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ZOONOTIC ASPECTS OF CRYPTOSPORIDIOSIS IN NEPAL

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Abstract

This article reviews cryptosporidiosis, a common protozoan disease in humans and animals in Nepal, acquired by ingestion of oocysts that were excreted in the feces of infected individuals. Contaminated water represents the major source of *Cryptosporidium* infections for humans and can be transmitted from person-to-person, from animal-to-person, animal-to-animal, by ingestion of contaminated water and food or by contact with contaminated surfaces. Being highly resistant to environmental and chemical processes and representing the only group of pathogen surviving chlorination, it has no effective chemotherapy identified for the treatment which makes cryptosporidiosis a debilitating and persistent disease with high potential of transmission among immune-compromised ones like children and AIDS patients. Various reports have been documented for potential transmission of the oocyst among humans and animals in Nepal through common niche like river water, tap water sources and also from wild animals through the interaction on buffer zones. Literatures reported prevalence rate of 16 % in Children, 11% in HIV infected patients in case of human; whereas, 14% in Calves, 19% in buffaloes and 12.5% in swamp deer in case of animals in Nepal. In conclusion, the persistent shedding of oocysts by reservoir hosts like calves, kids, poultry and wild animals like deer and monkeys possess great threat to the transmission to general public. The epidemiological studies of cryptosporidium and the knowledge of the pattern of the disease outbreak can guide therapy and effective preventive measures against this disease.

Keywords: *Cryptosporidium,* immuno-suppression, zoonosis.

Introduction

Cryptosporidium sp are common parasites of humans, domestic animals and wild vertebrates. Cryptosporidiosis has been considered to be a zoonotic disease for due to the wide host range of *Cryptosporidium* sp. The role of animals, especially farm animals and domestic pets, in the transmission of human cryptosporidiosis is nevertheless not clear. The coccidian protozoan parasite, *Cryptosporidium* is an oval or spherical in shape and measuring 2-6μ in diameter. It was believed to be an enteric pathogen with a worldwide in distribution. *Cryptosporidium* infection rates were predicted to be the highest in developing countries and in children (Snelling, 2007).

In Nepal, the first report of human Cryptosporidiosis was from a three years old boy with chronic diarrhea at Kanti Children Hospital, Kathmandu brought in relation to rota virus infection (Sherchand *et al.,* 1992). In Nepal, very little information is available on the prevalence of Cryptosporidiosis. It was reported that very high prevalence of *C. parvum* was found in the different parts of Nepal such as Jomsom (17%), Kathmandu valley (17.5%) and Chitwan (14.6%) (Sherchand *et al.*, 1995). Out of 31 outbreak cases of *cryptosporidium* from 2002-2004, 3 were school outbreaks (16 positive samples, 4 school trip outbreaks (8 positive cases), 3 religious ceremony outbreaks (7 positive cases) (Ghimire *et al*, 2005). An Infection caused by *C. parvum* occurred in people of all ages but most of cases were reported within children less than 5 years. Prevalence rates of *C. parvum* among patients with diarrhoea showed clinical reports with laboratory diagnosis from 1 to 20 percent (Sherchand and Shrestha,1996). Cryptosporidiosis in Nepal is more commonly seen during warm rainy season, which reflects the increased oocysts contamination of surface and domestic water supplies (Ghimire *et al.*, 2005)

The *cryptosporidium* in wild and domestic animals was also reported in Nepal. Among the wild animals the highest prevalence was observed in Deer which was 71% and followed by Rhinoceros which is 25%. The calves and buffalo calves were suffering from 34 and 37% respectively (Karna, 2010). *Cryptosporidium rynae* was observed in some zebu cattle and water buffaloes in the buffer zones of Chitwan national Park (Feng *et al.*, 2012).

