Risk Assessment Of Waterborne Cryptosporidium

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RISK ASSESSMENT OF WATERBORNE CRYPTOSPORIDIUM

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DEDICATION

First and foremost to God who is always with me. To my husband, Manuel, who with his love and patience helped me see this project forward. To my children who are my greatest inspiration in life and who always encouraged me to endeavor in this project, Rodrigo, Alonso, and Paloma. With lots of love to my mother, and my family who are always there for me. Last but not least, to my friends, and colleagues for always cheering me on and for helping me at various moments with this project.
RISK ASSESSMENT OF WATERBORNE CRYPTOSPORIDIUM

by

VICTORIA NORMA OCHOA

DISSERTATION

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The University of Texas at El Paso
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ABSTRACT

RISK ASSESSMENT OF WATERBORNE CRYPTOSPORIDIUM

In an effort to update the risk assessment of waterborne Cryptosporidium an additional and more complete human dose-response dataset of the parasite was used to calculate the risk of infection. The complete data studied contains larger human outcomes than the initial prior risk assessment evaluated by Haas et. al. in 1999. Quantitative analysis of the complete dose-response indicates that it no longer follows the Exponential Model used to estimate the risk for the initial data. The complete dataset appears to provide a better fit with the Beta-Poisson Model and even a dose independent constant risk model, which shows a considerable higher risk than the one previously calculated for the initial work. A comparative examination between the complete exponential, initial exponential and complete Beta-Poisson risk analysis is presented on this study including a recommended water treatment level based upon the complete dataset. Employing Quantitative Microbial Risk Assessment (QMRA) the complete Beta-Poisson dataset results and the Milwaukee outbreak of 1993 were compared, and the results observed produced an excellent agreement with the outbreak outcomes.

The impact of immune status was incorporated to the study in order to determine a possible reduction of human health risks associated with waterborne Cryptosporidium. The antibody status in the study volunteers’ and the type of isolate strain used in the human exposure are confounding factors that complicate the risk assessment. Antibody status was found to significantly influence infection rates. The type of isolate appears to be important but the dataset is too small to discern clear differences.

In addition, an annual comparative risk assessment examination between the complete constant, complete Beta-Poisson, and initial exponential risk analysis is presented on this study;
which reflects and reinforces the existence of a higher risk when complete constant and complete
Beta-Poisson models are employed in an order of 100 times greater risk than initial exponential
model.
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1.0 INTRODUCTION

1.1 Cryptosporidium Description

Cryptosporidium species were identified in 1983, as pathogenic parasites capable of surviving conventional water treatment and persisting in water that was traditionally considered safe. Since then, strategies have been proposed to evaluate and control the risks associated with Cryptosporidium in the environment. Human exposure can occur from various sources including contaminated drinking and recreational waters. Many species of Cryptosporidium infect humans and animals. Cryptosporidium parvum and Cryptosporidium hominis are the most common species that cause disease in humans [CDC, 2011]. These protozoan parasites belong to the apicomplexa phylum.

The life cycle of Cryptosporidium starts with sporulated oocysts that are 4-6 μm and contain four sporozoites. Oocysts are primarily transmitted through the fecal-oral route [CDC 2010, Kato et al., 2004]. After the ingestion of oocysts, the sporozoites become excited and are released, and subsequently parasitize the epithelial cells of the gastrointestinal tract or other tissues. In these tissues, the sporozoites hatch from the cyst and undergo asexual multiplication (schizogony and merogony) and sexual multiplication (gametogony), where the female (macrogamont) and male (microgamont) gametes are produced. Macrogamonts are fertilized by microgamonts, and develop oocysts that sporulate in the infected host. Two types of oocysts are produced; one thick-walled oocyst that is excreted from the host and a thin-walled oocyst that is involved in autoinfection. Infective oocysts are excreted with the capacity to be transmitted via the fecal-oral route and possibly inhalation.

1.2 Cryptosporidium Characteristics that Facilitate Waterborne Transmission
1.2.1 Size and Oocyst Wall Composition

*Cryptosporidium* oocysts have a small size (4-6 μm) compared to other protozoan parasites. The oocysts’ small size makes their removal less efficient during the passage in the process of filtration applied at some stage in the drinking water treatment. The structure and composition of oocyst walls are primary factors that determine the survival and hydrologic transport of *Cryptosporidium parvum* oocysts outside of the host [Jenkins et al., 2010].

According to Jenkins [2010], the *C. parvum* oocyst wall shows an outer electron-dense layer with a complex chemical composition under microscopy analysis. The layer components are: surface glycocalyx, lipid hydrocarbons, proteins, and structural polysaccharides. Based on these findings, Jenkins *et al.* [2010] propose a model of the oocyst wall which shows variable water-resistant properties and wall with the capacity to counterattack numerous environmental pressures, such the survival of chlorine disinfection process, high concentration of salt, and low and elevated temperature ranges [Fayer *et al.*, 1998, WHO 2009, EPA 2010].

1.2.2 Elevated Infectivity

Several studies and outbreaks information supports the fact that the ingestion of only one or a small number of oocysts provides a possibility of infection [Hass *et al.*, 1996, Chappell *et al.*, 1999, Craun *et al.*, 2001].

1.2.3 Elevated and Prolonged Oocysts Shedding

Numerous research reports state that during the acute phase of the infection, oocysts are shed in high numbers facilitating the auto-infection process. Humans excrete up to $10^{5-7}$ oocysts per gram of feces [Chappell *et al.*, 1999, Rose *et al.*, 1988, Madore *et al.*, 1987]. Bushen *et al.*, 2007] reported Brazilian children shed *C. hominis* oocysts of $3.5 \times 10^6$ and $1.7 \times 10^7$ per milliliter of *C. parvum*. In Peruvian children, shedding period was 13.9 days for the human
genotype of *C. parvum*, and the zoonotic genotype was excreted for 6.4 (P=.004) days [Xiao *et al.*, 2001]. Elevated numbers of oocysts shedding are reported in symptomatic subjects on days when they are not experiencing diarrhea [Chappell *et al.*, 1996]. In Belgium, Vanderberg *et al.*, [2012] reported that children in a day care center excreted *C. hominis* oocysts for more than fifteen days.

1.2.4 No Maturation Period is Required

*Cryptosporidium* oocysts are distinct to other members of the coccidian parasites; they do not need to pass by the maturation phase in order to be infective. After oocysts are shed, they instantly have the capacity to infect a host.

1.3 Occurrence

*Cryptosporidium* species can be found throughout the environment, including various water sources, soil, food, and human and animal hosts. Several physical and chemical environmental factors contribute to its prevalence, such as: oocysts’ survival capacity at temperatures ranging from freezing to very hot, UV exposure, microbial predation, and chemical antagonist conditions.

Although water is likely to be the most significant transmission vehicle, food has also been implicated. *Cryptosporidium* has contaminated food (meat and fresh milk, apple cider of farmhouse manufacture, fermented milk, uncooked vegetables and salads,) [Casemore *et al.*, 1997], and in some cases, outbreaks due to food handlers [Quinn *et al.*, 1998, Quiroz, *et al.*, 2000, WHO 2009]. The protozoan has also been found in oysters [Schets *et al.*, 2002, Fayer *et al.*, 2003, WHO 2009].
1.3.1 Occurrence in Water

Oocysts have been found in surface water, groundwater, drinking and recreational waters [WHO, 2009]. Surface water is considered the most important source of *Cryptosporidium* outbreaks [Fayer *et al.*, 2008]. Surface water becomes contaminated from the discharge of untreated and treated sewage, and animal wastes. Superficial waters (e.g., large rivers and lakes) are frequently contaminated from agricultural run-off due to rainfall transport of nutritional compounds and pathogens into surface waters. Furthermore, surface waters receive treated and untreated local wastewater. The concentration of oocysts in surface water ranges from 0.01-100 per liter [Fayer *et al.*, 2008].

In the United States, 12% of groundwater sources are contaminated with the parasite [Hancock *et al.*, 1998]. Often, groundwater contamination is caused by infiltration in the galleries and horizontal wells from contaminated surface water [Hancock *et al.*, 1998, Fayer *et al.*, 2008]. Several sources of literature have reported that the most frequent cause of drinking water *Cryptosporidium* outbreaks occur because the parasite often is found in surface water and groundwater [Craun *et al.*, 1992, Mackenzie *et al.*, 1994, Hunter *et al.*, 2001, USEPA 2001].

1.3.2 Survivability in Water

*Cryptosporidium* oocysts are capable of remaining alive for months in surface water. Studies also report survival for more than 12 weeks at 20 ºC in estuarine waters and 4 weeks in seawater [Fayer *et al.*, 1998, WHO, 2009]. Oocysts not only survive in antagonist physical situations, but also under aggressive chemical environmental conditions where chemical compounds are used for water treatment. Oocysts have also been shown to survive in a body of water treated with chlorine and chloramines [WHO, 2009]. Several outbreaks reported from swimming pools are associated with the incapacity of typical chlorine concentration levels used to destroy the oocysts.
together with an inefficient filtration system [USEPA 2001]. The ozone treatment has provided good inactivation levels, but not at low temperatures. An elevated concentration of chemicals cannot be used due to the fact that it generates toxic by-products formed with the chemical compounds found in the water despite the results of *in vitro* analysis which suggest a low oocyst inactivation [Ransom *et al.*, 1993]. *In vivo* studies report a high sensitivity of oocysts when exposed to UV [Rochelle *et al.*, 2004].

Due to high survivability of oocysts in water, it is required the generation of a more comprehensive approach to treatment. Methods of membrane filtration have shown higher levels of oocysts removal of more than 4 logs if the integrity of the filtration system is maintained in optimum conditions. The best recommendation is a combination of multiple physical and chemical stress conditions, which limits oocysts infectivity in environmental treatments [CDC. 2007—2008].

1.3.3 Detection in Water

The current method of *Cryptosporidium* detection approved by the EPA is Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA (USEPA 2001, Method 1623).

This procedure starts with filtration of a water sample, which traps oocysts and other external material on a filter and this matter is eluted. Oocysts’ elution is concentrated by centrifugation and the supernatant is eliminated by aspiration. Oocysts are then attached to magnetic beads conjugated with anti-*Cryptosporidium* antibodies using a magnet. The magnetized oocysts are separated from the rest of the material, and then the magnetic bead complex is removed from the oocysts. The oocysts are transferred onto well slides and stained with fluorescently labeled monoclonal antibodies and 4’, 6-diamidino-2-phenylindole (DAPI). Staining is followed by oocyst observation using the fluorescence and differential interference contrast (DIC)
microscopy. Each slide is reviewed for characteristic size, shape and fluorescent Cryptosporidium oocysts. Total oocysts are counted to obtain a quantitative analysis.

According to Weintraub et al., [2006], this method has a number of limitations. The recovery efficiency is variable which does not evaluate oocysts’ viability or infectivity and does not identify the Cryptosporidium species. Moreover, its overall detection has limitations related to water sample collection, filtration and transportation, which confers some degree of uncertainty.

1.4 Epidemiology

1.4.1 Occurrence in Humans

Cryptosporidium parasites are found in every region of the United States [CDC, 2011] with approximately 748,000 cases of cryptosporidiosis occurring each year [Scallan et al., 2011, Current and Garcia 1991] reported a prevalence of Cryptosporidium in 1-4% of Europe and North America and in 3-20% of Africa, Asia, Australia, South and Central America. Its incidence shows a maximum peak of prevalence during the summer [Van Asperen et al., 1996] and spring [Casemore 1990]. A high occurrence is demonstrated in developed countries in children less than 5 years of age and in young adults. It is common to find the disease in infants less than one-year- old and in elderly adults.

In the last ten years, 325 waterborne outbreaks have been reported throughout the world; 165 (50.8 %) out of 325 corresponded to Cryptosporidium. The outbreaks are mainly found in the USA, Canada, Australia, the United Kingdom, and Ireland affecting both adults and children. Global data of environmental and veterinary surveillance reports from the United Kingdom state that Cryptosporidium is found in the entire water treatment system. These data symbolize an undesirable health risk, especially for immuno-compromised people [Putignani and Menichella, 2010, Yoder et al., 2007]. Stool surveys have served to identify Cryptosporidium asymptomatic
carriers, which happen to appear at a rate of less than 1% even though several studies have reported a high incidence in nurseries [Lacroix et al., 1987, Crawford et al., 1988, Garcia-Rodriguez et al., 1989, Current et al., 1991].

1.4.2 Human Health Impact

*Cryptosporidium* is considered to cause significant health problems due to diarrhea in both immuno-compromised and immuno-competent persons. While for immuno-competent individuals cryptosporidiosis only causes watery diarrhea that usually is self-limited, sometimes the disease in healthy subjects is completely asymptomatic. People with weak immune systems can develop serious, chronic, and life-threatening illness. Complications of the disease may include: inflammation of the bile duct or gallbladder, hepatitis, pancreatitis, severe malabsorption, and wasting loss syndrome. The degree of disease development is determined by each person’s status of immune suppression. Humans become infected after accidentally swallowing or, by a less common route, inhaling the parasite. The parasite can be found in soil, food, water, or on surfaces that have been contaminated with the feces of infected humans or animals; *Cryptosporidium* is not spread by contact with blood [CDC 2013].

