

# The Prevalence of *Cyclospora cayetanensis* in Water: A Protocol for a Systematic Review and Meta-Analysis

## Authors

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## Registration

This protocol is available in the University of Guelph's repository and electronically published with Systematic Reviews for Animals and Food (SYREAF). The protocol can be accessed at <https://atrium.lib.uoguelph.ca/xmlui/handle/10214/10046> or <http://www.syreaf.org/contact/>. This protocol follows the outline of the PRIMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) 2015 checklist (1). The systematic review will be reported using the PRISMA statement guidelines (2).

## Author Contributions

The lead reviewer, TN, will be responsible for the title/abstract and full-text screening, data extraction, risk-of-bias assessment, and meta-analysis. The search strategy, screening questions, data extraction tool, and risk of bias assessment was developed by TN with input from JMS, AOC, CW, ST, and AG. All drafts of the protocol and manuscript will be prepared by TN in collaboration with all co-authors. This protocol has been read and approved by all authors.

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## Introduction

Rationale:

Globally, infectious diseases associated with water have resulted in high rates of morbidity and mortality. It is estimated that 4.0% of all deaths and 5.7% of disease burden (measured in disability adjusted life years) around the world can be attributed to water, sanitation, and hygiene practices (3). Waterborne pathogens can cause many types of infections, including enteric illness.

An example of a food-borne and water-borne infectious disease is cyclosporiasis, caused by *Cyclospora cayetanensis*, a protozoan parasite discovered in 1979 (4,5). Molecular methods are required to speciate *Cyclospora spp.* as *C. cayetanensis* and differentiate it from non-human

pathogenic parasites of the same genus (6,7). This parasite has a fecal-oral route of transmission and *C. cayetanensis* is the only species of *Cyclospora* known to cause human infection. It is shed through the feces of infected individuals and requires 1-2 weeks of maturation in the environment before becoming infectious to other people (5). Common clinical signs of disease include fever, diarrhea, abdominal cramping, nausea, and fatigue (8). Severity of illness differs between age groups, demographic region, and the presence of pre-existing health conditions (8). The majority of people who have healthy immune systems recover without any treatment or seeking medical attention (9). However, for others untreated infections may require hospitalization and can lead to prolonged illness lasting weeks or months (10,11).

All human populations are susceptible to cyclosporiasis; however, there is an increased prevalence in tropical and subtropical regions where the parasite is endemic. Outside these regions, sporadic cases of cyclosporiasis have been attributed to travel. Outbreaks in areas where *C. cayetanensis* is not native have mainly been associated with food imported from countries where the parasite is endemic (5). In both sporadic and outbreak cases, contaminated water has been identified as a source of infection (12). Since water is a potential source of contamination, this review will focus on determining the prevalence of *C. cayetanensis* in water (8).

Objective: The purpose of this protocol is to describe the methodology to review the literature to answer the following Population-Outcome question using systematic review and meta-analysis:

What is the prevalence of *C. cayetanensis* in different types of water?

## Methods

The study characteristics for this Population Outcome (PO) study are:

P: Water that has been tested for *C. cayetanensis*

O: The detected prevalence of *C. cayetanensis*

Eligibility Criteria:

In addition to the above characteristics, all types of water will be eligible (e.g. natural occurring water, wastewater, and water used for farming). The studies included in the review will be primary research studies published after 1979 (when the parasite was discovered) and will be limited to English-language publications.

Study design eligibility: All observational studies will be included except for case-control, case reports, and case series, because prevalence data cannot be extracted from these study designs.

Information Sources:

The databases and platforms that will be used to conduct this review will be: MEDLINE® (Web of Science™), CABI Direct (CABI), Agricola (ProQuest), Food Science and Technology Abstracts (EBSCOhost), and Water Resources Abstracts (ProQuest). These databases were selected because they are most likely to contain comprehensive literature on the environmental sciences.

