

# Detection of *Cryptosporidium* in the Bethlehem Wastewater Treatment Plant

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## INTRODUCTION

*Cryptosporidium* is an intestinal parasite that can exist in the environment as a hardy oocyst (Figure 1). When an oocyst is ingested, healthy individuals can experience cryptosporidiosis, a self-limiting diarrheal illness, while infected immunocompromised individuals are at risk of death. *Cryptosporidium* is highly resistant to environmental stresses and only a low dose is necessary for infection.

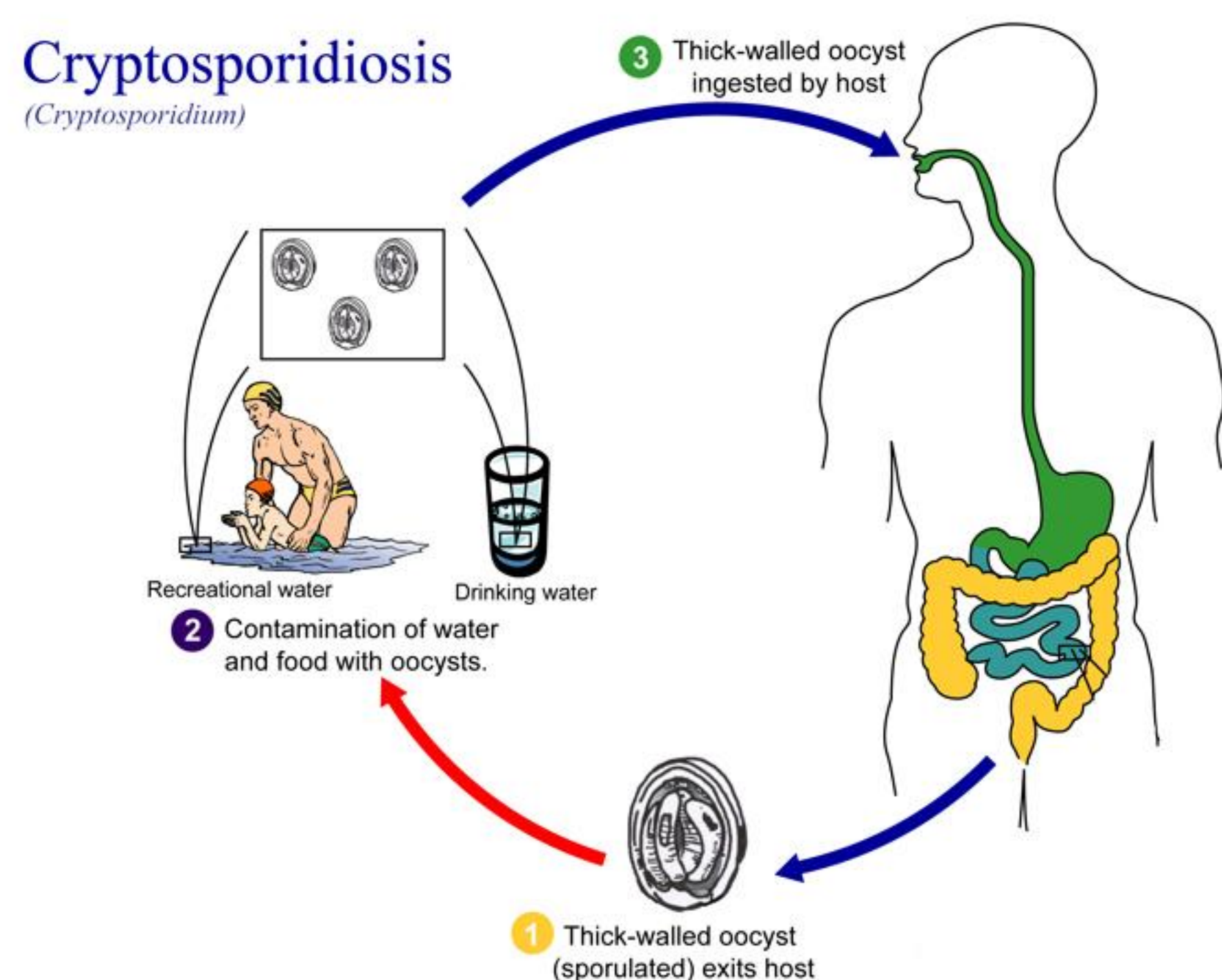


Figure 1

The life-cycle of *Cryptosporidium* and its infection of a host (Adapted from <http://phil.cdc.gov/phil/details.asp>).