1.4.2.1 Clinical Symptoms

The incubation period is between 2-10 days (an average of 7 days). The most common symptom is cholera-like diarrhea. Other symptoms consist of stomach pain and/or cramps, vomiting, nausea, fever and fatigue. Symptoms usually last about 1-2 weeks; in immuno-competent persons, the infection is self-limited and generally resolved within 10-14 days. In immuno-compromised individuals, the infection is severe, with frequent evacuations of 2-3 liters of diarrheic stool, significant dehydration, malnutrition, and profound weight loss. In Acquired Immuno- Deficiency Syndrome (AIDS) patients the disease becomes chronic and can cause
death. Sporadically, people may experience the reappearance of symptoms after a short period of recuperation before the illness ends. In some cases symptoms can reappear and disappear for up to 30 days, although other infected persons would not present any symptoms. In these types of subjects Cryptosporidium is also capable of infecting other areas of the digestive or respiratory tract [Putignani and Menichella, 2010, CDC 2013].

1.4.2.2 Treatment

Immuno-competent people will recover without treatment with diarrhea being managed by drinking abundantly fluids to prevent dehydration. People with poor health or weak immune systems are more susceptible to dehydration as a result of diarrhea and should drink plenty of fluids while the symptoms persist. Constant and profuse re-hydration is recommended in babies due to the fact that in infants the loss of water might be life-threatening.

Nitazoxanide is the anti-microbial approved by the Food and Drug Administration (FDA) for treatment of diarrhea caused by Cryptosporidium in people with healthy immune systems; it must be prescribed by a physician. The effectiveness of nitazoxanide in immuno-suppressed individuals is unclear and requires more trials for its investigation. Since nitazoxanide treatment has not been well documented in non immuno-competent persons, the use of fluids, electrolyte replacement and anti-motility drugs is the only suitable treatment choice [Abubakar et al., 2007]. Thus, for individuals with a weak immune-system, it is highly recommended to avoid exposure to the protozoa due to the absence of effective therapy.

In persons with AIDS, anti-retroviral therapy improves the immune status and decreases or eliminates symptoms of cryptosporidiosis. In this type of population, even though symptoms disappear, cryptosporidiosis is regularly not curable and the symptoms might come back as promptly as the immune status worsens. In addition the profuse administration of parenteral
or and oral fluids and electrolytes replacement is recommended [CDC 2010]. The highly active antiretroviral therapy (HAART) in Human Immuno-deficiency Virus – positive (HIV-positive) children helped to reduce the mortality death rate due to Cryptosporidium infection from 2.6% in the period of 1994-1996, to 1.5% for the years of 1997-2000 and 0% for 2001-2006 [Brady et al., 2010].

1.4.3 Morbidity and Mortality

As per the Center of Disease Control (CDC), the number of reported Cryptosporidium cases has increased from 1.0 case per 100,000 populations in 1999 to greater than 3.0 cases in 2008 [Yoder et al., 2010]. Most of the cases reported were children from 1-9 years old and adults 25-39 years old. A ten-fold increase in transmission of cryptosporidiosis occurs during the early summer to the early fall, consistent with exposure to recreational water by young children during vacation periods; this is a well-known condition that increases the risk [USEPA 2001, CDC 2010].

The estimated mean mortality risk of Cryptosporidium in immuno-competent persons is 1 in 100,000 for symptomatic cases based on a waterborne outbreak in Milwaukee [Dusseldorp et al., 2007]. However, Hunter et al., [2001] calculated that the Milwaukee outbreak risk of infection developed was of 1-10, these Milwaukee numbers were re-calculated after they obtained higher values in an outbreak analysis of North West England which reported 306 confirmed cases of cryptosporidiosis.
1.4.4 Major Outbreaks

1.4.4.1 Milwaukee

In April of 1993, the Milwaukee department of Health contacted the Wisconsin division of Health after they had received several reports of widespread acute watery diarrhea within hospital employees, school teachers and students of the Milwaukee area [Mac Kenzie et al., 1994], due to these reports, a study was conducted to discover the cause of the outbreak. The investigation was initiated in 14 clinical laboratories located in the county. From March 1 to April 16, the laboratories received 1,051 stools to detect Cryptosporidium. In the results, 342 out 1,051 were positive to the parasite, which represent a 30%. Because most of the gastrointestinal infections are acquired by fecal-oral route, the next step in the investigation was proceeded to review the city water suppliers. They found that Lake Michigan was the water source for the Milwaukee Water Works (MWW) that simultaneously provided treated-water to the population, commerce and companies of Milwaukee city, as well as to nine towns located in its vicinity. The city had two treated-water plants located in the northern and southern parts of the area. The water turbidity records of both water plants were investigated, and the southern plant presented an abnormal elevate turbidity from March 25-April 9, 1993.

Mac Kenzie et al., [1994] determined the outbreak magnitude collecting the following data: Cryptosporidium stools’ analysis results provided by the local clinical laboratories, oocysts/liter count of ice blocks prepared with water of the outbreak days from the southern plant and information gathered from telephonic surveys from persons who exhibited water diarrhea during the epidemiological problem period. Using this data, calculations were made considering in those numbers the 1,610,000 habitants of the Milwaukee area; researchers reached an estimate of more than 400,000 persons were affected, with watery diarrhea due to this outbreak. Mac Kenzie et al.
[1994] findings demonstrated that *Cryptosporidium* infections can occur during large periods of time and with different degrees of severity. Their results implied that several measures must be applied, such as the increase of public health surveillance and the application of better methods for prompt and timely detection of *Cryptosporidium*. In addition, treated-water plants’ design and its methods of disinfection have to be improved by including a constant turbidity monitoring of treated water, especially on filter effluent and particulate size.

The CDC [Corso *et al.*, 2003] reported that the Milwaukee outbreak had a total cost of $ 96.2 million and Hoxie *et al.*, [1997] estimated that the outbreak caused 68 deaths in persons with AIDS, due to an increase, in the number of elderly patients hospitalized during this epidemic, the CDC considered that the elderly population was the second group most affected during this event; these patients were from the central and southern area of Milwaukee [Naumoba *et al.*, 2003].

1.4.4.2 Las Vegas

From 1992 to 1994 Las Vegas, Nevada reported several *Cryptosporidium* laboratory confirmed cases; in 1992 the first 3 cases in the state were described, 23 cases in 1993 and 78 in 1994. From these 78 cases, 61 subjects corresponded to HIV-infected adults and the cases occurred during the first fourth months of the year, more than 90% of them presented Cдо cell counts lower than 100 cells/mm [CDC1996 , MMWR of 1993-1994d; from them 31 (29%) presented diarrhea that last in a median of 60 days. By June of the same year 32 (52.5%) out of 61 cases had died. The death certificates of 20 out of 32 (62.5%) indicated cryptosporidiosis [Goldstein *et al.*, 1996]. The CDC never found the cause of this outbreak, but the CDC suspected of drinking water because it is the most common cause.
1.4.4.3 Carrollton

In 1983, *Cryptosporidium* affected an estimated 13,000 persons in Carrollton, Georgia. The outbreak was the first spread by the municipal water system that met all municipal and federal water standards available for that time. More than a 61% of the 489 participants in the survey of the water-exposed household members declared to have the disease. High levels of immunoglobulin G to *Cryptosporidium* were reported in the persons that became ill compared to the absence of antibodies in the population of that area.

The raw water was exposed to the conventional treatments; no positive samples for coliforms were detected and no elevation of turbidity was reported. Chlorine concentration was found between the limits dictated by the USEPA, Carrollton City and County Water Utilities. The only finding was a treated water sample that presented large particles of 100 μm. Oocysts were found in treatment-water plant samples; it is though that the outbreak causes were low level of *Cryptosporidium* from cattle in the water-line and manure runoff that contaminated the surface-water sources [Hayes et al., 1989].

1.4.4.4 Swimming Pools and Recreational Water Outbreaks

From 1988 to 2006, 136 outbreaks associated with swimming pools and recreational waters have been reported in the United States and the United Kingdom. Greater than 90% of these outbreaks correspond to disinfected waters and 9% to freshwater [Fayer et al., 2008]. In the annual reports of the CDC, *Cryptosporidium* species are found as the causal agent of 82.9% of recreational water associated outbreaks [CDC.MMWR 2007-2008].

During the summer of 2007, the state of Utah reported 5,700 outbreaks; 1,506 cases had confirmed cryptosporidiosis and 1,209 (80%) individuals declared that at least they used once the 450 recreational water places [CDC. MMWR 2008-2009].
In the summer of 2011, the Indiana department of Homeland Security reported to the Indiana State department of Health (ISDH) a gastrointestinal outbreak that affected firefighters of one fire station. This outbreak also happened in two Michigan fire stations which responded to control the same fire of a calf’s barn located along the border of Indiana and Michigan. From the three fire stations, a total of 20 firefighters of the 34 individuals who responded to control the fire presented gastrointestinal problems that where self-limited or controlled with medication in four days. The exception was one immuno-competent man who was hospitalized due to acalculous cholecystitis that was produced as a consequence of cryptosporidiosis. The water used to control the fire was collected from a local hydrant and a swimming pond. The pond water and barn calves were analyzed and were positive for Cryptosporidium [CDC.WWMR.2011]

1.4.5 The Disease in Special Sub-populations Immuno-compromised

Immuno-compromised persons have weak immune systems and may include: the elderly, infants, children less than 6 years old, pregnant women, malnourished individuals, persons with inherited diseases that affect the immune system, cancer patients, patients in immunosuppressive drug therapy, organ transplanted patients, and persons with AIDS[ CDC, 2011]. These individuals’ immune systems may not be able to easily combat infections for diverse reasons, such as a poor response, medications’ effects, insufficient immune response, age, or the protection gradient favoring the fetus in pregnancy. Immuno-compromised individuals may experience chronic severe diarrhea, which can lead to malnutrition and significant weight loss, and they may develop a chronic state that sometimes can be fatal such as in the case of AIDS patients. The risk of developing severe disease may differ depending on each person’s degree of immune suppression [CDC, 2011].
1.4.5.1 Persons with HIV and AIDS

_Cryptosporidium_ infection might be found in HIV-infected patients with different symptoms, ranging from a self-limited diarrheic illness at the beginning of the disease to severe, life-threatening diarrhea in patients with elevated states of immuno-suppression. Although the type of diarrhea is a non-inflammatory type it is important to focus on the group because in an immuno-compromised state, infectious diseases like cryptosporidiosis can easily progress to a chronic state or into one of the complications already mentioned in the human health impact section.

Patients with CD$_4$ T cell counts below 300/ml demonstrated an incidence of approximately 1% of the infection per year. HIV infected patients may experience up to 30-50% of cryptosporidiosis in the course of their life. A reduced risk can be created by avoiding contact with human and animal stools and avoiding the ingestion of water from lakes and rivers [WHO, 2006].

In AIDS patients, cryptosporidiosis causes a prolonged life-threatening diarrhea to which there is no effective treatment. [Guerrant _et al._, 1997]. For these patients, the disease can be extended throughout any part of the intestinal tract and even reach other parts of the body such as the pancreas, gallbladder and the respiratory tract [WHO, 2002]. In AIDS individuals, the invasion of the respiratory tract can contribute to death. _Cryptosporidium_ is considered the most common causal agent of diarrhea that is highly associated with the late stages of AIDS [Laurent _et al._, 2005].

1.4.6 Immuno-globulins Levels in the Population

For more than two decades, _Cryptosporidium_ oocysts have been reported in several water sources. Hence, the population has been exposed to the protozoan. Some of the cases have been
reported; others have not due to the absence of symptoms or because the infection was self-limited. Other possibilities exist and must be taken into consideration, for instance, the fact that oocysts in water sources are not viable and/or infective. In other cases the population is frequently exposed to low viable oocyst numbers and the risk of infection is low because of the protective action of the immune system. Consequently, many individuals should have a certain level of antibodies in circulation.

The serological detection of Cryptosporidium antibodies has been performed by different methods: Enzyme Immuno-Assay (EIA), Western Blot (WB) and Enzyme Linked Immunosorbent Assay (ELISA). In most of them the antigens tested are 15/17-kda antigen and 27-kda antigen. The antibodies found by different investigators in the U.S and other countries are immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA).

Several groups of researchers have evaluated the antibody prevalence in the population exposed to different water sources. Frost et al., [1997, 1998, 1999, 2001, 2003] have found a stronger serological response in individuals with a recent infection, especially in persons that use a surface water source; they also encountered in people that have suffered frequent infections, producing high levels of antibodies persisting for a long period of time.