Search Strategy:

To maximize sensitivity and identify all relevant literature, a broad search strategy was developed. The two main concepts for this search are *Cyclospora cayetanensis* and water. The search will be conducted using the key words, *C. cayetanensis* and water along with synonyms. Given the simplicity of the search, a library scientist was not consulted when developing the search strategy. The specific search strategy used, and the results compiled from MEDLINE® are outlined in APPENDIX 1. The search will be restricted to include studies published after 1979. To verify the results of the search, the reference lists of selected review articles will be used to determine if they were identified by the search.

#### Study Records:

##### Data management

Results from the searches will be compiled in Endnote® X8 Desktop where identical papers will be deduplicated. To ensure all articles are unique, manual deduplication will also be performed. Then, citations will be uploaded into DistillerSR® (Evidence Partners, Ottawa, ON, Canada), which will be used for review management.

##### Selection Process

The selection process will consist of two stages.

- 1) Title and Abstract screening
- 2) Full-text screening

Through both stages of selection, two reviewers will independently assess the citations or articles to ensure they meet the eligibility criteria. If there is a disagreement between reviewers and a consensus cannot be met, then a third reviewer will make a final decision. For the title/abstract screening the following questions will be asked; if the response for any question is “No”, the publication will not proceed to the next stage of the review. Fifty references from stage 1 and five references from stage 2 will be used to pretest the forms before starting the review.

For the title and abstract screening, the following questions will be asked; if the response for any question is “No”, the publication will not proceed to the next stage of the review.

- 1) Based on the title/abstract is this a study about *Cyclospora* (unspiciated or *C. cayetanensis*) and water?
  - a) Yes (proceed to Q2)
  - b) No (exclude)
  - c) Unclear (proceed to Q2)
- 2) Is this a primary research study?
  - a) Yes (proceed to full-text screening)
  - b) No (exclude)
  - c) Unclear (proceed to full-text screening)

Next, citations that pass the first stage will be reassessed for relevance based on the full text. The full text of the citations will be obtained using resources available at the University of Guelph. Below are the questions that will be used to assess eligibility at the full-text level, and if “No”, is answered for any of the questions the paper will not proceed to the next step of the

review. For studies that are evaluated based on the full text, reasons for exclusion will be reported.

- 1) Is the full-text available in English?
  - a) Yes
  - b) No (specify language)
- 2) Does the full-text describe a study on *Cyclospora* (unspeciated or *C. cayetanensis*) and water?
  - a) Yes
  - b) No
- 3) Does the full-text describe a primary observational study (not including case report, case series, or case-control)?
  - a) Yes
  - b) No
- 4) Does the full-text report the prevalence of *Cyclospora* (unspeciated or *C. cayetanensis*) or information sufficient to estimate prevalence (e.g. number of positive samples, the total number of samples, data from figures)?
  - a) Yes
  - b) No

#### Data collection process

All articles selected for data extraction and risk-of-bias assessment will be independently assessed by two reviewers. The data extraction form and the tool used for the risk-of-bias-assessment will be pre-tested on the same five references. The following section outlines the data extraction process.

#### Data items:

Full-text articles that passed the full-text screening will move to data extraction. Below is the set of questions that will be used to extract the data:

- 1) In what time frame was this study conducted (year/s and months)?
- 2) In what country was this study conducted?
- 3) What type of water was examined?
  - a) Natural, source water
  - b) Drinking
  - c) Drinking water treatment plants
  - d) Well water
  - e) Waterworks, water pumps, water tanks
  - f) Irrigation
  - g) Wastewater
  - h) Sewage
  - i) Sludge
  - j) Vegetable processing water
  - k) Post-harvest water
  - l) Recreational
  - m) Other
- 4) Was this an outbreak study?