## OBJECTIVE

Determine the seasonal threat to public health by detecting and source-tracking *Cryptosporidium* in the Bethlehem Wastewater Treatment Plant

## METHODS

Samples were collected every two weeks from the plant influent and effluent from March 2006 to October 2008. After elution and removal of the supernatant, the remaining pellet was processed via immunomagnetic separation. DNA was isolated and extracted from the samples and then amplified through a nested polymerase chain reaction (PCR). The PCR product was viewed under UV light after electrophoresis on a gel to determine if *Cryptosporidium* was present (Figure 2). Positive samples were cloned to identify multiple genotypes, and selected clones were sent to the University of Pennsylvania for sequencing.

## METHODS (CONT.)

Sequences were aligned using MacClade 4.06, and neighbor-joining and parsimony trees were created using Phylogenetic Analysis Using Parsimony (PAUP), beta version 4.0.

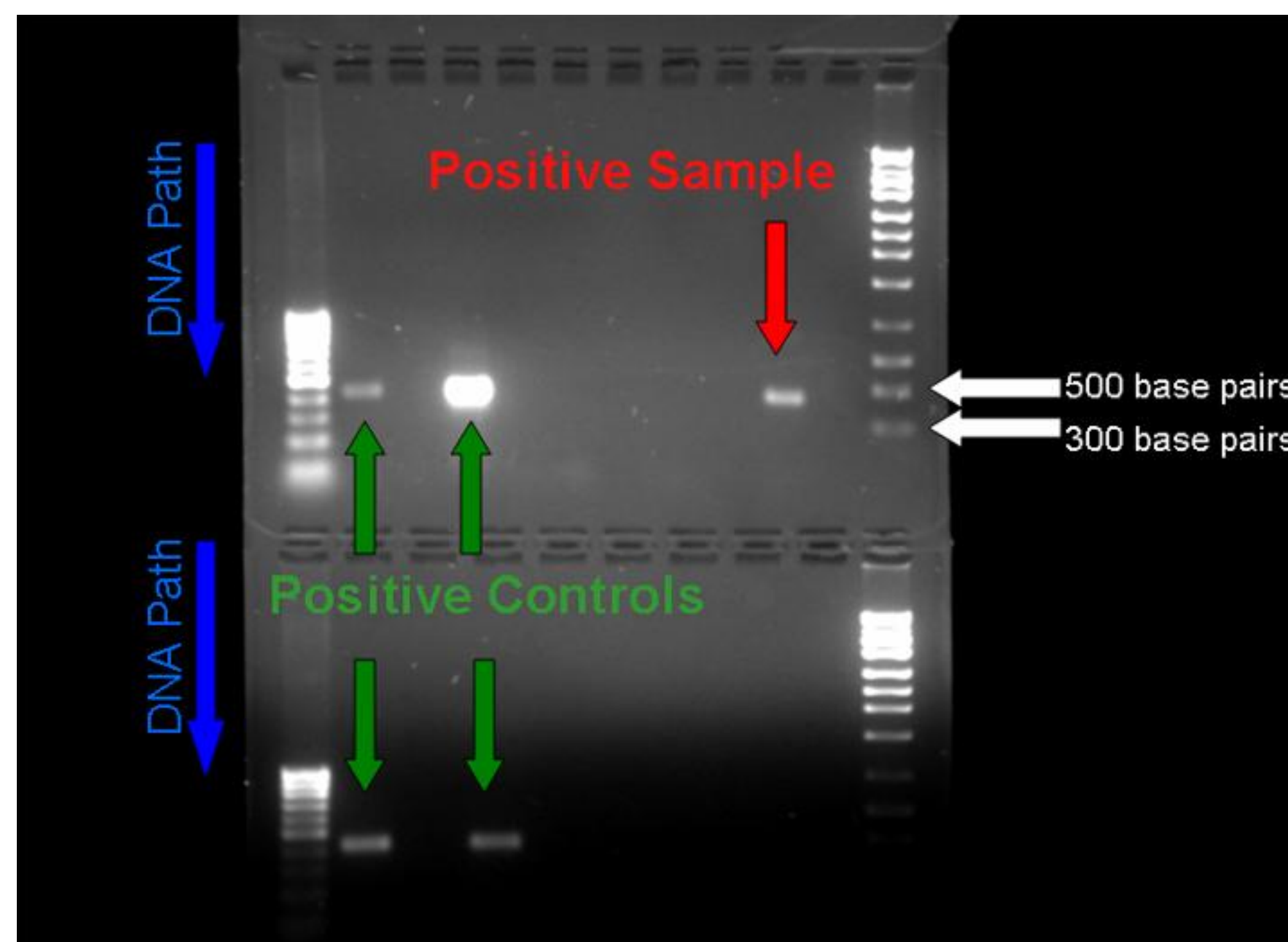


Figure 2

A picture of a gel viewed under UV light. One sample is positive for *Cryptosporidium* because it is aligned with positive controls and is approximately 434 base pairs in length.