In 2002, the prevalence of antibodies found in blood donors ranked from 11-40% of positive results against the 15/17 –kda antigen and around 18-38 % to 27-kda antigen.

Okhyusen et al., [1998] demonstrated that 19 healthy human volunteers re-challenged with oocysts presented less severe symptoms and more prompt recovery. Tenuis et al., [2002] found in a dose-response analysis performed in humans’ feedings that the infectivity was low in people with high preexisting anti-Cryptosporidium IgG-levels. Chappell et al., [2006] have reported serological studies that demonstrated ≤ 25% of the U.S. population has antibodies to
Cryptosporidium. In developing countries, higher numbers of antibodies are found due to reduced sanitation, and water quality, crowding communities and animals reservoirs in close proximity to the house. In Mexico, 6.4% -11.4% of sero-prevalence is found in children less than one- year -old [ Enriquez et al., 1997], while Israel reports that 4%-91% of children between 6-23 months old present serum antibodies against the parasite [Robin et al., 2001, Sureshbadu et al., 2011] have reported for the developing nation a 64% of antibodies against Cryptosporidium. The WHO [2009] reported an elevated sero-prevalence of Cryptosporidium in developed counties of 25-35%, and 60% in countries with low levels of hygiene. The prevalence of positive serological individuals and their relationship with the development of Cryptosporidium infection is not easy to explain, but it is indicative that some protection is conferred after the exposure to non-pathogenic strains or repetitive exposures to low oocyst concentration.

1.4.7 Occurrence of Cryptosporidium Species in Animals.

There are thirteen valid Cryptosporidium species. Eleven have been observed in animals. C. hominis, which has been detected only in humans and C. parvum in cattle, are the two species that predominantly cause infections in humans and are associated with waterborne outbreaks [Ryan et al., 2004]. Zoonotic infections due to Cryptosporidium are very common. Several outbreaks have been reported in children or students after they were exposed to calves or lambs [Casemore 1990, Casemore et al., 1997], as well as in occupational exposure to calves [Current 1994, Casemore et al., 1997]. According to Sulaiman et al., [1998] only C. parvum exhibits zoonotic transmission. The high prevalence of C. parvum in cattle and sheep plus the elevated oocysts excreted by infected animals, especially newborns, makes them one of the most important sources of environmental contamination that may infect humans with the parasitosis.
Contact with horses and horse manure is considered an indirect risk factor of cryptosporidiosis [Casemore, 1990]. *C. meleagridis* is found worldwide in turkeys. Turkey farms are one of the many major causes of *Cryptosporidium* environmental contamination [Mcdougald et al., 1998]. *Cryptosporidium* can infect common pets like cats and dogs, but do not appear to be an important source to cause human infections in immuno-competent persons [Casemore et al., 1997, Glaser et al., 1998]. *C. felis* (cats) and *C. canis* (dogs) are rarely encountered in humans [Pedraza-Diaz et al., 2001]. Olson et al., [2004] have reported prevalence 2.4-8.2% in cats and 1.5-45% in dogs. These numbers suggest that the two species may be considered an important source of infection in immuno-compromised people [Olson et al., 2004]. Genotyping studies have identified unique genotypes of *Cryptosporidium* in many species of wildlife [Olson et al., 2004]. The oocysts of these specific *Cryptosporidium* species are shed into the environment, increasing the total load of the parasite in drinking water sources. This especially occurs as young calves, horses, and lambs release their excretions containing a high number of oocysts to the watershed, providing an important source of environmental contamination [Xiao et al., 1994, Casemore et al., 1997, Olson et al., 2004].

1.4.8 Sensitivity and Challenges of Epidemiological Studies

1.4.8.1 Sensitivity

One of the most significant challenges that are faced in cryptosporidiosis is the fact that the methods of diagnosis have limited sensitivity; additionally the majority of people who exhibit diarrhea do not generally request medical attention, therefore these cases are reported and their risk is not assessed.

Although the standard methods currently existing for detection of *Cryptosporidium* in environmental water are simple, they must become more efficient and trustworthy. The highly
sensitive molecular techniques are important for their capacity of low detection levels, species identification, and even the determination of possible source contamination. But, these specific and sensitive techniques do not evaluate virulence and viability/infectivity of waterborne pathogens. Further, they are expensive or not convenient for ordinary detection of the waterborne parasites. [Quintero- Betancourt. et al., 2004]. Even the current USEPA approved Method 1623 does not assess the viability and infectivity in the parasites detected [Weintraub, 2006]. Thus, it is necessary to invest and develop new technologies that would be rapid, sensitive, specific, and adaptable to any type of environmental samples in order to establish real databases.

1.4.8.2 Challenges

Epidemiological studies demonstrate some imperfections such as inaccurate associations from the beginning to the end of the process, and as a result various assumptions have been included and they are used to calculate risks. These studies occasionally are not done on time, or not conducted at all due to the amount of time that it is required to perform them, their elevated cost, lack of financing, or poor logistics.

Epidemiological revisions have to take into consideration aspects such as the evaluation of all possible sources of infection like soil, fomites, person-to-person transmission, aerosols, vomit, and any type of water source. Furthermore, most of these epidemiology revisions do not consider the possibility that a low concentration of 10 oocysts of Cryptosporidium can be infective in healthy adults, as it was demonstrated by Okhuyusen and Chappell [2002]. Then, several facts reflect the need to generate and improve quantitative microbial risk assessment using updated valuable information.
1.5 Quantitative Microbial Risk Assessment

Quantitative microbial risk assessment (QMRA) is a method used to calculate the chances of someone becoming infected or ill from microbial exposures. QMRA intends to provide the best available information to allow the understanding of the possible effects of a microbial exposure. In the risk estimation, the most significant step is formulating risk as a mathematical model that directly answer the requirements of the decision makers, and /or to address with the important concerns of the communities interested.

The steps of microbial risk assessment are: hazard identification, dose-response assessment, exposure assessment, and risk characterization [Haas et al, 1999]. Hazard identification describes the type of microorganism and the associated diseases, including the effects in human health such as disease severity, symptoms, death rate, and identification of the most affected individuals due to the illness. Dose-response establishes the relationship between the different doses (number of microorganisms) provided and the frequency of the health effect. Data obtained from human and animal studies permit the creation of mathematical models used to predict the dose-response. Exposure assessment describes the microbial route(s) used to access the human population and cause the infection; it indicates the size and duration of exposure by route. Further, it calculates the type and size of people exposed. Risk characterization serves to integrate the information from exposure, dose-response, and human health effects into a mathematical model that calculates the risk of infection, illness and/or death, and it estimates the variability and uncertainty [CAMRA 2010, Hass et al., 1999].

This information can be used by persons responsible for establishing the standards for drinking water, water suppliers, those that evaluate the quality of drinking water, and those that propose
the implementation of a system to control infectious diseases. The beauty of this approach is that it can be applied through a simple screening to complicated statistical data.

The governmental agencies involved in the evaluation of risk assessment are: 1) the Consumer Product Safety Commission for legislation of consumer products (CPSC), 2) the Environmental Protection Agency (EPA) for legislation of the programs of the Clean Air Act (CAA), Clean Water Act (CWA), Safe drinking Water Act (SDWA), and Resource Conservation and Recovery Act (RCRA), 3) the Food and drug Administration (FDA) for the legislation of the Food Additive Program, and 4) the Occupational Safety and Health Administration (OSHA) in worker exposure and permissible exposure levels.

In any QMRA, the following aspects must be taken into consideration:

a. Sensitivity and limitations of epidemiological studies;

b. Data are obtained from use of animals to extrapolate their effects to humans;

c. The nature of mathematical models employed in the extrapolation of high and low infective doses;

d. Approaches for managing uncertainty in the estimates, and

e. Limitations in the use of real-world exposure, e.g., humans’ models.

1.5.1 Risk Characterization.

Prior to the integration of risk assessment with risk management, it is necessary to combine the data produced after the administration of several doses and determine the prevalence of health effects. The incorporation of exposure assessment, dose-response, and hazard identification facilitate the estimation and magnitude of undesirable problems caused by contact of individuals with the microorganism, as well as the evaluation of variability and uncertainty in the process or risk characterization. The risk characterization is formed by one or several quantitative estimates
of risk. In this estimation each study has to choose from the risk results which are the most relevant for decision makers.

Some of the significant aspects of microbial risk assessment are: expected risk of infection in a normal individual, the expected amount of diseases in the population, upper confidence limits of illness to “high exposed” individuals, upper confidence limits to probable number of diseases, the maximum amount of diseases presented at any moment, and many more examples that can be cited. But, each amount calculated is represented by an arithmetical value that is called a point estimate. The numerical point estimated can be substituted in the dose-response equation to obtain the risk of a single exposure. Also the multiple and continuous exposures have to be evaluated, due to people’s daily consumption of water. Essential third parameters that must be calculated are the interval estimate; which offers a probable distribution that gives an exact idea from how the risk has been estimated.

1.5.2 Dose-response Model

The dose-response assessment has the purpose of demonstrating a connection between the level of microbial exposure and the probability of an undesirable result. In general, the odds of illness occurrence depend on three conditional probabilities:

a. The probability that the infectious agent is consumed; this information is obtained from the exposure assessment, which is previously calculated and depends on the model adopted.

b. The probability that the infectious agent stayed viable after its ingestion, and initiated infection.

c. The probability that the host develops illness once he/she is infected.

The dose–response analysis describes the microbial disease process, including the following factors:
a. The organism characteristics; e.g., resistance to the specific and non-specific immune response attack, virulence factors, and the mechanism of pathogenicity.

b. The weakness of the host; e.g., immuno-competence, malnourished condition, elderly and children.

c. The characteristics in the food or drink where the organism is transported; foods rich in lipids will protect the infectious agent from the acidic stomach pH.

A second form to develop a dose-response function will examine how the infectious process happens, and convert that information into a mathematical function. In the development of the dose-response function, it is assumed that the infectious process occurs and the information is converted into a mathematical function. The pathogenic microorganism survives and passes through all of the barriers in order to produce the illness (response), a fact that implies the existence of some finite probability that the pathogen will be successful; thus, the concept can be transformed into probability statements and then into mathematical functions.

Haas et al., [1999] proposed a dose-response model using a mathematical derivation. The model summarizes as follows: in any infectious event there is a probability (P1) to ingest “j” organisms when a person is exposed to a mean dose “d”. This probability is indicated by:

\[ P1 (j \mid d) \]  \hspace{1cm} \text{Equation 1} 

This can be interpreted as the probability of “j” cells being ingested in a dose that contained an average of “d” cells. Further, once pathogens are ingested, only some “k” organisms will have the probability to survive and begin an infection or illness. Now the probability is represented by P2, which includes the host and microbe interaction and can be written as:
Equation 2 indicates the probability of “k” cells surviving given that “j” cells were ingested. Considering that both processes, P1 and P2, are independent, and then the general probability can be written as [Hass et al., 1999]:

\[
P(k) = \sum_{j=1}^{\infty} P_1(j \mid d) P_2(k \mid j)
\]

\[
P_{resp(d)} = \sum_{j=k_{min}}^{\infty} \sum_{j=k}^{\infty} P_1(j \mid d) P_2(k \mid j)
\]

The infection or response will take place if some critical \( k_{min} \) organisms survive.

Two hypotheses can be applied to explain how infection and illness occur once the pathogen is ingested. The first, called “cooperative interaction” or “threshold assumption,” states that the organisms cooperate in a group to pass the barriers. The human body exhibits multiple barriers that ingested pathogens have to pass in order to access correct infection sites where the infectious process and illness can be initiated. In this case the response occurs with minimum dose, assuming that \( k_{min} > 1 \). The second hypothesis is known as “independent action” and assumes that the organisms operate alone and no threshold exists. For this other condition, the minimum dose to obtain a response is 1, so \( k_{min} = 1 \).

At the present time, the second hypothesis of the independent action is used to establish the model, because only one surviving cell is capable of reproducing and generating a response. A previous assumption of the pathogenic infection is that there is no non-threshold for pathogenic infection; in that case, depending on the assumptions associated with P1 and P2, and considering the especial type of probability and its distribution, it is possible to make some
mathematical operations and obtain diverse mathematical functions. The most common functional forms utilized in dose-response are the exponential and beta Poisson function.

1.5.2.1 Exponential Dose-Response Function

The exponential dose-response function assumes the following:

a. One cell is capable of causing the infection and there is no threshold.

b. Random distribution of organisms in the serving, P1, is explained by the Poisson procedure.

c. A constant relationship between the host-pathogen is represented by P2.

Adopting the prior assumptions, the exponential dose-response function is derived by Hass et al., [1999] as follows:

\[ P_{\text{response}} = 1 - \exp(-rd) \]  
Equation 5

where “d” is the dose administrated and “r” represents the event or characteristics in the process of the dose-response function, which is interpreted as the probability of one cell to effectively start a response or infection.

