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Cryptosporidium species: Preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa

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Abstract

In the present study, the prevalence and species distribution of *Cryptosporidium* among school children and hospital patients in the Venda region of South Africa was determined. Real time PCR (qPCR) was used for initial screening to detect positive samples while a nested PCR followed by restriction fragment length polymorphism was used to determine the species genotype. From a total of 244 stool samples tested, 44 (18%) had *Cryptosporidium* with no significant difference ($\chi^2 = 0.04$; $P = 0.841$) between samples collected from patients attending hospitals 36/197 (18%) and the samples from primary schools 8/47 (17%). The age groups most affected were those from 2 to 5 years old (28.6%) and 50 to 59 years old (50.0%). *Cryptosporidium* was detected in 4 (12.5%) of the 31 HIV positive individuals. Fifty-seven percent of the *Cryptosporidium* positive samples were diarrheic and 26 (59.1%) had elevated lactoferrin content. *C. hominis* (82%) was more common than *C. parvum* (18%). This study has demonstrated the high prevalence of *Cryptosporidium* infections in the Venda region and its implications in causing diarrhea and inflammation.

Index Descriptors and Abbreviations: *Cryptosporidium hominis*; *Cryptosporidium parvum*; Diarrhea; Epidemiology; Protozoa; South Africa; Limpopo; Venda; HIV, Human immunodeficiency virus; PCR, Polymerase chain reaction; qPCR, Real time PCR; RFLP, Restriction fragment length polymorphism

1. Introduction

During the last decade, *Cryptosporidium* has emerged as an important enteric pathogen and has defied water and health authorities by its ability to withstand chlorine disinfection and filtration. It has been the cause of multiple diarrhea outbreaks in the United States (MacKenzie et al., 1994), Sweden (Insulander et al., 2005) and in other developed and developing countries. *Cryptosporidium species* are common protozoan pathogens with worldwide distribution, and has been known to cause severe and life-threatening diarrhea in immunocompromised hosts. Although this protozoan is more common in immunocompromised patients, it also induces diarrhea in immunocompetent persons, even though it might be for a short period. In developing countries, diarrhea caused by *Cryptosporidium parvum* early in childhood may be associated with subsequent impaired physical and cognitive development (Guerrant et al., 1999).

Cattle are known to be a source of *Cryptosporidium* infections, but environmental waters are also an important source of *Cryptosporidium* (Smith and Rose, 1998). The prevalence of *Cryptosporidium* varies widely from country to country and from one region to another. In Korea, for example, Lee et al. (2005) reported a prevalence of 1% (among HIV patients) while in Tanzania Houpt et al. (2005) described a prevalence of 17.3% amongst HIV patients. In Guinea Bissau, *Cryptosporidium parvum* had a prevalence of 7.7% and

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was the second most common parasite with a marked seasonal variation, with peak prevalences found consistently at the beginning of or just before the rainy seasons, May through July. In South Africa, studies by Kfir et al. (1995) indicated that Giardia cysts and *Cryptosporidium* oocysts were found in all types of water tested including surface water, sewage or treated effluents. Studies by Moodley et al. (1991) in Durban, South Africa showed that *Cryptosporidium* was the second most common enteric pathogen isolated from children admitted to hospital with gastroenteritis with infection rates varying between 1.2 and 20.9% according to season with the highest prevalence in the summer months and 10% of the children infected with *Cryptosporidium* died. However the prevalence of *Cryptosporidium* infections is not known in Limpopo Province and particularly in the Venda region.

The genus *Cryptosporidium* was recently described and named species that are currently considered valid species now include *C. andersoni* (cattle), *C. baileyi* (chicken and some other birds), *C. canis* (dogs), *C. felis* (cats), *C. galli* (birds), *C. hominis* (humans), *C. meleagridis* (birds and humans), *C. molnari* (fish), *C. muris* (rodents and some other mammals), *C. parvum* (ruminants and humans), *C. wrairi* (guinea pigs), *C. saurophilum* (lizards and snakes), and *C. serpentis* (snakes and lizards) (Xiao et al., 2004). Among all these species, *C. hominis* (previously known as the *C. parvum* human genotype or genotype I), is the species that almost exclusively infects humans. *C. parvum* (previously known as the *C. parvum* bovine genotype or genotype II) can infect not only humans but also ruminants and perhaps a few other animals. Other species that also infect human but less commonly include *C. meleagridis*, *C. felis*, *C. muris*, *C. canis*, and *C. suis*. Recent literature have also described *C. hominis* as the most common species isolated from human stools from HIV infected and non-infected individuals (Houpt et al., 2005) as well as diarrheic and non-diarrheic stools (Peng et al., 2003). However, genotype distribution data for *Cryptosporidium* in South Africa is scanty. Thus this study sought to determine the prevalence and species distribution of *Cryptosporidium* among school children and hospital patients in a semi-urban area of the Republic of South Africa where published data are not available.

2. Material and methods

2.1. Ethical clearance

This study was approved by the research and ethical committee of the University of Venda and the Department of Health and Welfare and the Department of Education in Polokwane, Limpopo Province, South Africa, before the initiation of the study.

2.2. Study site and sample collection

The Vhembe district, the site of the study, represents what used to be known as Venda, which is a previous black homeland in the apartheid South Africa and became part of the country after the first free elections in 1994. Thohoyandou, the site of the University of Venda, is the headquarters of the Vhembe district and is the tenth most populated city in the country with 584,469 people while the population of the region is approximately 1.2 million. Stool samples submitted for analysis were collected from the three main public hospitals (Donald Frazer, Elim, and Tshilidzini hospitals) in the Vhembe district. Stool specimens were also collected from pupils attending two primary schools situated in Wuwani, locality situated at about 6 km from the Tshilidzini hospital. At the primary schools, the objectives of the study were explained to the parents in a meeting with the authority of the schools who then distributed the collection bottles to the pupils whose parents had agreed to the study and signed a consent form. The pupils then brought the collection bottles home and with the help of their parents collected the stool in the bottles. The samples were collected the following morning from the schools and transported without any further delay to the Laboratory of Microbiology, University of Venda. The samples were further aliquoted in 1.5 ml Eppendorf tubes without dilution for diarrheic (soft) samples or diluted in sterile saline for non-diarrheic (hard) stools. Demographic information such as age and sex as well as HIV status was also collected. Stool samples were stored at −80 °C until needed.

2.3. C. parvum and C. hominis

Cryptosporidium parvum oocysts (Iowa strain) were obtained from a commercial source (Pleasant Hill Farms, Troy, ID). This strain was originally isolated from a calf by Harley Moon. It has been passaged through calves and purified from fecal material by ether extraction, followed by a one-step sucrose gradient. *C. hominis* oocysts were kindly provided by Dr. Saul Tzipori, Tufts University School of Veterinary Medicine, Grafton Ma.

2.4. DNA purification

Genomic DNA was purified from oocysts by a modified version of the method described by Haque et al. (1998). Fifty microliters of 1 M KOH and 18 µl of 1 M dithiothreitol were added to 250 mg or 250 µl of stool. The samples were mixed thoroughly by stirring with a pipette tip, followed by brief shaking. After incubation at 65 °C for 15 min, the samples were neutralized with 8 µl of 25% HCl and buffered with 80 µl of 2 M Tris–HCl (pH 8.3) and the suspension was mixed by briefly vortexing. The genomic DNA was then purified from the suspension using the QIAamp DNA Stool Mini Kit from Qiagen (Valencia, CA, USA) following the manufacturer's instructions.

2.5. Real time PCR (qPCR)

A real time PRC protocol based on the amplification of a specific sequence of the 18S rRNA gene was used to