Global scenario

Out of the 71 *Cryptosporidium*-linked outbreaks described in the decade 2000-2010, 40 (56.3%) appear to be correlated to waterborne diseases, with a distribution almost constant throughout the years, marked by picks in 2000, 2001, 2002, and 2007 (Putignani and Manichella., 2010). Geographically, the outbreaks seem to be concentrated in the USA, Canada, Australia and Europe, especially in the UK and Ireland and appear to affect both adults and children. Worldwide environmental and veterinary surveillance data reveal the presence of *Cryptosporidium* sp in the entire water treatment system, which represents an unacceptable health risk, particularly in sensitive (pregnant women, children) and immunocompromised populations (HIV-positive and transplanted patients. In Asia, the emerging need to facilitate the characterization of the endemic transmission of cryptosporidiosis has recently provided a large study on genotype distributions of *Cryptosporidium* oocysts in domestic waste water in China (Putignani and Menichella, 2010). Various reports for the occurrence of Cryptosporidium causing human disease in developing countries are listed in table-1.

***Cryptosporidium* species in humans**

Cryptosporidium parvum was once considered to be the only *Cryptosporidium* species to infect humans and these eventually became *Cryptosporidium hominis*, both infectious for immuno-competent and immuno-compromised persons (Xiao *et al.*, 2004). It is likely that other *Cryptosporidium* species can infect humans under certain circumstances. *Cryptosporidium muris*-like oocysts were found in two healthy Indonesian girls (Katsumata *et al.*, 2000). Many cases of *Cryptosporidium* in human has already described in introduction section in this review, whereas their modes of transmission are described below.

Table 1: Occurrences of human-pathogenic *Cryptosporidium* species in humans in developing countries

Location	Patient type	Reference
Brazil	Children	Bushen *et al.* (2007)
China	Children	Peng *et al.* (2001)
Colombia	HIV+adults	Navarro-i-Martinez *et al.* (2006)
Guatemala	Children	Xiao *et al.* (2004b)
Haiti	HIV+adults	Ngouanesavanh *et al.* (2006)
India	HIV+adults	Muthusamy *et al.* (2006)
India	Children	Gatei *et al.* (2007)
India	Children	Ajjampur *et al.* (2007)
Kenya	HIV+adults	Gatei *et al.* (2003)
Kenya	Children	Gatei *et al.* (2006)
Malawi	Children	Peng *et al.* (2003)
Malawi	Children	Morse *et al.* (2007)
Nepal	HIV+children	Amatya *et al.* (2011)
Nepal	Children +chronic diarrhea	Sherchand *et al.*, 1992
Nepal	Children +chronic diarrhea	Sherchand *et al.*, 1995
Nepal	children	Ghimire *et al*, 2005
Peru	HIV+adults	Cama *et al.* (2003)
Peru	Children	Xiao *et al.* (2001)
Peru	Children	Cordova Paz Soldan *et al.* (2006)
South Africa	HIV+children	Leav *et al.* (2002)
South Africa	All	Samie *et al.* (2006)
Thailand	4 HIV+children, 25 HIV+adults	Tiangtip and Jongwutiwes (2002)
Thailand	HIV+adults	Gatei *et al.* (2002)
Uganda	Mostly HIV+children	Tumwine *et al.* (2005)
Uganda	Children	Tumwine *et al.* (2003)
Venezuela	HIV+adults	Certad *et al.* (2006)

Transmission by Drinking water

Among the parasitic protozoa, *Cryptosporidium* oocysts have the greatest potential for transmission through drinking water because (a) human infective oocysts are widely distributed in the environment, (b) oocysts can penetrate physical barriers in water treatment processes and are disinfectant resistant and (c) *Cryptosporidium* have a low infectious dose for humans, although some human isolates are less infectious or cause different clinical signs and symptoms than others. The contributors to environmental contamination include infected human, livestock and feral hosts as well as transport hosts. Contaminated water represents the major source of *Cryptosporidium* infections for humans and can be transmitted from person-to-person, from animal-to-person, animal-to-animal, by ingestion of contaminated water and food or by contact with contaminated surfaces (Ghimire *et. al.*, 2005). The prevalence of *cryptosporidium* was reported to be 13% in sewage water 2% in river water and 0% in pond and well water and 1% in municipal water supplies from 2002 and 2004. The total prevalence of *cryptosporidium* in different water sources was 4.4% oocysts voided in faeces can contaminate water directly, or indirectly, and the disposal of human and animal wastes remains a significant public health issue that has yet to be assessed or controlled in many parts of the world (Ghimire *et al.*, 2005) . The rate of fecal contamination of drinking water samples collected from different sites in Kathmandu valley was 75% in Kathmandu valley and 86% in rural hilly villages (Matsumura *et al,* 1998). Transmission of *Cryptosporidium* through drinking and recreational water is well documented, as are outbreaks of cryptosporidiosis and giardiasis following the consumption of contaminated water.

