosis. Information in this article will give physicians and young scientists a comprehensive overview on cryptosporidiosis and this article will be helpful in controlling and prevention of cryptosporidiosis.

Related reports

This article has selectively reviewed 132 articles selected from a large number of original articles and review articles

on cryptosporidiosis and its pathogen. This article has updated the information on cryptosporidiosis compared to other review articles.

Innovations and breakthroughs

In this article the authors have summarized information on cryptosporidiosis including classic microbiology, epidemiology, clinical manifestations, diagnosis and treatments for cryptosporidiosis. The information is based on articles published from 1907 to 2013.

Applications

This article provided information on the research on cryptosporidiosis. The updated information in the article including pathogenesis, diagnosis and treatment of cryptosporidiosis will be very helpful to physicians who do not have time to go through large quantities of articles.

Peer review

This is a nice review article that has summarized information on cryptosporidiosis including classic microbiology, epidemiology, clinical manifestations, diagnosis and treatments based on articles published from 1907 to 2013. Information in this article will give physicians and young scientists a comprehensive overview on cryptosporidiosis and this article will be helpful in controlling and prevention of cryptosporidiosis.

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