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CRYPTOSPORIDIOSIS-AN EMERGING ZOONOSIS

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Cryptosporidiosis,a disease of neonates, is caused by the species of *Cryptosporidium*-an eukaryotic coccidian protozoan which parasitises a wide range of vertebrates including human beings.Profuse mucoid or haemorrhagic diarrhoea characterises the disease. The present communication attempts to highlight different aspects of this emerging disease including application of molecular and immumological techniques in understanding its virulence and pathogenesis.

INTRODUCTION

Cryptosporidiosis is a disease of neonates and responsible for significant morbidity and mortality (Ajjampur *et al*., 2008). The disease is caused by several species of *Cryptosporidium*, an eukaryotic coccidian protozoan parasite which has prevalence in a wide range of vertebrates including humans worldwide. The disease is characterized by profuse mucoid or haemorrhagic diarrhoea which may last for about a month. The infection is self-limiting in immunocompetent individuals; however, it can be quite serious and potentially life-threatening in immunodeficient patients. *Cryptosporidium muris* and *C. parvum* were the first species described (Tyzzer,1907; 1912). The association of *C. meleagridis* with morbidity and mortality in turkey (Slavin. 1955) and *C. parvum* with clinical diarrhoea in lambs and calves (Panciera *et al*., 1971), the veterinary importance of *Cryptosporidium* was established. Since then several species of *Cryptosporidium* have been identified in a wide variety of animals ranging from fish to humans. The first human case of Cryptosporidiosis was reported in 1976 (Meisel *et al*., 1976; Nime *et al*. 1976) and thereafter it is now recognized as one of the important causes of diarrhoea in humans. The organism has attracted special attention with the increase in number of HIV infected individuals. Through application of molecular and immunological techniques, considerable advancement has been made in our understanding of *Cryptosporidium* virulence and pathogenesis in recent years.

LIFE CYCLE

The cryptosporidia follows a typical monoxenous coccidian life cycle with a marked difference of not invading the enteric epithelium. The sporulated oocysts of *Cryptosporidium* are voided through the faeces of the infected host. Each oocyst contains four sporozoites which are free and not restricted within a sporocyst. The infection is acquired through ingestion of sporulated oocysts in contaminated drinking water or through faeco-oral route. The sporozoites emerge from the oocyst and attach to intestinal epithelial cells. In sharp contrast to other coccidia, *Cryptosporidium* sporozoites do not invade the enteric epithelial cells; instead they induce the fusion of the microvilli and thereby creating a niche for itself extracellularly surrounded by a double membrane of host origin. The location is in fact a junction formed between the parasite and the host enterocyte termed as 'feeder organelle' or the 'adhesion zone'. The sporozoite eventually transforms into a trophozoite which divides asexually (merogony) and produce 4-8 merozoites which are released into the intestinal lumen. The merozoites form new adhesion zones using fresh uninfected intestinal epithelial cells and undergo additional rounds of merogony. The increased severity of the disease in immunocompromised patients is due in part to their inability to limit these additional rounds of merogony.

Following few cycles of merogony, a sexual reproduction is initiated. The merozoites develop into either macro- or microgametocytes following the infection of an enterocyte. Microgametogenesis involves several rounds of replication followed by the release of numerous microgametes into the intestinal lumen. The microgametes fertilize macrogametes still attached to the intestinal epithelial cells leading to formation of zygotes. The resulting zygote undergoes sporogony and the sporulated oocysts are excreted through the feces (Fayer *et al*. 1997). An autoinfection is also possible which may contribute to the increased disease severity in immunocompromised patients.

EPIDEMIOLOGICAL IMPLICATIONS

Cryptosporidium parvum and other species are important from zoonosis point of view. The monoxenous lifecycle, wide host range of the parasite and excretion of large number of oocysts (up to 100 billion from a single infected calf) contributes immensely in the spread of the disease. Several ecological and parasitological factors, *viz*. close association between human and animal hosts, excretion of large number of infective sporulated oocysts, low infective dose and comparatively higher resistance of oocysts to disinfectants, *viz*., chlorine, further contribute favourably to the process of transmission. Besides transmission through contaminated drinking water, horizontal human transmission is no less important. Since the asymptomatic infected children are important source of infection in the household, secondary cases of infection in households are high. Therefore, widespread infections are not uncommon in hospitals, institutions and day care centers where

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the surroundings favour faeco-oral transmission. It is appropriate to refer here the outbreak of cryptosporidiosis in Milwaukee during the spring of 1993 when about 400,000 people developed symptomatic cryptosporidiosis (MacKenzie *et al*. 1994). The prevalence of *Cryptosporidium* in the Indian livestock is quite high. A recent PCR based survey revealed a 30.2% infection among neonatal bovine calves across three different regions of India with *Cryptosporidium* spp (Paul *et al*. 2009; 2008).