## RESULTS

The detection limit for influent samples was variable. 10,000 oocysts have been detected in spiked influent samples 60% of the time; 1,000 and 500 oocysts have both been detected in spiked influent samples 33% of the time. Effluent detection limit testing is in progress.

As seen in Figure 3, *Cryptosporidium* was detected at different rates for each season. During the winter, the lowest detection rate of 15% was observed; the highest detection rate of 29% occurred during the summer.

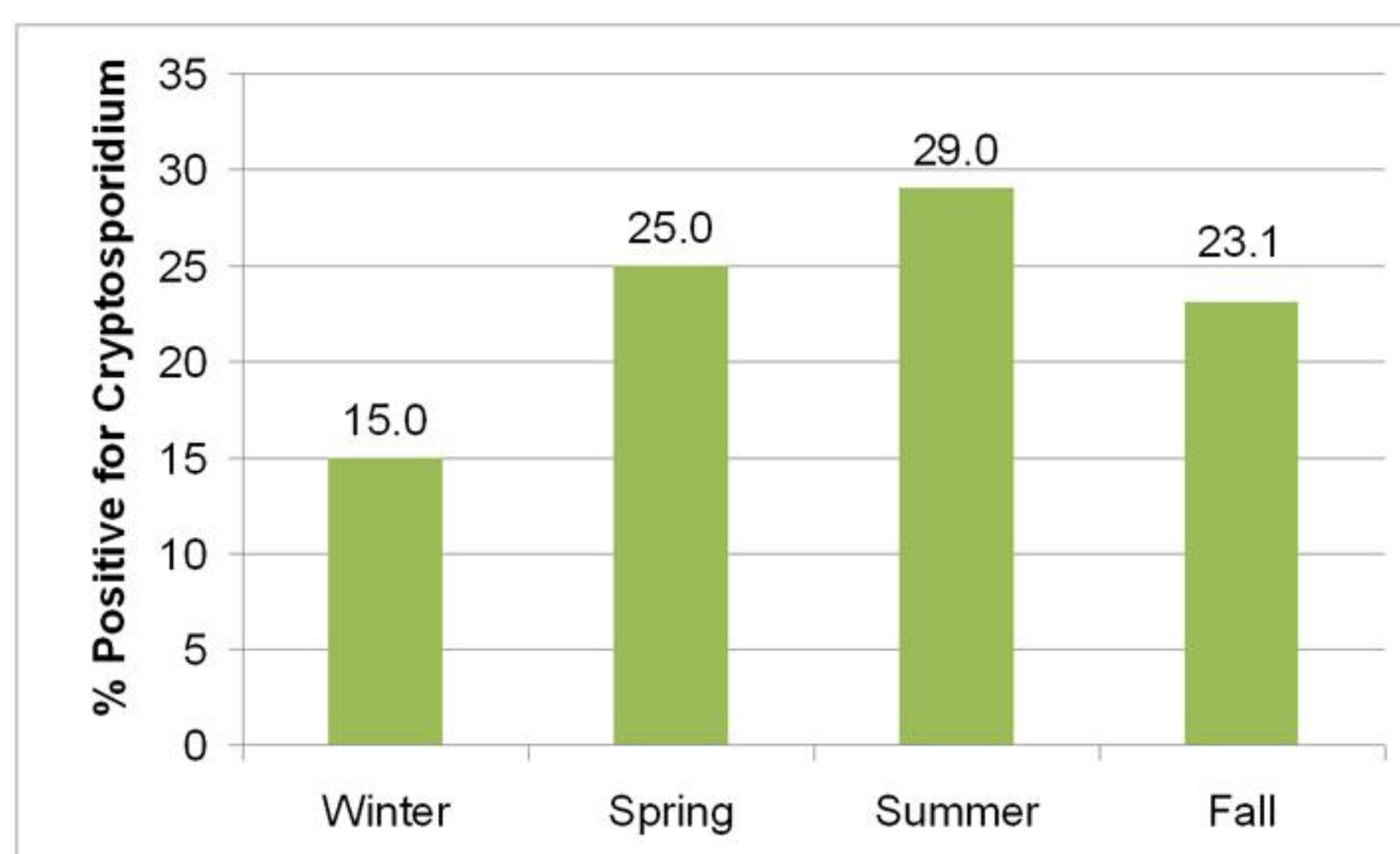


Figure 3

The seasonal detection trends of *Cryptosporidium*.

## RESULTS (CONT.)

Figure 4 illustrates that the percentage of detected *Cryptosporidium* genotypes that are human pathogens ranged from 71 – 86% seasonally. During the spring and summer months, the remaining 20 and 29%, respectively, of detected genotypes were pathogens that have been associated with human disease. In the fall and winter months, the remaining 14 and 25%, respectively, of detected genotypes are pathogens that have not been associated with human disease.