The median infection dose \( (N_{50}) \) is given by

\[ N_{50} = \ln(0.5)/-r \]  
Equation 6

If \( K = 1/r \), also the probability of response can be calculated with:

\[ P_{\text{response}} = 1 - \exp(-d/K) \]  
Equation 7

1.5.2.2 Beta-Poisson dose-Response Function

The beta-Poisson dose-response function includes variations in the success rate. In human hosts, the variations can be different human response, diversity of pathogen competence, or both. The beta-Poisson dose-response functions presuppose the following assumptions:

a. One cell is capable of causing the infection and there is no threshold.

b. Random distribution of organisms in the serving, P1 is explained by the Poisson procedure.
c. The finite probability that the organism will pass all the barriers and effectively initiate infection (independent action) - Binomial.

d. The finite probability of bypassing each barrier to allow the contact host-pathogen is beta distributed, P2 or beta distribution, where d is the dose, and β and α, are the two parameters of the beta distribution that explains the host-pathogen relation. The beta-Poisson dose response can be calculated using a simplified version of the distribution (Haas. et al., 1999).

\[
P_{\text{infection Beta - Poisson}} = 1 - \left[\frac{(2^\alpha - 1)}{\beta} d + 1\right]^{-\alpha}
\]

Equation 8

Where d = dose, α and β are the parameters to fit the model. Any change in the two parameters produces a different effect in the dose-response model. Then, when beta is changed and alpha is fixed, the changes in the curve are analogous to those observed in the exponential model. But, when the beta parameter is changed, it generates a change in the slope of the curve.

In general, the beta-Poisson model is more flexible to describe data, and it is used to obtain results when more data are added to the projects. All models accepted should be examined by goodness of fit.

1.5.3 Cryptosporidium Risk Assessments

QMRA has been applied to evaluate the risk of different outbreaks caused by other types of microorganisms, including Cryptosporidium. Examples are Escherichia coli 0157:H07, Salmonella species, Shigella species, Yersinia enterocolytica, Norovirus, and Giardia lamblia.

A brief survey of some applications of the QMRA models demonstrates its usefulness in understanding outbreaks, though only in conjunction with other public health measures.
1.5.3.1 QMRA in Other Countries

*Cryptosporidium* QMRA is used as an important tool around the world for multiple purposes and under different conditions. Since 2002, the QMRA model has been used to evaluate different water sources. QMRA has been employed in The Netherlands, Sweden, Bangladesh, Australia, Thailand, Mexico, and the United States. In The Netherlands, *Cryptosporidium* QMRA assisted policymakers in the generation of alleviation strategies, and in establishing the priorities and assessment of helpful protective methods that integrate it as a component of water safety plans [Schijiven et al., 2011]. QMRA provided data of estimates of swimmer exposure, thus reducing uncertainty in assessing the risk of infection with the waterborne pathogens and allowing the identification of risk groups [Schetz et al., 2011]. These are examples of how *Cryptosporidium* QMRA is employed to increase the degree of community protection, but also for recognition of a population with a higher risk of exposure.

QMRA modeling in other countries has further demonstrated its usefulness in cases where treatment has not yet begun or there is limited data. In Sweden, the QMRA method has been employed to evaluate the risk reduction of *Cryptosporidium* in the river water before treatment to determine water status and improve its treatment [Aström et al., 2007].

It has been used in countries such as Bangladesh with limited data, where the results still offered important information for the regulation of water supplies [Howard et al., 2006]. The Bangladesh study confirms the fact that QMRA can be successfully applied either abundant or reduced data exist for the assessment. Australia used *Cryptosporidium* QMRA to apply a hypothetical water supply system and quantify the risk reduction in a routine monitoring of *Cryptosporidium* and the response to oocysts’ detection [Singor et al., 2006]. The method serves for the application of information for a real situation and as an important prevention tool.
The Monte Carlo simulation was employed in the Thailand QMRA of *Cryptosporidium*, *Giardia* and diarrheic *Escherichia coli* to approximately calculate the human health risks related with the use of canal water for recreation purposes in a tropical area close to the city. The three canals that were investigated accepted municipal, agricultural and a high load of industrial wastewater. An elevated number of *Cryptosporidium* and *Giardia* lamblia were found, in which *Giardia* presented a higher estimated risk. The study results provided an estimated annual risk of diarrheal infection up to 120-fold greater than those previously reported values in the zone of study and in the country [Diallo *et al.*, 2008]. Thailand QMRA results proved the adaptation capacity of the protocol of risk assessment using different statistical methods that calculate risk probability.

In summary, *Cryptosporidium* QMRA has been successfully applied in several countries with the purpose of generating strategies that increase the degree of protection in a water system. Furthermore, the method can be used to recognize populations that could be more affected in the case of a failure in the water disinfection process. In addition, QMRA can be adapted to diverse conditions such is the case of scenarios where the information available is reduced and different mathematical models can be employed to aid the process of risk assessment.

1.5.3.2 QMRA in the United States

Other QMRA studies have demonstrated the usefulness of this model with particular attention to the public health concerns of the United States. In Texas QMRA was used to evaluate the public health impact of the protozoan in vegetables irrigated with water contaminated with *Cryptosporidium* and *Giardia*; it was not only used to evaluate the microbial risk, but also the same assessment could be used to offer a base for development guidelines [Mota *et al.*, 2009]. *Cryptosporidium* and the waterborne pathogens Norovirus, Rotavirus, Adenovirus, *Giardia*
*lamblia, Campylobacter jejuni, Salmonella enterica*, and *Escherichia coli* O157:H7 were utilized to make a QMRA study from the Great Lakes during 2003 and 2004 [Sollar et al., 2010]; the study estimated the probability of pathogen-induced adverse health effects in swimmers exposed to fecal contamination in these recreational waters. The numbers obtained from this study reflected that Norovirus was the major cause of gastrointestinal illness, thus QMRA revealed the agent that had more impact in the risk study [Soller et al., 2010]. Thus, QMRA can ultimately be a primary tool in the overall public health measures in targeting and remediating water contamination.

In 2009, the World Health Organization published a document that contains a guide on a system assessment for *Cryptosporidium* as one of the microbial hazards encountered in drinking water, where *Cryptosporidium* is used as pathogenic enteric protozoan of reference. The WHO considers the QMRA an important instrument to calculate the risks related with a source of water. According to them, the QMRA explains the information that all water providers have to present in order to assess the safety of their water supply system starting from the catchment until the moment that is received by the consumer. The information received is converted into a numerical value that represents how safe the water is at the moment that it is consumed by a certain population, as well as an aid to manage and reduce the risk.

1.5.3.3 Validation of Milwaukee Outbreak

One example of how QMRA has been successfully used is evident in relation to the Milwaukee Outbreak, whose data was validated by Hass et al., [1999]. The research group analyzed the dose-response relationship for *Cryptosporidium parvum*, using the human data provided by DuPont et al. [1995d] and the numbers obtained from the Milwaukee outbreak [Mac Kenzie et al. 1994]. DuPont et al., [1995] data are shown in the Table 1.
The Milwaukee outbreak provided the following information:

a. It is believed that the outbreak produced more than 400,000 cases of illness.

b. Supported on the cases’ distribution, the contamination had a duration time (t) of approximately 21 days, with a probable range of 15-30 days.

c. The attack rate calculated for the region was 0.21, exhibiting a normal distribution with a standard deviation of 0.01.

Because there was no evidence of the amount of water ingested, the distribution of water ingestion proposed by Roseberry et al., [1992d was used. The average daily water intake rate (q) is of 1948 mL, with one standard deviation of 827 mL. An oocysts’ concentration with a corrected geometric mean of 0.79 L\(^{-1}\) by applying the correction of 90% loss of oocysts during the process of freezing and thawing.

The oocysts’ concentration daily dose result was of 1.54 (1,948 X .079 L\(^{-1}\)). Then a single exposure was estimated with the use of exponential best-fit dose-response data to obtain the infection risk as

\[ P_{infection} = 1 - \exp \left[ -(-0.00419) \times 1.54 \right] = 0.0064 \]

Employing the parameter of illness risk already calculated and published by Haas et al., [1996] of 0.39, which indicates that disease risk is independently from the dose, therefore the risk of illness from a single exposure resulted in 0.0025 (0.0064 x 0.39).

Based on the assumptions previously mentioned, each day of exposure represents the same individual risk. The total amount of subjects affected by the disease in 21 days of exposure was calculated as follow
\[ P_{\text{morbidity}} = 1 - (1 - 0.21)^{1/21} = 0.052 \]

Formerly the estimated illness proportion result was 5.2 %. Validation outcomes values differed from the 21% previous reported by the epidemiological studies; because according to Haas these calculations were produced using as a base several assumptions and potential uncertainty conditions. Still, the computed and observed attack proportion showed analogous numbers and in order to improve the comparison between both attack predictions the confidence levels must be assessed by other mathematical methods, which have more precise and accurate operations that lead finally to conclude that the \textit{Cryptosporidium} dose-response validated the Milwaukee outbreak [Haas et al., 1994, 1999]

1.5.4 Effect Assessment

Even as QMRA is needed within a comprehensive public health plan, its effects assessment can be further improved.

\textit{Cryptosporidium} is one of the few pathogenic microbes that have been studied in human trials; the effect assessment was evaluated in its natural environment, showing how variable it is in its infectivity and pathogenicity depending on the species. \textit{C. parvum} is the species best studied; \textit{C. parvum} Iowa isolate, which was original derivate from a calf in the University of Iowa, the Iowa isolated produced significant response with a median dose (\textit{Id}_{50}) of 132 oocysts [DuPont et al., 1995]. Chappell et al., [2006] have revised the dose response of \textit{C. hominis} (TU502) in healthy human adult volunteers. The research group estimated a 50% infectious dose (\textit{Id}_{50}) of 10-83 oocysts; at the same time they found that \textit{C. hominis} generated an increased Ig G serological response under a dose of \( \geq 30 \) oocysts. It is undeniable that some differences exist in response
effect depending on the species. Genotypic studies are essential in order to improve the understanding of the infectivity, taxonomy and the capacity *Cryptosporidium* transmission. The effect assessment must be reviewed using more isolates in human trials. Further information is required to establish a superior human dose- response assessment and avoid the use of surrogated organisms that do not have an analogous infectivity and pathogenicity. A variety of genetic markers have been found in the pathogenic strains; new statistical systems have to be developed in order to comply with the variety of genetic sources.

1.5.5 Dose-response Model Used by USEPA

The dose–response for *Cryptosporidium* QMRA used by the United States Environmental Protection Agency (USEPA) is based on the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR)[USEPA, 2005a]. The model employed by LT2ESWTR is the exponential with \( r=0.09 \) and the infectious dose \( 50 \) (ID\(_{50}\)) of 8 oocysts. Due to uncertainty in the dose-response model, the model parameter is calculated within a uniform range that varies from 0.04 to 0.16. As per recommendation of the Science Advisor Board (SAB) and influenced by the values reported by Okhuysen *et al.*, [1999] about the highly infective *Cryptosporidium* strains TAMU and IOWA, the board advised USEPA to re-analyze the existing models and six more and make a comparative study. Based in the models’ analysis results, the USEPA estimated that the infectivity obtained was consistent with infectivity values estimated from the Milwaukee outbreak [USEPA 2005a and USEPA 2010].

1.5.6 Uncertainty and Variability

It is important to re-examine QMRA with attention to uncertainty and variability because these issues can greatly impact the validity of the data.
1.5.6.1 Uncertainty

Uncertainty is a state of having limited knowledge to predict a possible outcome. When uncertainty is applied to the QMRA, it is an uncertain risk of having undesired effect or significant loss. Because sometimes the mathematical model does not truly measure a real-life situation, most of them only measure approximations.

In 1990, Finkel described and classified many sources of uncertainty and divided them in parameters and models. Further, Finkel made the following distinctions to understand uncertainty; the parameters are measurement, sampling, and systematic errors:

a. Measurement. Measurement error occurs when there are physical limitations that do not permit the precise determination on some important factor, e.g., in a waterborne outbreak, the amount of water consumed by the population exposed is obtained from surveys.

b. Sampling. Random errors emerge from the use of a small number of subjects for the dose–response assessment.

c. Systematic. The systematic failure happens due to error at the time of gathering the data, conducting to favoritism in the direction to determinate information, e.g., only considering the populations that might be more harmfully affected by the infection disease for the study.