- a) Yes
  - b) No
- 5) What laboratory method was used to identify the organism (check all that apply)?
- a) PCR
  - b) Light microscopy (without staining)
  - c) Staining technique (including acid-fast technique)
  - d) Fluorescent (auto fluorescent) microscopy
  - e) Other
- 6) Was the organism of interest confirmed to be *C. cayetanensis*?
- a) Yes
  - b) No
- 7) Did the authors report an expected analytical detection limit?
- a) Yes
  - b) No
- 8) What was the basis for the limit?
- a) Reference
  - b) In-house testing of analytical sensitivity
  - c) Other
- 9) What sampling method was used (check all that apply)?
- a) Filtration (type if available)
  - b) Flocculation
  - c) Other
- 10) How many liters of water were collected?
- 11) What was the total number of positive samples?
- 12) What was the total number of samples?
- 13) What was the reported prevalence?
- 14) What is the type and result of the reported variability metric?
- a) Interquartile range
  - b) Standard deviation
  - c) Variance
  - d) Confidence interval
  - e) Other
- 15) Was the reported prevalence adjusted?
- a) Yes
  - b) No
- 16) If yes to Question 12, what covariates were reported?

#### Outcomes and Prioritization:

The primary outcome is the prevalence of *C. cayetanensis* in water. However, it is anticipated that not all studies will speciate, studies including prevalence estimates at the genus level (i.e. *Cyclospora* spp.); these unspciated estimates also will be eligible. Reported prevalence is preferred; however, if prevalence is not reported, raw data will be extracted and the proportion will be calculated. Also, unadjusted prevalence will be prioritized over adjusted prevalence.

#### Risk of Bias in Individual Studies

For risk-of-bias of individual studies, the criteria of Hoy et al. RoB checklist was modified to fit the needs of this review and can be found in APPENDIX 2 (13). This tool was originally developed for assessing the risk of bias for reviews about the prevalence of health outcomes. The results of the assessment will be presented in a risk of bias table and the results will be used to explore possible sources of heterogeneity.

#### Data Synthesis:

Meta-analysis will be performed using R Studio (Version 1.1.456). A random effects model will be used to summarize the observed prevalence reported from all studies. Differences of prevalence in various sources of water will be explored between naturally occurring water (lakes, rivers, and, ponds), water used for farming (irrigation or post-harvest water), drinking water (water treatment plants or bottled water), and types of wastewater (sewage, or sludge). To normalize and stabilize the variance, prevalence estimates from individual studies will be transformed using the double arcsine transformation (14).

The Q and  $I^2$  statistics will be used as measures of the between studies variability. Possible sources of heterogeneity between studies could be attributed to differences in water type, the methods for data collection, laboratory methods for organism identification (e.g. speciated and unspeciated studies), and differences between countries. Therefore, if at least two studies per category are eligible, a subgroup meta-analysis will be performed on data collection and organism identification methods (including studies that speciated *C. cayetanensis* and ones that did not), water type, and different regions, given the seasonality of *C. cayetanensis* and the possible moderation due to location (12,15).

#### Meta-bias(es):

Not applicable

#### Confidence in Cumulative Evidence:

Not applicable

## References

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## APPENDIX 1

The following table reports the results from a preliminary search conducted in MEDLINE® (Web of Science) on 2020/07/04

#	Search String	Results
1	TS= (Cyclospora cayetanensis OR C. cayetanensis OR cyclosporiasis OR cyclospora OR C cayetanesis OR cayetanensis) Indexes=MEDLINE Timespan=1979-2020	694
2	TS= (water OR sewage OR pond OR sludge OR lake OR river) Indexes=MEDLINE Timespan=1979-2020	777, 350
3	#1 AND #2	150

## APPENDIX 2

### Modified Hoy et al. RoB checklist

Criteria	Question
#1	Was the primary objective of the study to measure the prevalence of <i>Cyclospora cayetanensis</i> or was the prevalence estimate an additional component of the study? a) Yes b) No
#2	Was the same method of data collection used for all samples? a) Yes b) No c) Not reported
#3	Was the same method of organism identification used for all samples? a) Yes b) No c) Not reported