FERNANDA PINTO FERREIRA

PESQUISA DE PATÓGENOS EM ÁGUA, SOLO E HORTALIÇAS CRUAS

Londrina
2019

FERNANDA PINTO FERREIRA

PESQUISA DE PATÓGENOS EM ÁGUA, SOLO E HORTALIÇAS CRUAS

Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Estadual de Londrina como requisito parcial para a obtenção do título de Doutor.

Orientador: Prof. Dr. Italmar Teodorico Navarro

Co-orientadora: Prof. Dra. Roberta Lemos Freire

Londrina
2019

FERNANDA PINTO FERREIRA

PESQUISA DE PATÓGENOS EM ÁGUA, SOLO E HORTALIÇAS CRUAS

Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Estadual de Londrina como requisito parcial para a obtenção do título de Doutor.

BANCA EXAMINADORA

Orientador: Prof. Dr. Italmar Teodorico Navarro
Universidade Estadual de Londrina - UEL

Prof. Dr. João Luis Garcia
Universidade Estadual de Londrina - UEL

Prof. Dr. Luiz Daniel de Barros
Universidade Estadual de Londrina - UEL

Prof. Dra. Fabiana Maria Ruiz Lopes Mori
Secretaria de Educação do Estado do Paraná - SEED-PR

Prof. Dra. Luciane Holsback Silveira Fertonani
Universidade Estadual do Norte do Paraná - UENP

Londrina, 23 de abril de 2019.

Dedico à minha família: Sueli, Jair, Alessandra, Jaqueline, Emerson, Raffael, Maria, Arthur, Heloisa, Katherina, Davi e Baltazar

AGRADECIMENTOS

Agradeço, primeiramente, a Deus, por me dar forças sempre.

À minha vózinha, Maria Alves Pinto (*in memorian*), que há 22 anos não está mais presente em minha vida, em pessoa, mas sempre em minha memória, quem me ensinou valores humanos e espirituais, a quem eu tenho eterna admiração e amor.

À minha mãe, Sueli Pinto Ferreira, minha rocha onde eu me sinto segura, obrigada por todos momentos dedicados, pelas palavras, conselhos, honestidade, amizade, carinho, apoio incondicional, por me fazer sempre uma pessoa melhor, jamais deixarei de amá-la.

Às minhas irmãs Jaqueline Pinto Ferreira e Alessandra Pinto Ferreira, pelo companheirismo, incentivo, por me mostrarem que nunca estou sozinha nesta caminhada, por serem muito mais que irmãs e por me apoiarem sempre.

Ao meu pai, pela dedicação à família e por nunca me deixar faltar nada, mesmo em meio a dificuldades.

Ao meu esposo Emerson Simões Martins, por ser meu braço direito, por me aconselhar, ouvir, ajudar e amar.

Aos meus lindos sobrinhos Arthur, Maria Victória e Raffael José por darem esperança e felicidade para minha vida, por me mostrarem sempre o quanto posso ser feliz com tão pouco.

Aos meus "filhos" Heloiza, David, Baltazar e Katherina por me fazerem sentir amada todos os dias.

Ao meu orientador e pai científico, Prof. Dr. Italmar Teodorico Navarro, pelo acolhimento, orientação, confiança, valiosos ensinamentos (não apenas científicos), e sobretudo pela amizade ao longo desses anos. Jamais esquecerei de nossas conversas, dos seus conselhos e apoio.

À professora Dra. Roberta Lemos Freire, minha eterna orientadora, quem me fez apaixonar por epidemiologia e por saúde pública, a quem admiro muito.

À professora Dra. Regina Mitsuka Breganó, pelo incentivo, pelas oportunidades e por ser muito mais que uma professora, uma amiga.

Às amigas Aline Kuhn Sbruzzi Pasquali e Eloiza Teles Caldart por me aguentarem, por serem confidentes, solidárias, por me fazerem sentir bem, mesmo em momentos difíceis, vocês são muito especiais para mim, tenho orgulho de ser amiga de pessoas esforçadas, humildes e de boa índole, como vocês.

Às amigas Aline Ticiani e Juliana Bernardes pelo companheirismo, momentos de

diversão, de reflexão e apoio e por fazerem parte da minha caminhada.

A amiga Roberta Toledo por fazer parte do meu crescimento como pesquisadora, pela amizade e ensinamentos.