Model uncertainty comes up from associations made or the operational equations employed in the development and evaluation of microbial exposure or dose-response. The models of uncertainty are composed of:

a. Surrogate conditions. Surrogate conditions are when there is a possible difference between the concerned amount and the size of it that is used in the model, e.g., the use of pure microbial cultures with high viability for the risk assessment when in the environment the evaluation of microbial viability is very inexact or it is not possible to be determined.
b. Excluded variables. Sometimes the mathematical model employed does not include factors that represent a significant impact in the assessment, e.g., the microorganism’s food survival depends of environmental conditions like pH, water activity, and temperature. These conditions affect the level of pathogens at the point of exposure.

c. Abnormal conditions. These conditions are related to the excluded variables; in this case an unusual, but possible catastrophic event has to be considered because it may contribute to the risk, which could be the case of serial failures in the treatment system that compounds a water plant.

d. Incorrect model form. Uncertainty in the model of dose-response may appear if there are several models that fit with the data. In that case, each model will show a diverse behavior at the moment that low-doses are extrapolated.

In the case of Mac Kenzie’s model, the concentration of oocysts in the water was calculated using uncertain measurable information in order to estimate the population affected. It was caused by the disadvantage of lack of appropriate measurable samples to quantify Cryptosporidium in the contaminated water. They used the ice prepared with contaminated water to calculate the oocyst concentration. A correction was made due to the loss of oocysts during the process of freezing and thawing, thus the exposure assessment was uncertain. Another uncertain value used in Mac Kenzie’s model is the prevalence of gastrointestinal illness in the US was 0.12 per person per month. Hunter et al., [2001] reported a higher prevalence value of 1.2 – 1.4 per person per month. If the lower rate was used this value would decrease the estimated magnitude of the Milwaukee outbreak, this level of uncertainty through the estimated data demonstrates an underlying need to remodel the problem formulation in QMRA.
1.5.6.2 Variability

Variability is defined as the factor to find variations in a determinate situation; in QMRA for *Cryptosporidium*, the sources of variability emerge from special characteristics that can produce different dose-response and distinct exposure. The first one is the amount of water consumed that differs depending on age and gender. The dose-response sensitivity also is affected by the variability with respect to the severity of the disease (acute or chronic response). In general, elderly, and children are more aggressively attacked by pathogens. Similarly, immuno-compromised individuals such those with AIDS, in immunosuppressant therapy, and malnourished will show a severe response. These areas where variability shows up in the data demonstrate that the QMRA model must be further adapted.

1.5.7 Interval Estimates

In the process of risk characterization, it is necessary to establish the interval estimates for a risk, offering a range or a possible distribution that gives an idea of how accurate the risk was calculated. The first requirement to make the intervals, it is determine if the inputs are accurate or not, or if they can be obtained from a range value. In this evaluation also it is important to distinguish and consider uncertainty and variability.

1.5.8 Limitations with dose-Response Risk Assessment.

In the dose-response assessment procedure, the doses consist of microscopic counted oocysts that are administrated by oral route to the participants; oocysts are ingested by healthy adult humans with an immuno-competent system. Participants must be screened to detect antibodies against the pathogen. The procedure usually makes use of less virulent strains. In ideal conditions multiple exposures have to be estimated.
The following limitations of the method of dose-response assessment have been considered at the moment of the calculations:

a. The dose provided is only an oocysts count, which includes viable and non-viable protozoa. Therefore, the dose administrated does not reflect oocysts’ viability.

b. The employ of less virulent strains; it is important to remember that high pathogenic strains can survive better and have the possibility to be in contact with the population.

c. Only immuno-competent individuals receive the dose. The general population is also composed of persons of different or low, deficient and suppressed immune response. Thus, these types of individuals can generate a more severe response. Due to ethical principles it is difficult to quantify the nature of response in this kind of population. The data available for this concept is obtained only from some animal studies.

d. With the exception of the dose-response assessment of *Cryptosporidium* studies, few researchers [DuPont *et al.*, 1995 and Okhuysen *et al.*, 1999] have evaluated the response to multiple exposures.

e. Method should measure the oocysts shedding time generated by the participants in the study in order to determine their risk impact in environmental waters sources.

f. More limitations are found in the application of the dose-response mathematical model that extrapolates high to low oocyst doses. The current model uses a high dose of 500 oocysts that were administrated in human feedings; due to ethical issues, human trials cannot be exposed to elevate doses such more than 1,000 oocysts. Thus, high dose effects serve as a base to evaluate the risk assessment and they are used to estimate the effect of low oocysts doses.

In addition, most of the information to evaluate several doses has been produced in animal models and their effects also are extrapolated to humans in order to estimate the risk.
1.5.9 Integrating Risk Assessment Results with Risk Management

Water companies and the authorities in charge of water policies have to make decisions based on the information from QMRA/epidemiology studies, which is complex and has a degree of uncertainty.

QMRA data provide valuable tools that could assist to indicate a degree of safety in different water sources rather than a fixed assessment of health risk. Further, QMRA provides significant systematic information that can facilitate and generate a better understanding of the pathways and obstacles that pathogenic microorganisms such as Cryptosporidium have to pass in order to cause an infection and/or disease. Gale et al., [2002] found that the risk assessment information has some gaps and weak points. He considered that the QMRA is a chain of mathematical calculations with a fragile connection, in which frequently there is a component sustained in reduced or insufficient information and with an elevated level of uncertainty. For this reason QMRA is a concept that requires more investigation, especially in those weak areas that require analysis with a more efficient base.

In order to control the Cryptosporidium risk in a source of water, some common actions are taken in risk management, such as monitoring increase, improving catchments, producing better filtration systems and launching supplementary procedures in water treatment. When risk management actions are taken, some important issues must be addressed like financial cost, manufacturing, and legal and political concerns that are involved.

In 2009, the WHO found two main areas of concern; these areas have a priority in investigation. The concerning areas are the exposure and effect assessment.

In the exposure assessment, where detection and quantitative methods of Cryptosporidium need to be improved, the crucial areas of concern are:
a. Produce accurate methods of oocysts recovery that includes confirmation of viability and infectivity.

b. In the water treatment more site-specific QMRA data are required. With the exception of ozone most water suppliers generally do not have site-specific records of where and how much *Cryptosporidium* is removed from the water in treatment. In any well-operated treatment procedure those rare but significant moments of reduced performance can occur and in that case, they increase the health risk. It is well known that most of the outbreaks are due to deficiencies in the treatment e.g., ultra violet rays treatment has high sensitivity at low doses. But, some oocysts sub-populations have demonstrated resistance due to a possible DNA repair mechanism. More investigation is necessary to understand the oocysts ability of UV repair mechanism.

c. In the distribution it is important to assess the possibility that *Cryptosporidium* might come into the dispersal network, like in those moments when there is no or low pressure, leaks and/or leak repairs. Records of all of these conditions must be kept, including frequency and degree of the event and if *Cryptosporidium* was found in the contaminated material in the system. Since QMRA is part of a comprehensive plan and has some limitations, it is necessary to evaluate it more closely.

1.5.10 Future Research

USEPA and WHO agree that future research is necessary since there are many data gaps that still exist in the health effects areas of prevalence in the water sources, risk assessment, and treatment of cryptosporidiosis. Additionally, amplification of the pathogenic mechanism produced by *Cryptosporidium* will offer valuable information.
The risk assessment for predisposed populations must be characterized as well as their
determination of infective dose and virulence. Serological methods should be improved,
additional epidemiological studies have to be conducted to create a serological database, and the
models used for risk assessment must be more accurate.
2.0 DISSERTATION AIMS

2.1 Aim I

To compare the predictions from the current Cryptosporidium risk assessment methodology with laboratory-generated dose-response output, proportions of Cryptosporidium infections (empirical) will be calculated for each level of exposure. In addition, the theoretical risk for each exposure level will be calculated using the conventional Cryptosporidium risk model by Haas et al., [1999]. The set of proportions of infection will be compared to the set of risks estimated by the current risk model.

2.2 Aim II

To determine the impact of immune status on Cryptosporidium infections; proportions of Cryptosporidium infections (empirical) will be calculated for each level of exposure taking in consideration the antibodies concentration. The calculated proportions will be stratified in the base of antibodies levels. The theoretical risk for each exposure level and antibodies concentration will be calculated using the conventional Cryptosporidium risk model by Haas et al., [1999].

2.3 Aim III

To estimate the impact of Cryptosporidium on drinking water treatment standards employing the results produced in aim I
3.0 AIM I

3.1 Materials and Methods

3.1.1 Materials

Aim I was to determine differences in outcomes of the dose-response compared to the previous initial studies conducted by Haas et al., [1999]. It was necessary to obtain two legal documents, a Memorandum of Understanding (MOU) and institutional authorization, to conduct and collect laboratory dose-response data from human subjects. The MOU was made with Dr. Cynthia L. Chappell et al. from the University of Texas Health Science Center at Houston’s School of Public Health and the second document was obtained from the Institutional Review Board (IRB) within The University of Texas El Paso (UTEP).

The MOU was drafted between the author, Co-chair/advisor, Statistician and data Owner which established the agreements, terms, goals, objectives, conditions, patient confidentiality and cost of dose-response research. The IRB document (387643-1) was approved by the UTEP IRB Committee on December 07, 2012. Once the two legal requirements were completed and authorized for this study, a complete laboratory generated dose-response data were provided by the owner Dr. Cynthia L. Chappell to be used as a baseline for the risk assessment research protocol.

The data was composed from the dose-response outcomes of 159 volunteers. Of these, 29 belonged to the first human dose-response published in 1995 from the owner and collaborators (Table 1). The 29 results were given the name “initial”. The additional 130 dose-response outcomes from human volunteers were produced by investigators during the last eighteen years. A second group of participants were given the name “complete” consisted by merging these data of the 29 and 130 to have a dose-response data of 159 volunteers, composed by the initial and the
new. These data sets selected contained the infection response of 159 volunteers who tested normal for complete blood count, blood chemistry panel, urinalysis, T-cell subgroups, chest radiography, and electrocardiography as well as negative stool tests for occult blood and parasites. Furthermore, no antibodies were found for them from the serological tests of hepatitis B surface antigen, syphilis, and the human immunodeficiency virus. An additional requirement for the participants was they all needed to be positive for the skin delayed reactivity to the control antigens of *Trichophyton*, mumps, and *Candida* as well as a negative tuberculin skin test. Once the previous tests were conducted, volunteers were exposed to different infective tested oocysts doses [DuPont *et al.*, 1995, Chappell *et al.*, 1996, Okuyusen *et al.*, 1998]. The parasite exposure was called “a challenger” by the owner.

Table 1. Cryptosporidium Oocysts Infectivity in Human Volunteers by Oral Dosing. [DuPont *et al.*, 1995]

<table>
<thead>
<tr>
<th>Dose Administrated (Number of oocysts)</th>
<th>Number of Positive Volunteers</th>
<th>Total Volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>300</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>500</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>1,000</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10,000</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>100,000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1,000,000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>29</td>
</tr>
</tbody>
</table>
3.1.2 Methods

3.1.2.1

Observed proportions were calculated by dividing the number of individuals which developed the infection over total participants. The infection was established in human volunteers that excreted oocysts on the stool.

3.1.2.2

Using the SAS® (9.3) program and Equation 5 of the mathematic exponential model recommended by Haas et al., [1999 and USEPA, 2012], the r-values were computed for the initial and complete dose-response data sets. Each value of “r” was named according to its corresponding data.

\[ P_{infection} = 1 - \exp (-r \cdot d) \]  

Equation 5

3.1.2.3

Theoretical proportions were obtained using Equation 5 of conventional exponential mathematic model and employing on each calculation their respective value of “r” previously estimated. The exponential infection proportions for initial and complete datasets were computed using Microsoft Excel (2010).

3.1.2.4

A goodness of fit test was evaluated for the exponential model employing the Chi-Square \( (X^2) \) test estimated by Microsoft Excel (2010) and Mathematica® software Version 9.0. Critical values of chi-square were found using Table F from Wayne [2009] and results are displayed in Table 5.
3.1.2.5

The datasets were computed with the Beta-Poisson mathematical model using Equation 8 and were fitted to the Nonlinear Model Fit command with Mathematica® software. The chi-Square ($X^2$) test for the Beta-Poisson infection proportion was assessed for goodness of fit using Microsoft Excel (2010) and Mathematica® software. Critical values of chi-squares were found using Table F from Wayne [2009].

3.1.2.6

Observed infection and morbidity proportions were computed and graphed with Mathematica® software (Figure 2). Volunteers were considered infected when they excreted the oocysts in feces and disease was established for participants who presented one or more of the following symptoms: diarrhea, stomach pain, and/or cramps, vomiting, nausea, fever, and fatigue.

3.2 Aim I Results

Table 2 includes the infection proportion of 159 volunteers challenged with viable *C. parvum* and *C. hominis* oocysts; these outcomes were employed to make the comparative study between the initial and complete dose-response datasets, where initial dataset presented an observed infective response in 18 (62%) out of 29 subjects and complete dataset 71 (45%) of 159.
Table 2. *Cryptosporidium* oocysts Infectivity of 159 Human Volunteers by Oral dosing.