Foodborne Transmission

C. parvum oocysts can survive for several weeks in fields amended with livestock wastes, and have the potential for contaminating subsequently grown food crops, via run-off and/or contamination of water courses used for crop irrigation. Oocyst contamination of herbs, surface waters used for salad crop irrigation, and wash water at packing houses has been described. Mechanical transmission to crops or foodstuffs, in the faeces and on the bodies of filth flies or in the faeces of refuse eating and coprophagous birds and mammals has also been described. Livestock faecal and slurry discharges and run off from spreading farmyard manure can play a major role in oocyst contamination of shellfish in fresh water, estuarine and marine, coastal environments. Heat treatments used for surface decontamination of beef carcasses are effective in inactivating *C. parvum* oocysts which reduces the risk of transmission following the consumption of inadequately cooked meat, contaminated at slaughter.

In Nepal, River water is the main source for cleaning raw vegetables before marketing in Nepal. Polluted water used for the cleaning purpose worsen the situation by contaminating the food itself. Vegetables are suspicious since they are often ingested raw or under cooked. They are easily contaminated and provide organism with an optimal environment for the survival prior to host ingestion. The *cryptosporidium* were detected in 16.7% in washing of radishes, 3.3% in cauliflower, 13.3 % in washing of mustard leaves with the frequency (Ghimire *et al*, 2005) this has been attributed to the use of livestock manure in the agriculture fields and water contamination with animal feces.

HIV patients

Cryptosporidiosis in immunocompetent hosts is usually mild, selflimiting and recovers within a few weeks. In contrast, the infection may have a severe, chronic and even fatal clinical course in immunocompromised individuals, such as those with AID. Laboratory-confirmed cryptosporidiosis of greater than one month's duration in an HIV-infected person is an AIDS defining condition (Castro *et al.*, 1992).The prevalence was found to be 6.8% in non-HIV/AIDS individuals in Nepal (Sherchand and Shrestha, 1996).An overall prevalence of 10.7% of cryptosporidiosis was noted in HIV/AIDS patients during this study. Sherchand and Shrestha, (1996) screened three hundred and fifty-four soft, loose or watery stool specimens from patients in Nepal with acute diarrhea were for the presence of Cryptosporidium oocysts. A modified Ziehl Neelsen with DMSO staining method was used for detecting Cryptosporidium oocysts in the stool samples. The oocysts were identified in 24 (6.8%) samples, while 46 samples (13%) showed mixed infections. Amatya *et al.*(2011) identified 4.4% prevalence in the HIV seronegative children from eastern region of Nepal.

Children

Persistent diarrhoea is the leading cause of death in children younger than five years of age in developing countries, where it accounts for 30 to 50 percent of childhood mortality (Snelling *et al.*, 2007). It was found that the prevalence rate for *C. parvum* infection was 10.4% in the children aged between 4-6 years were mostly infected and was highest in the month of July (14.2%) and lowest in the month of October(4.7%) (Dhakal *et al.*, 2004).

In Nepal, a study of acute diarrhoea in 160 children aged five years and below found that *Cryptosporidium* was detected in nine cases (5.6%), and all 50 control children were negative. Another study in western Nepal was conducted to find the association between protozoal agents and persistent diarrhoea in children younger than age 5 years. Stool samples were collected from 253 children with persistent diarrhoea, from 155 children with acute diarrhoea (disease controls) and from 100 healthy children from the community (normal controls). Of 253 children with persistent diarrhoea, 90 (35.5%) had protozoal infections, 63 (24.9%) helminthic infections, 32 (12.6%) had bacterial infections and 16 had mixed infections (Snelling *et al., 2007)*. But in the Eastern Nepal, 4.4 % of the children brought were diagnosed as *Cryptosporidium* (Amatya *et al.*, 2011).

Transmission through Swimming pools

Wallowing in buffaloes is a typical behavior and can be potential cause for the outbreak of cryptosporidiosis. Common wallowing pool used by the animals causes them to transmit the organism to healthy ones.