Molecular genotyping has revealed the existence of two primary genotypes in humans. Genotype 1 of *Cryptosporidium* sp. has only been isolated from humans and shown to be non-infective for mice and calves, whereas genotype 2 has been isolated from both animals (bovine and ovine) and humans and is infective for mice and calves. Lately, based on molecular genotyping studies and existence of some other biological differences in the population, it has been proposed to rename genotype 1 as *Cryptosporidium hominis*. However, some other species and genotypes of *Cryptosporidium viz, C. felis*, dog-like genotype, etc., have been isolated from AIDS patients and infrequently from immunocompetent humans (Morgan *et al*. 2000). Genetic evidences are suggestive of involvement of two different populations of *Cryptosporidium, viz., C. hominis* and *C. parvum* in two distinct transmission cycles in humans. *C. hominis* has an exclusively anthroponotic (i.e., human-to-human) cycle, whereas *C. parvum* has a zoonotic cycle. The zoonotic cycle may initially involve transmission from animals, *viz*., cartle or sheep to humans and then subsequently human-to-human transmission and possibly a human-to-animal transmission. Both of the species have been incriminated with the waterborne outbreaks of cryptosporidiosis. *C. hominis* linked waterborne outbreaks may be associated with contamination of water with human sewerage, whereas waterborne outbreaks associated with *C. parvum* are likely due to contamination of water with cow or sheep feces.

CLINICAL SIGNS

The most common clinical manifestation of cryptosporidiosis is a mild to profuse watery diarrhoea. This diarrhoea is generally self-limiting and persists from several days up to one month.Recrudescence is common. Abdominal cramps, anorexia, nausea, weight loss and vomiting are additional manifestations of the disease which may occur during the acute stage. The disease may be much more severe in immunocompromised persons where diarrhoea is chronic and may be lasting for months or even years. Some AIDS patients exhibit a fulminating cholera-like illness where intravenous rehydration therapy becomes imminent. The fatality rate can be quite high in fulminating infections.

DIAGNOSIS

Cryptosporidiosis is primarily a disease of newborns. Pathognomonic sign for disease diagnosis is absent and therefore, laboratory confirmation is essential. The infection is confirmed by demonstration of sporulated oocysts in the feces (Fig.1). Absence of sporocyst is characteristic and four free sporozoites are present in the oocyst. Acid-fast staining is the preferred method for microscopical examinations which stains *Cryptosporidium* bright red.

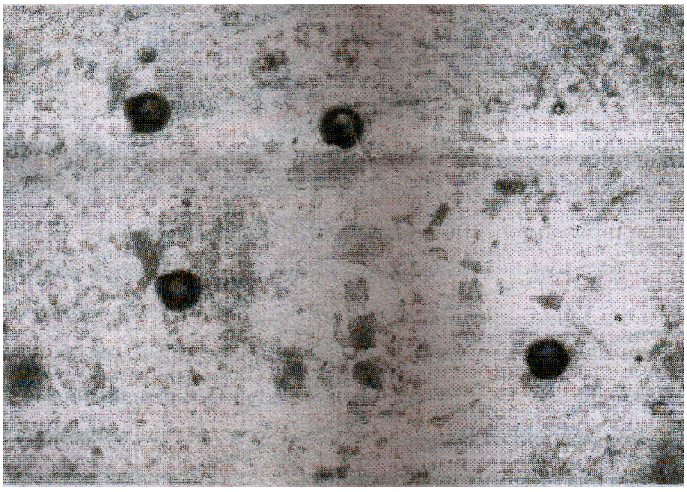

Fig. 1. Acid fast staining shows oocysts of *Cryptosporidium parvum* in the diarrheic faeces of calf o with clinical signs of profuse watery diarrhoea (1000X)

Serological assays for oocyst antigen capture or immunochromatographic lateral flow (ICLF) assays are currently used (Jex *et al*., 2008); however, positive reactions need to be confirmed by using a suitable confirmatory test (Chalmers *et al*., 2010). PCR based detection of Cryptosporidium DNA has also been reported (Bouzid *et al*. 2008)

TREATMENT

Chemotherapeutic treatment against *Cryptosporidium* is generally not satisfactory because of the 'extracytoplasmic' location of *Cryptosporidium*. Several anti-coccidials have been tried with variable results. Paromomycin has been used for the treatment of cryptosporidiosis; however, its efficacy is questionable. Controlled studies revealed that paromomycin can suppress parasitemia in immunocompromised individuals. Supportive therapy, *viz*., rehydration and nutritional support, in acute cryptosporidiosis is extremely important.

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