<table>
<thead>
<tr>
<th>Dose Administered Numbers of oocysts</th>
<th>Initial Positive Subjects</th>
<th>Initial Total Subjects</th>
<th>New Positive Subjects</th>
<th>New Total Subjects</th>
<th>Complete Positive Subjects</th>
<th>Complete Total Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>6</td>
<td>11</td>
<td>6</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>17</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>8</td>
<td>11</td>
<td>27</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>300</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>500</td>
<td>5</td>
<td>6</td>
<td>10</td>
<td>27</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>1000</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td></td>
<td>3</td>
<td>13</td>
<td>3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>10 000</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>12</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>50 000</td>
<td></td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>100 000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1 000 000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>29</td>
<td>53</td>
<td>130</td>
<td>71</td>
<td>159</td>
</tr>
</tbody>
</table>

Table 3 displays the “r-values” calculated by the SAS® (9.3) for the exponential model of both datasets. The r-value of initial dataset was 0.00419, which was identical to the r-value reported by Haas et al., [1999] based on the same human outcomes. The complete r-value was 0.00137, which comprises the amalgamation of initial and new dose-response data. This unification was possible because these data came from the same research group of Dr. Chappell and both studies were conducted under the same laboratory controlled conditions, as well as the combination has more statistical representation. Table 3 shows the reduced r-values of the complete dataset when the number of participants increased.
Table 3. Estimated r-values calculated by SAS® (9.3). Initial dose –response of 29 volunteers (p-value= 0.00375), and complete with 159 subjects (p-value= 0.000025).

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Estimated r-value</th>
<th>Approximate 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.00419</td>
<td>0.00143</td>
</tr>
<tr>
<td>Complete</td>
<td>0.00137</td>
<td>0.000745</td>
</tr>
</tbody>
</table>

Table 4 shows the observed infection proportion and exponential model results of initial and complete datasets. Comparing the values of both data sets, the initial exponential model results reflected the previous reported and expected trend, in which exposure to higher parasite doses produced an increased the infection proportion and the observed values fit well with the model; while complete dataset does not follow the exponential distribution because: a) the infection proportion does not fall towards zero as the dose decreases down to 10 oocysts, and b) the infection proportion does not asymptote towards 1.0 as the dose increases. Datasets and exponential model were tested for goodness of fit with Chi-Square test ($X^2$).

Table 5 indicates the complete exponential model Chi-Square test values with a computed $X^2 = 303$ and critical value of chi-square ($X^2$) of 19.675 with $\alpha= 0.05$ and 11 degrees of freedom (d. f.), since the computed $X^2$ value was greater than the critical $X^2$, exponential model was rejected.

With the statistical rejection of exponential model, the complete dataset was computed with the Beta-Poisson model to produce parameters of $\alpha = 0.0446769$ and $\beta= 3204.17$ followed with the theoretical estimation of the dataset infection proportion which produced a more adequate correlation between observed infective response and Beta–Poisson. Posteriorly, Chi-Square test was performed for the goodness of fit of the Beta-Poisson. Table 6 indicate the
Table 4. Observed and exponential model values from infection proportion of initial [Haas et al., 1996] and complete data sets. Initial r-value =0.00419 and complete r-value 0.00137.

<table>
<thead>
<tr>
<th>Oocysts dose</th>
<th>Initial Observed</th>
<th>Initial Exponential</th>
<th>Complete Observed</th>
<th>Complete Exponential</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.2</td>
<td>0.118</td>
<td>0.545</td>
<td>0.014</td>
</tr>
<tr>
<td>30</td>
<td>0.375</td>
<td>0.342</td>
<td>0.318</td>
<td>0.040</td>
</tr>
<tr>
<td>100</td>
<td>0.667</td>
<td>0.715</td>
<td>0.400</td>
<td>0.128</td>
</tr>
<tr>
<td>300</td>
<td>0.833</td>
<td>0.877</td>
<td>0.455</td>
<td>0.337</td>
</tr>
<tr>
<td>500</td>
<td>0.800</td>
<td>0.877</td>
<td>0.455</td>
<td>0.496</td>
</tr>
<tr>
<td>1000</td>
<td>1</td>
<td>0.985</td>
<td>0.400</td>
<td>0.746</td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>0.750</td>
<td>0.984</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td></td>
<td>0.231</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>10000</td>
<td>1</td>
<td>1</td>
<td>0.667</td>
<td>1</td>
</tr>
<tr>
<td>50000</td>
<td></td>
<td>0.667</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>100000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1000000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

values used to compute $X^2$ with 10 degrees of freedom and $\alpha=0.05$ for the complete Beta-Poisson model, where computed $X^2$ was of 5.23 and critical $X^2$ of 18.307 for the reason that computed $X^2$ was lower than $X^2$ critical, the complete dataset and Beta-Poisson model fits better.

A better perception of comparative results are exhibited in Figure 1 which displays the initial observed infection proportion of 29 volunteers represented by blue circles and the complete observed proportion of 159 volunteers represented by red circles. The size area of each circle is directly proportional to the number of volunteers exposed to each dose. The first red circle corresponds to 11 volunteers exposed to a dose of 10 oocysts in which 6 (55%) out 11 participants presented a positive response. The second red circle indicates the exposure of 22 subjects to 30 parasites, with only 7 (31%) out of 22 responded to infection. The third red circle represents 35 individuals exposed to 100 oocysts, in which 14 (40%) out of 35 were positive, shows the result of 11 participants, 5 (45%) out of 11 were infected with 300 oocysts. The fifth
Table 5. Exponential model Chi-Square values, with 11 degrees of freedom. Computed $X^2 = 303.11$ and critical $X^2 = 19.675$.

<table>
<thead>
<tr>
<th>Oocysts Dose</th>
<th>Observed (Oi)</th>
<th>Expected (Ei)</th>
<th>$(Oi - Ei)^2 / Ei$</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>6.000</td>
<td>0.150</td>
<td>229.759</td>
<td>11</td>
</tr>
<tr>
<td>30</td>
<td>7.000</td>
<td>0.886</td>
<td>42.252</td>
<td>22</td>
</tr>
<tr>
<td>100</td>
<td>14.000</td>
<td>4.480</td>
<td>20.23</td>
<td>35</td>
</tr>
<tr>
<td>300</td>
<td>5.000</td>
<td>3.706</td>
<td>0.451</td>
<td>11</td>
</tr>
<tr>
<td>500</td>
<td>15.000</td>
<td>16.361</td>
<td>0.113</td>
<td>33</td>
</tr>
<tr>
<td>1000</td>
<td>4.000</td>
<td>7.458</td>
<td>1.603</td>
<td>10</td>
</tr>
<tr>
<td>3000</td>
<td>3.000</td>
<td>3.934</td>
<td>0.221</td>
<td>4</td>
</tr>
<tr>
<td>5000</td>
<td>3.000</td>
<td>12.986</td>
<td>7.679</td>
<td>13</td>
</tr>
<tr>
<td>10000</td>
<td>10.000</td>
<td>15.000</td>
<td>1.666</td>
<td>15</td>
</tr>
<tr>
<td>50000</td>
<td>2.000</td>
<td>3.000</td>
<td>0.333</td>
<td>3</td>
</tr>
<tr>
<td>100000</td>
<td>1.000</td>
<td>1.000</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1000000</td>
<td>1.000</td>
<td>1.000</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 6. Beta-Poisson model values and results of the Chi-Square ($X^2$) test. Computed $X^2$ of 5.21 and critical $X^2 = 18.307$ with 10 degrees of freedom and $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Oocysts Dose</th>
<th>Observed (Oi)</th>
<th>Expected (Ei)</th>
<th>$(Oi - Ei)^2 / Ei$</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>6.000</td>
<td>3.883</td>
<td>1.154</td>
<td>11</td>
</tr>
<tr>
<td>30</td>
<td>7.000</td>
<td>8.447</td>
<td>0.248</td>
<td>22</td>
</tr>
<tr>
<td>100</td>
<td>14.000</td>
<td>14.568</td>
<td>0.022</td>
<td>35</td>
</tr>
<tr>
<td>300</td>
<td>5.000</td>
<td>4.886</td>
<td>0.003</td>
<td>11</td>
</tr>
<tr>
<td>500</td>
<td>15.000</td>
<td>15.072</td>
<td>0.000</td>
<td>33</td>
</tr>
<tr>
<td>1000</td>
<td>4.000</td>
<td>4.733</td>
<td>0.114</td>
<td>10</td>
</tr>
<tr>
<td>3000</td>
<td>3.000</td>
<td>1.994</td>
<td>0.507</td>
<td>4</td>
</tr>
<tr>
<td>5000</td>
<td>3.000</td>
<td>6.628</td>
<td>1.986</td>
<td>13</td>
</tr>
<tr>
<td>10000</td>
<td>10.000</td>
<td>7.872</td>
<td>0.575</td>
<td>15</td>
</tr>
<tr>
<td>50000</td>
<td>2.000</td>
<td>1.673</td>
<td>0.064</td>
<td>3</td>
</tr>
<tr>
<td>100000</td>
<td>1.000</td>
<td>0.571</td>
<td>0.322</td>
<td>1</td>
</tr>
<tr>
<td>1000000</td>
<td>1.000</td>
<td>0.613</td>
<td>0.244</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 1. Observed initial infection proportions represented by blue circles and observed complete infection proportions characterized by the red circle. Circles areas are directly proportional to the number of volunteers exposed to each oocysts dose. Blue line indicates exponential model of initial dataset, red line the complete dataset and green line represents complete dataset with Beta-Poisson model.

red circle symbolizes 33 volunteers exposed to the dose of 500, with 15 infected (45%) of the 33. The sixth red circle indicates 10 participants, 4 (40%) of them infected with a dose of 1000. The seventh red circle exemplifies 4 subjects challenged with 3000 parasites, 3 (75%) out of 4 result positive. The eighth red circle represents an infective response of 3 (23%) out 13 volunteers exposed to 5000 dose. The dose of 10,000 is characterized by the ninth red circle resulted with 10 (66%) positive of 15. The tenth red circle symbolizes 3 volunteers that received 50,000 oocysts with 2 (66 %) of them being positive. The eleventh and twelfth red circles denote the elevated doses of 100,000 and 1,000,000 with the participation of only one volunteer per dose.
and the infection result was positive in both cases. As it is shown in Figure 1, the complete observed proportions showed constant fluctuations in the response with an average of 45% of the positive result for the doses between 30 to 1,000 oocysts, which implies that despite volunteers fed with a low dose of 30 or a higher of 1,000, in general 45% of them developed an infection. This trend is demonstrated by a high concentration of red circles in the area around 45% where 111 subjects presented an infective response representing 69.8% of the total participants. In doses above 3,000 parasites, the response was around 72%. However, it is important to notice that in elevated doses of 100,000 or 1,000,000, the number of volunteers was only one in each case.

Figure 1 also shows the application of the initial (blue line), and complete (red line) datasets to the exponential model and the complete dataset with a linear Beta-Poisson response (green line). It is distinguished that initial exponential produced the expected pattern of higher doses yield a stronger infective response, but when the exponential model was applied to the 159 responses of the complete dataset it cannot be denied that the dataset does not follow the exponential model and the model is not the most appropriated to represent it. It is perceived that despite the low dose of 10 or a higher of 1000 oocysts, there is a constant response to infection that does not follow an exponential trend. When doses above 3000 oocysts were administered, the infection response was abrupt as a result of a human body defense mechanism overwhelmed with massive parasite doses.

It can be perceived in Figure 1 that the complete dataset followed better the Beta-Poisson model, which is demonstrated with most of the observed infection proportions (red circles) around the Beta-Poisson green line in 9 of 12 doses, even though it is important to remember that the last two doses only exposed one volunteer.
Figure 2 displays the complete observed infection proportion and morbidity of the 159 volunteers, where red circles represent an infection proportion mean of 45%; orange circles indicate disease proportion reflecting an average of 35%; circle area size is proportional to the number of volunteers exposed to each oocysts dose with a disease/infection ratio of 80%.

![Figure 2. Complete observed morbidity and infection proportion of 159 volunteers. Observed infection = 45% and observed disease 35%. Circles areas are directly proportional to the number of volunteers exposed to each oocysts dose. Disease/Infection ratio= 80 %.](image)

3.3.0 Conclusion and Discussion of Aim I

It was possible to compare initial dose-response data of Cryptosporidium with a more complete laboratory dose-response data resulting in the production of different outcomes. The results demonstrated a remarkable difference between initial and complete observed infection proportion; as well as the initial data set fitted well with the exponential model but no the complete dataset that practically was forced to enter into the model. In fact, this is reinforced with the statistically rejection of the goodness of fit model. Results demonstrated that the
exponential model cannot be used to assess the risk of complete dataset; moreover, it is detected that 70% of volunteers presented a constant infective response around 45%, and an overwhelmed response in elevated doses above 3000; moreover at low doses of 10 oocysts, the human response was positive which was already been mentioned by the WHO [2009], and USEPA [2010]. Complete dataset and Beta-Poisson model seems to have more adequate correlation, which is reinforced with a good Chi-square test result. Even though the best model for complete dataset is unknown without the need of a mathematical model observed the results indicated a constant infective response of 45% in volunteers exposed, a morbidity of 35%, and an increased morbidity/infection ratio of 80% superior to 39% reported in 1996 [Haas et al.,] from the initial dataset, which implies a higher risk in the healthy population used in this study.