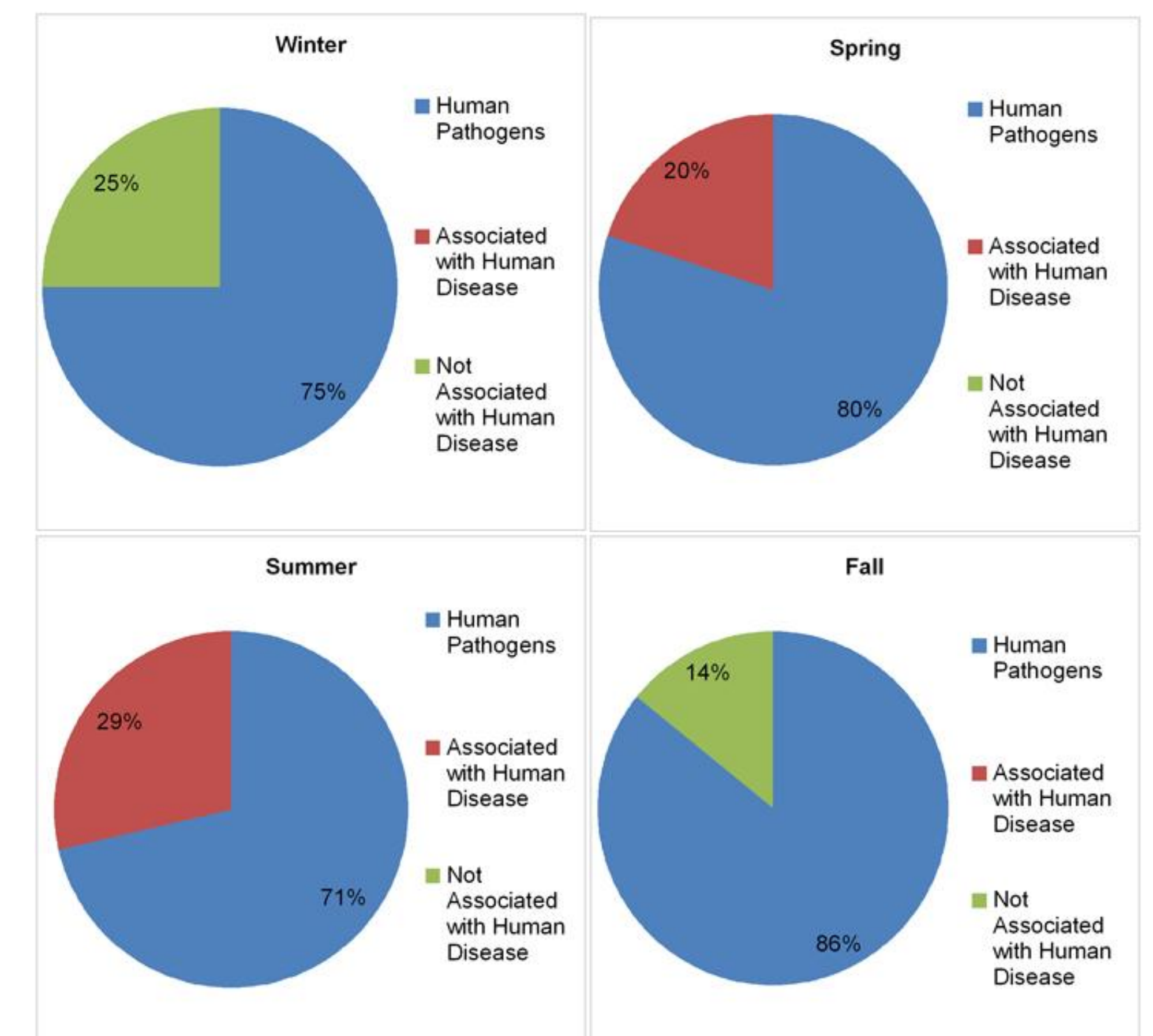


Figure 4

The seasonal trends of detected genotypes of *Cryptosporidium* grouped by their threat to human health.

## DISCUSSION

- The majority of *Cryptosporidium* genotypes detected year-round are human pathogens
- Cryptosporidium* genotypes that do not infect humans were detected only during the winter and fall months
- The highest occurrence of *Cryptosporidium* was in summer months
- Due to variable detection limits, it is probable that *Cryptosporidium* was present more often than was detected

## FUTURE WORK

- Finish effluent detection limit tests
- Investigate the effects of alkalinity, BOD, and turbidity on the detection of *Cryptosporidium*

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