Swimming in humans in the public swimming pools has also demonstrated the transmission between them. The oocysts are very hardy and survive for prolonged periods in the environment. It can retain viability and therefore infectivity under moist and cool conditions for several months. *Cryptosporidium* has also been associated with chlorine treated water in swimming and wading pools. Chlorination of water supplies does not appreciably kill the oocysts of *Cryptosporidium.*

There are street human faeces as the point sources of the coccidian transmission. Surface rain water acts as the medium of coccidia transmission in different places. Besides, the oocysts carried by sandals, may be carried by surface rain water and might contaminate the pool. Insects, mice, birds may act as the mechanical vectors to carry oocysts from point sources to swimming pools. Another possibility is the transmission of coccidia by carrier swimmers through their anal parts (Ghimire *et al.*, 2010).

***Cryptosporidium* species in animals**

Wild animals like deer and rhinoceros have been found to be affected with *cryptosporidium*. The analysis of feces from the livestock of buffer zones of the CNP has leaded us to conclude that there has been transmission in between them (Karna, 2010). This can be any way interconnected when human being comes in contact with them. Nearly 50 *Cryptosporidium* genotypes have been described in animals and new genotypes are continually being discovered (Xiao *et al.*, 2004). Each group contains parasites of mammals, birds and reptiles. Surveys conducted in cattle, sheep, pigs, kangaroos, squirrels, wild mammals, Canada geese, and reptiles have shown that most animal species are infected with only a few host-adapted Cryptosporidium species or genotypes (Guselle *et al.*, 2003; Jellison *et al.*, 2004; Power *et al.*, 2004; Zhou *et al.*, 2004; Langkjaer *et al.*, 2007; Feng *et al.*, 2007a, b). The zoonotic aspects of Cryptosporidium transmission are described below.

Zoonotic aspects of *Cryptosporidium* transmission

The zoonotic transmission is dependent on species of parasite that infect both human and animal hosts, their occurrence in the environment, their potential for survival and their potential for subsequently infecting humans. Prior to the development of molecular epidemiological tools, descriptive epidemiology, based on investigations of outbreak and sporadic cases, indicated that livestock was an important zoonotic source for human cryptosporidiosis. The advent of appropriate molecular epidemiological tools for species determination, disease and source tracking has provided further evidence that C. parvum transmission can be zoonotic. However, recent evidence suggests that not all C. parvum are zoonotic. Glycoprotein 60 (GP60) gene sequencing has revealed C. parvum variants that are predominantly or exclusively associated with human, but not animal, infections in defined geographic areas. Similarly multilocus genotyping of C. parvum isolates, based upon mini- and micro-satellite typing, revealed that within the 5 C. parvum groups identified, two appeared to be human-specific, in that none of the livestock isolates analyzed were represented. On the other hand, *Cryptosporidium hominis* is primarily an infection of humans, although reports of experimental and natural infections in livestock and other non-human hosts. Similarly, *Cryptosporidium meleagridis*, *Cryptosporidium muris*, *Cryptosporidium suis*, *Cryptosporidium felis* and *Cryptosporidium canis* and the *Cryptosporidium cervine* and monkey genotypes also infect humans, and are named after the host species from which they were isolated. Person-to-person transmission is assumed for C. meleagridis, C. muris, C. suis, C. felis and C. canis and the *Cryptosporidium* cervine and monkey genotypes; however, less is known about their zoonotic potential.

Conclusion

The small and non-filterable size of the oocysts (3-5 μm), their resistance to chlorine disinfection and their low infective dose are the major infective potential of *Cryptosporidium*. The persistent shedding of oocysts by reservoir hosts like calves, kids, poultry and wild animals like deer and monkeys possess great threat to the transmission to general public.

This is not an organism that falls under routine scheduled check up at hospitals. Hence, all the diarrhoea cases should include *Cryptosporidium* as differential diagnosis. Furthermore, AIDS patient should have routined check up for *cryptosporidium*. The epidemiological studies of *cryptosporidium* and the knowledge of the pattern of the disease outbreak can guide therapy and effective preventive measures against this disease. Primarily focus should be made to avoid sewage and feces that mix into water source and recreational water should be prevented from fecal contaminations.

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