Outcomes reflected the risk assessment analysis of more than five times the human volunteers published in the initial dose-response study. In the same way the calculations were made with no gaps of information or with no need of an animal’s risks and the extrapolation of data because the evidences came from healthy human adults.

From the epidemiological point of view, the results of the complete dataset implied a constant infective response to Cryptosporidium. At least for the complete dataset, the exponential model is not the best model anymore to evaluate the parasitosis risk. Although the Beta-Poisson model is more adequate, with the conclusion of this study, it is still not known; which is the best model to analyze the complete dataset indicating the need of additional studies to re-assess Cryptosporidium QMRA that will be reflected on drinking water risk.

In addition to this, the comparative study revealed that each dose exposure assessment requires its own determination of the “r” parameter due to diverse conditions exist in each risk assessment (i.e. varied environments, geographic circumstances, human race, population size, people
immune status, source and type of water and treatment types). During recent years, several
investigators around the world have continued using the exponential model such as in the case of
Teunis et al., [2000 and 2002] and Sweets P. et al., [2007] in the Netherlands, Cummings et al.,
[2010] in Ireland, Agullo´-Barcelo´ et al., in Spain [2012], Sokolova et al., [2012] and Westrell
worked with models like the Monte Carlo stimulation employed by Pouillot et al., [2004] in
France; in China, Xiao et al., [2012] ran a hydrodynamic and microbiological model; while in
Australia, Longanthan et al.,[2012] computed the risk with SPPS software. Similarly, Messner
et al., [2006, 2010, and 2012] in the U.S. has reported three r-values produced using the
exponential model. Despite which mathematical model was selected to measure the risk
assessment, evaluators in the U.S. and worldwide have calculated their own value of “r”
depending of their distinctive conditions which has resulted in multiple scientific publications
related with QMRA.

3.4.0 Aim I Study Impact

The results of this study indicates the exponential model which has been used since 1996 in
several risk assessments of Cryptosporidium is not a suitable model for the complete human
laboratory generated dose-response. Additionally, the results demonstrated that the human
infection response observed followed a constant positive pattern of infection proportion which
represented a higher risk of develop the parasitosis. It was also demonstrated that the Beta-
Poisson model can be used to assess the parasitosis risk inferring a higher threat that the one that
has been managed for more than 20 years. Therefore, if the human exposure to the parasite
denotes a constant or higher risk, water polices must be re-evaluated to evade and/or reduce the
risk of cryptosporidiosis in the population.
Another point must be taken into consideration, if a higher risk was estimated for healthy adults with their competent immune systems having the ability to reduce the probability of infection then immuno-compromised persons would have a greater risk. Unfortunately, for ethical reasons, it is not possible the risk evaluating these types of populations. In support of ethical considerations for immuno-compromised people, it is important to review, modify, and perhaps increase the rules and regulations for drinking water as dictated by USEPA and WHO [2009]. This recommendation can help prevent the possibility of affecting sensitive people as demonstrated by an increased mortality rate in the immuno-compromised population that lived in the Milwaukee area reported by Hoxie et al. [1997] after the major *Cryptosporidium* outbreak.
4.0 AIM II

4.1 Materials and Methods

4.1.1 Materials.

Table 7 represents the infection response of 159 healthy human volunteers exposed to infective and viable [Okhuysen et al., 2001, Chappell et al., 2006] oocysts of Cryptosporidium. Volunteers were challenged with seven different geographic isolated strains of C. parvum. The Iowa strain isolated originally from a calf at the University of Iowa, Ames (Duppont et al., 1995, Okhuysen et al., 1999]. A UCP calf secreted strain from the Uniformed Services University of the Health Sciences, Bethesda, MD [Okhuysen et al., 1999]. A Texas strain received from a veterinary student that acquired the parasite while practicing a necropsy of an infected foal from Texas A&M University, College Station, TX. The Moredun isolate obtained from a deer and facilitated by the Moredun Research Institute, Glasgow, Scotland [Chappell et al., 2004]. Finally, isolates from human outbreaks that occurred in 1997 in the country of Peru, and 16W from the 1998 Washington, D.C. outbreak. Also included is a child isolated called TU502 of C. hominis [Chappell et al., 2006]. As was detected, the laboratory generated dose-response data of Table 7 was complex to analyze due different reasons, isolates were from the different geographical locations, which also came from diverse animal sources, and range of infective response. These responses varied from 33% to 75%, and for these reasons it was decided that for the purpose of aim II a more simple examination was needed which resulted in the formation of three groups of data. The first contained 34 volunteers with previous positive anti-Cryptosporidium antibodies called complete +, a second set of 96 volunteers with negative anti-Cryptosporidium antibodies named complete –, and a third set which included the 157 volunteers with and without anti-Cryptosporidium antibodies entitled complete – and +.
Prior to the parasite exposure challenge, all volunteers were previously examined. The selected volunteers presented normal results for complete blood count, blood chemistry panel, urinalysis, T-cell subgroup, chest radiography, electrocardiography as well as negative stool tests for occult blood and parasites, as well as no antibodies for hepatitis B surface antigen, syphilis, and the human immunodeficiency virus [DuPont et al., 1995; Chappell et al., 1999; Okhuysen et al., 1999]. Microsoft Excel (2010) was used to prepare the datasets and SAS® (9.3) was used for statistical analysis.

Table 7. Infection response of the 159 healthy human volunteers exposed to the six isolates (Iowa, UCP, Texas, Peru, Moredun and 16W) of *C. parvum* and one of *C. hominis* (TU502).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Iowa</th>
<th>UCP</th>
<th>Texas</th>
<th>Peru</th>
<th>Moredun</th>
<th>16W</th>
<th>TU502</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocysts dose</td>
<td>Infected</td>
<td>Non Infected</td>
<td>Infected</td>
<td>Non Infected</td>
<td>Infected</td>
<td>Non Infected</td>
<td>Infected</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>3</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>100</td>
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<td>5</td>
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<td>4</td>
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<td></td>
</tr>
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<td>300</td>
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<td>1</td>
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<td>2</td>
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<td></td>
</tr>
<tr>
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<td>5</td>
<td>4</td>
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<td>3</td>
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<td>0</td>
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<td></td>
<td></td>
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<td>25</td>
<td>24</td>
<td>7</td>
<td>14</td>
<td>12</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>

Infected/total: 25/49, 7/21, 12/26, 2/3, 8/16, 8/18, 926

| Infection Proportion | 0.510 | 0.333 | 0.462 | 0.667 | 0.500 | 0.444 | 0.346 |

4.1.2 Methods

4.1.2.1

A primary comparative table (Table 8) was prepared using Microsoft Excel (2010) which included the complete observed infection proportion of volunteers with complete -, complete +, and complete – and + anti-*Cryptosporidium* antibodies.
4.1.2.2

Statistical analysis was used to determine significant relationships for the observations of Table 8. Data were divided into strata according with the dose provided to the subjects and antibody status. These data were evaluated using the Conchran-Mantel-Haenszel, Fisher Exact, and Chi-square statistical tests using SAS® (9.3).

4.2 Aim II Results

Table 8 displays the dose-response of 159 human volunteers, the oocysts dose provided, the number of individuals who responded positive to infection, the total volunteers per dose, their antibody status (indicated by the negative or positive sign), and the observed infection proportion in relation with the parasite doses administrated. Doses without volunteers are reported as blank. Almost half (48%) of the 125 volunteers with complete negative antibody status presented a positive response while the complete positive volunteers showed a reduced 32% of infectivity (Table 8). Of the total of 159 volunteers, composed by complete negative and positive, 45% had positive infective response (Table 8).

Statistical analysis results showed significance with the Conchran-Mantel-Haenszel test with a p-value of 0.0211, but the Fisher Exact and Chi-square tests did not demonstrate statistical significance (p-value >.05).
Table 8. Observed infection dose-response of volunteers with complete negative, complete positive, and complete negative and positive anti- *Cryptosporidium* antibodies

<table>
<thead>
<tr>
<th>Oocysts dose</th>
<th>Complete - Positive</th>
<th>Total</th>
<th>Observed Proportion</th>
<th>Complete + Positive</th>
<th>Total</th>
<th>Observed Proportion</th>
<th>Complete - and + Positive</th>
<th>Total</th>
<th>Observed Proportion</th>
</tr>
</thead>
<tbody>
<tr>
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<td>10</td>
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<td>0.285</td>
<td>6</td>
<td>11</td>
<td>0.545</td>
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<td>35</td>
<td>0.4</td>
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<td>1</td>
<td>3</td>
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<td>5</td>
<td>11</td>
<td>0.454</td>
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<td>Total</td>
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<td>11</td>
<td>34</td>
<td>71</td>
<td>159</td>
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</tr>
</tbody>
</table>

4.3 Conclusion and Discussion of Aim II.

The datasets presented in Table 7 are not easy to analyze together because the volunteers’ infection proportions are the results of exposure to seven isolates of various geographic locations that come from different animal or human sources and with a variable response. This fact was already evidenced by Teunnis *et al.*, [2002], who reported a more aggressive response of Texas isolate compared to Iowa and UCP isolates which presented a similar infective reaction.

However, when the datasets are analyzed just taking into consideration the presence or absence of anti- *Cryptosporidium* antibodies, the results demonstrated the presence of antibodies in the complete positive volunteers. This conferred them a protective immunological status versus the parasite for doses below 10,000 oocysts and evidently overwhelmed responses to the huge dosage.
It is known that immuno-competent persons that have had contact with an infectious agent such as *Cryptosporidium* normally developed cells with memory and protected antibodies levels that help them to evade the parasite infection; per this immunological protection they can show less severe signs and symptoms and a prompt recovery [Okuyser *et al.*, 1998, Chappell *et al.*, 1999]. Sometimes other people with protective antibodies do not present apparent symptomatology because it has been thought that the immune system guards them [Chappell *et al.*, 2004, 2006]. The observed outcomes of the datasets confirmed that pre-existing antibodies confer a protective status versus the parasite; such results were supported by the statistical significance of the Conchran-Mantel-Haenszel test. In addition, when the exponential model was used to estimate the infection proportion, the subjects with antibodies presented a reduced response compared with volunteers without antibodies. Once again outcomes established the precedent of protection that already was proposed and published by Chappell *et al.*, [1999, 2004], Moss *et al.*, [1998], Teunis *et al.*, [2002].

The results of Aim II contribute to increase the understanding of the immunological response in immuno-competent volunteers against cryptosporidiosis, which it is not well understood yet. Nevertheless it seems that previous exposure to the parasite leads to a certain degree of immunity.

4.4 Aim II Study Impact.

The results not only increased the immunological knowledge of cryptosporidiosis, but represent a way to control the disease which affects overall immuno-compromised people. These results reflect a future promise that can help sensitive populations evade or reduce *Cryptosporidium* infections if a vaccine is developed. Currently, vaccines have only been developed and proved effective in animals such is the case of Liu *et al.*, [2010] which reported a good cellular immune
response on mice using a divalent peptide vaccine. Besides, advances in the parasite genetic information reflect the existence of antigenic proteins that produce an effective humoral and cellular immune responses, as it was proposed by Manque et al., [2011]. In Bangladesh, a study performed in children with cryptosporidiosis, demonstrated that the presence of anti-p23 protein antibodies in their serum produced a reduction of the diarrheic process compared to the control group. The p23 protein is present in most of the parasite infective strains and could be another good candidate vaccine [Borad et al., 2012]. The outcomes of protective status experiments will help to understand how some healthy persons exposed to the parasite do not show any sign or symptoms.
5.0 AIM III

5.1 Materials and Methods

5.1.1 Materials

The dataset from the results of Aim I which include initial exponential, complete constant, and complete Beta-Poisson models. The parameters were also included and used to assess the risk of Cryptosporidium infection, as well as the values of observed infection proportion, morbidity and disease/infection ratios of the complete and initial datasets.

5.1.2 Methods

5.1.2.1 Daily Risk Assessment

Daily risk assessment of infection and disease was calculated using Excel for a hypothetical city with a population of 100,000, assuming 2 liter of daily water ingestion [Roseberry and Bunmaster, 1992]. The following steps were made in order to estimate the risk and also compare the use of complete constant and initial exponential models:

5.1.2.1.1

Random oocysts concentrations per liter were used with a minimum range of 1 X 10^{-10} and maximum of 1. These concentrations were adjusted to a population of 100,000, which consume 2 liters of water daily.

5.1.2.1.2

To compute the daily constant model infection, each oocyst’s concentration was multiplied by constant infection proportion found in Aim I of 44.6541%. The constant disease proportion was estimated with the ratio of 80% obtained in Aim I. Previous information was used to obtain the fraction of the population that can develop the disease considering oocyst/city/day.
5.1.2.1.3

To find the initial exponential infection proportion, the exponential model Equation 5 was used with the r-value of 0.00419 and the oocyst dose. These values are displayed in Table 11. The initial disease proportion was computed using the 39% reported by Haas et al., [1996]. The initial model fraction of the population that has the probability to present the illness was estimated considering the oocyst/city/day.

5.1.2.2 Annual Population Infection Risk Assessment

Using Mathematica®, a log linear plot graph was made, including the datasets from the initial exponential, complete constant and complete Beta-Poisson.

The graph displayed in the abscissa drinking water oocysts concentration in $10^6$ liters and in the ordinate axis the population risk of infection per person per year. For the generation of the liner plot graph the following conditions were assumed:

a. A person ingests 2 liter/day.

b. Aim I slopes results were used to calculate the daily probably of infection, employing for the complete constant the infection observed mean value of 0.4465 and for the complete Beta-Poisson 0.2829.

To obtain the daily probability of infection each slope value was multiplied by the concentration of oocysts in water. Afterwards, the linear exposure result was scaled to population risk. Additional information obtained was applied to produce an annual risk with the Binomial Equation 9.

\[
P(\text{one or more infections per year by each individual}) = 1 - (1-p)^{365.25} \tag{Equation 9}
\]

where \(p = 2 \text{ liters/day} \times \text{risk slope} \times \text{concentration (oocysts/liter)}\) in drinking water.
For comparative purposes, the initial dataset was included in the graph with the same linear approximation at low dose.

5.1.2.3 Milwaukee Validation

The data published by Haas et al., [1999] of the initial exponential model, complete constant, complete Beta-Poisson results of Aim I along with the epidemiological values reported from the Milwaukee outbreak of 1993 [MacKenzie et al., 1994] were employed to validate the results of this study.

5.1.2.3.1

According to Hoxie et al., [1997] during the Milwaukee outbreak of 1993, over the span of approximately two weeks, 403,000 of an estimated 1.61 million residents in the Milwaukee area (of which 880,000, were served by the malfunctioning treatment plant) became ill with stomach cramps, fever, diarrhea and dehydration caused by the pathogen.

5.1.2.3.2

The values reported by Haas et al., [1999] were based in the only water source recovered from the outbreak which was ice prepared with contaminated drinking water, where it was found 0.079 oocyst/L. Haas assumed for the his calculations a 90% of oocysts loss from freezing – thawing process, converting the oocysts concentration into 0.79 per liter. A daily ingestion of 1.948 L/day was considered and an exposure duration of 21 days.

Then Equation 5 of the exponential model was applied using 1.54 (0.79 X 1.94) as the oocysts daily mean dose and the r-value of 0.00419 to obtain the daily probability of infection.

Using the disease rate reported by Haas et al., [1996] of 39%, the single exposure risk of illness was computed. Finally, values were calculated using Equation 9 to determine the probability of disease according with the exposure time.
5.1.2.3.3

To validate the complete Beta-Poisson results from the Milwaukee data of 0.079 oocysts /L, a water consumption of 1.958 L/ day and an exposure time of 17 days reported by Hoxie [1997] were used. With these numbers the oocyst daily mean dose was calculated. The values were applied to Equation 8 with the Beta-Poisson parameters produced on Aim I of \( a=0.045 \) and \( b=3204 \), to obtain the probability of daily infection of complete data set and Beta-Poisson.

A range of probability of disease was computed with 80% disease/infecting ratio produced from Aim I. The range comprised a minimum of a single exposure in seventeen days and a maximum of a daily exposure during the whole period.

5.1.2.3.4

Complete constant model validation was made by dividing the 71 volunteers with infective response by the 159 participants and then multiplied by 80% of the disease/infection ratio produced in Aim I.

5.2 Aim III Results

5.2.1 Daily Risk Assessment

Table 9 shows the risk assessment probability of infection and disease for the complete constant and initial exponential models results. If the oocysts’ concentration of \( 1 \times 10^{-4} \) is analyzed because it is closer to the EPA limit of risk of \( 10^{-4} \), with the complete constant model results, 8.9 of 100,000 persons had a probable infection and 7.2 of them could present the disease representing \( 7.2 \times 10^{-5} \) of the entire affected population (Table 9). In the exponential model for the same oocysts concentration in water 0.084, less than 1 person in 100,000, of the exposed subjects will have the probability of infection, 0.033 of them will develop the disease, and a fraction of illness in the population is zero. However, in the case of a daily concentration of 1
oocyst per liter, it was observed that the complete constant model values can produce a probability of infection in 89,000 persons, and 72 % of a population could develop the disease; whereas the application of the initial exponential model will produce that 830 subjects present a probable infection, from where 330 persons will develop the disease, resulting an illness population fraction of $3.3 \times 10^{-3}$. It is obvious the results demonstrate a great difference between the applications of both models with a higher risk with the complete constant model.
Table 9. Daily Risk Assessment of Cryptosporidium in a population of 100,000 consuming 2 liters of drinking water employing the constant model [This study] and initial model [Haas et al., 1999]. Constant infection proportion of 44.65% and disease/infection ratio 80% with initial exponential infection proportion computed with r-value of 0.00419 and 39% illness rate.

<table>
<thead>
<tr>
<th>Oocysts/ liter</th>
<th>Oocysts/ city/day</th>
<th>Constant Model Infection</th>
<th>Constant Model Disease</th>
<th>Constant Model Fraction of Population Disease</th>
<th>Initial Exponential Model Infection</th>
<th>Initial Exponential Model Disease</th>
<th>Initial Exponential Model Fraction of Population Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>.00E-10</td>
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<td>8.9E-06</td>
<td>7.2E-06</td>
<td>7.2E-11</td>
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5.2.2 Annual Population Infection Risk Assessment

The probability of infection of Cryptosporidium infection has been established by the ingestion of at least one oocyst. As supported of the results of Aim I, the complete constant model data showed an infection mean of 0.4465. However, the Beta-Poisson model that does not have the properties of the exponential, it cannot be extrapolated to fractional dose since the probability of infection depends on > 1 ingested oocyst as demonstrated by Teunis and Havelaar [2000]. If only one organism is used in the Beta-Poisson fit for this analysis, the probability of infection is 0.2829. Since this study did not determine a perfect model, the results of both models are presented as follows. Assuming the average person drinks 2 liters of water per day and a population of a million, the daily dose is determined by the number of people which ingest an
oocyst in 2 liters per day multiplied by the concentration of oocysts in the water. The probability of infection results is the slope (0.4465 or 0.2829) times the average oocysts ingested per day. Due to the exposure equation being linear, it can be scaled to population risk, and then to an annual risk using the Binomial (Equation 9) to determine the odds of one or more infections per year.

\[ P_{\text{one or more infections per year by each individual}} = 1 - (1-p)^{365.25} \]  

Equation 9

where \( p = 2 \) liters per day multiplied by risk slope multiplied by concentration (oocysts/liter) in drinking water.

For comparison, the initial dataset with the exponential model was graphed with the same linear approximation at low dose. In the initial exponential calculation, as the dose goes to zero the slope is limited by the r-value (0.00419), as well as, in the resulting risk graph, the Binomial has the limiting value of \( P = n*p = 365.25*p \) when \( p \) is low; which is why the graph is linear until infection becomes more certain.

Figure 3 displays the results of the population risk per person per year of complete constant, complete Beta-Poisson and initial exponential models; the upper bound of the ordinate axis is the probability of one or more infections per person per year. An annual risk of \( 10^{-4} \) is recommended as permissible by the USEPA for safe drinking water.

In a simple risk assessment analysis of Figure 3, solving for the concentration of Cryptosporidium oocysts providing the allowable USEPA risk of \( 10^{-4} \), shows the initial dataset exponential model requires 32.7 oocysts/million liters to overpass the recommended permissible values; while with complete Beta-Poisson dataset model 0.48 and 0.31 oocysts/million liters for
complete constant dataset model are enough to pass the regulations generating with them a population risk of approximately 100 times greater as for the initial exponential dataset.

![Graph showing concentration of Cryptosporidium oocysts in 10^6 L and population risk](image)

**Figure 3.** Linear plot association between the concentration of *Cryptosporidium* oocysts in 10^6 L and the population risk (considering infection per person per year); complete constant (black line), complete Beta-Poisson (red line) and initial exponential (blue line) of Haas *et al.* [1996].

5.2.3 Milwaukee Validation

5.2.3.1 Milwaukee Outbreak Results

The epidemiological studies of the 1993 Milwaukee outbreak reported (Mackenzie *et al.*, 1996) an infection proportion of 45.8% (403,000/880,000).
5.2.3.2 Validation Results of Haas et al., and Initial Exponential Model

The probability of infection in a single exposure with the exponential model reported by Haas et al., [1999] was 0.0064. Considering a disease ratio 0.39%, the single exposure risk of illness is 0.0025. Applying these values to Equation 9, the $P_{\text{Disease}}$ in 21 days is 5.2%. Calculation is as follows:

$P_i = 1 - \exp\left[-(0.00419)(1.54)\right] = 0.0064$

Single exposure risk of illness $= 0.0064 \times 0.39 = 0.0025$

$P_{\text{Disease}} = 1 - (1 - 0.0025)^{21}$

$P_{\text{Disease}} = 5.2\%$

5.2.3.3 Validation Results of Complete Beta-Poisson Model

The complete dataset using Beta-Poisson computed an oocyst daily mean dose (x) of 2.62 ($0.079 \times 1.948 \times 17$) which produced a range of probability of disease of 25.1 – 96.3%. Calculation is as follows:

$P_i \text{Beta-Poisson} = 1 - \left[(2^{1/2.62} - 1) \times 2.62 / 3204 + 1\right]^{-2.62}$

for a daily exposure in 17 days

$P_{\text{Disease}} = 1 - (1 - \text{single exposure risk of illness})^{17} = 0.963$

for an exposure on only one day of the outbreak duration

$P_{\text{Disease}} = 1 - (1 - \text{single exposure risk of illness})^1 = 0.251$

5.2.3.4 Validation Results of Complete Constant Model

Complete observed calculation produced a probability of the disease of 35.7%.
\[ P_{\text{Disease}} = \left( \frac{71}{159} \right) 0.80 = 35.7\% \]

5.2.3.5 Final Validation

Comparing the Milwaukee disease rate reported of 45.8\% against the complete observed of 35.7\%, the numbers are closer to those numbers reported in 1993; against a 5.2\% of the exponential model that according to Haas et al., [1999] which can be adjusted using other methods. On the other hand, the Beta-Poisson comprises the Milwaukee illness rate described in its range of 25.1 – 96.3\%.

5.3 Conclusion and Discussion of Aim III

Application of complete constant model values of infection, disease, and infection/disease ratio in the daily QMRA of cryptosporidiosis for a hypothetical city with a population of 100,000, showed a higher risk. This could be compared to a lower or no risk in the population exposed when the initial exponential model is employed in the quantification.

The higher annual risk per person was found with complete constant and complete Beta-Poisson models, and lower risk with initial exponential dataset models. The results in Figure 3 not only demonstrated a substantial difference between the models, they reinforced Aim I results, as it is observed a marked increased risk of the order of 100 times more than the already shown by the initial exponential model. This implies that mathematical models recommended by the USEPA to assess cryptosporidiosis must be reviewed in order to find the most appropriated analysis to assess a true representation of the risk evaluation for communities to have access to safe drinking water.
The complete constant and Beta-Poisson models were validated in this study. These produced results of illness rate values close to numbers reported by the Milwaukee outbreak of 1993 in the case of the complete constant, as well as a disease rate that falls within the Beta-Poisson model range.

5.4 Aim III Studies Impact

Analysis of the complete data set reflects that an elevated and dangerous risk exists and it suggests the necessity of re-evaluate mathematical models to assess the parasitosis risk. Also, the rules and regulations for drinking water must be modified to prevent massive outbreaks similar to those that have been historically seen and to prevent future outbreaks, especially in sensitive populations which could result more persons dramatically affected.
6.0 REFERENCES


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7.0 CURRICULUM VITA

Victoria N. Ochoa was born in El Paso, Texas. She graduated with Honors from the National University of Mexico working in her bachelor degree thesis entitled “Detection of soluble antigens of *Cysticercus cellulosae* in Cerebrum Spinal Fluid’. During more than 22 years she was working in the clinical field. In July of 2005 she obtained a Master in Biological Sciences from University of Texas at El Paso, working with, “Selection of Chlorine Tolerant *Echerichia coli* O:157H: 7”.

In the last 16 years she has been working as instructor for El Paso Community College in the areas of Parasitology, Mycology, Serology, Hematology, Urinalysis, General Biology I and II for Science Majors, Microorganisms and disease, Anatomy and Physiology I and II.

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