Aos amigos Andressa Rorato e Ricardo Matos, pelas conversas, conselhos e amizade.

Ao amigo José Mauricio Ferreira Neto e Paulo Romanelli, irmãos científicos, a quem tenho grande afeto e admiração.

À minha banca maravilhosa que aceitou prontamente fazer sugestões importantes a esse trabalho: Prof. Dr. João Luis, Prof. Dr. Luiz Daniel, Profa Dra. Fabiana Mori, Prof. Dra. Luciane Holsback.

Agradeço a todos que fizeram ou fazem parte da minha vida científica: Keila Clarine, Juliana Petruske, Marielen de Souza, Maira Moreira, Carol Constantino, Winni Ladeia, Marcelle Mareze, Jonatas Campos de Almeida, Fernanda Evers, Ana Carolina Miura, Luiz Daniel, Mércia de Seixas, Felippe Martins, Vanessa Holsbach, Thais Agostinho, Thais Cabral, Ana Flávia, João Pedro Sasse, Aline Benitez, Renata Dias, Beatriz Nino pelo convívio no dia a dia do laboratório.

Aos secretários Helenice e Valdecir, pela disposição em ajudar nos momentos em que

precisei.

À Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa de doutorado.

A todos que contribuíram direta ou indiretamente, a ajuda de todos vocês foi imprescindível para a realização desse trabalho.

“E ainda estou confuso só que agora é
diferente, estou tão tranquilo e
tão contente...” Meu amado
Renato Russo

FERREIRA, Fernanda Pinto. **Pesquisa de patógenos em água, solo e hortaliças cruas**. 2019. 75 f. Tese (Doutorado em Ciência Animal) – Universidade Estadual de Londrina, Londrina, 2019.

RESUMO

Doenças relacionadas à ingestão de alimentos contaminados afetam um terço da população mundial, sejam de origem viral, bacteriana, fúngica ou parasitária. Além de grande importância na saúde pública têm importância econômica devido aos gastos com tratamento. A toxoplasmose é uma zoonose causada pelo *Toxoplasma gondii*, que assim como a giardíase (*Giardia intestinalis)* e criptosporidiose (*Cryptosporidium* spp.) estão associados à surtos de origem hídrica e alimentar. No que diz respeito à toxoplasmose, analisando seu histórico por meio da revisão sistemática, foi a causa de 35 surtos mundialmente publicados nos últimos 50 anos e as principais vias de transmissão foram: água, frutas, verduras, carne crua ou pouco cozida e leite de cabra. A ocorrência de surtos conforme a forma biológica do parasita foi: esporozoíto (oocisto) e bradizoítos (cisto) 45,7% (16/35) cada; enquanto para os taquizoítos 8,6% (3/35). Nas décadas de 1970 e 1990, os surtos ocorreram principalmente por meio da ingestão de cistos na carne e seus derivados; nos anos 80, leite contaminado com taquizoítos; em 2000, oocistos na água; a partir de 2010, houve um aumento na ocorrência de surtos devido a oocistos em vegetais crus. Observou-se uma mudança na epidemiologia dos surtos nos últimos 20 anos. Os vegetais tiveram seu consumo aumentado, esses alimentos quando não higienizados de forma adequada podem conter estruturas parasitárias, para detecção de parasitas, utilizou-se Reação em Cadeia da Polimerase (PCR), Hoffman, Willis and Faust e foi observado contaminação por *T. gondii* em 12,9% (8/62), *G. intestinalis* em 25,8% (16/62), *Cryptosporidium* spp. em 11,3% (7/62), e outros parasitas em 45,23% (19/42) dos vegetais analisados. Além disso, por meio do Kit Comercial Colilert de análise de contaminação de água, observou-se que 81,0% (19/21) amostras de água de irrigação apresentavam *Escherichia coli.* O uso de água de rio na irrigação demonstrou um papel importante na contaminação dos vegetais, em contrapartida a suplementação do solo com calcário contribuiu positivamente para a manutenção de hortaliças mais seguras. Em 2015, houve um surto de toxoplasmose em uma instituição de pesquisa em Londrina, Paraná. Um total de 20 indivíduos apresentaram sintomas e sorologia compatíveis com a infecção aguda. Para análise de fatores de risco foi estruturado um estudo de caso e controle na razão 1:2,25. Todos os participantes da investigaçãoresponderam a um questionário epidemiológico. Foram coletadas um total de 29 amostras ambientais, todas negativas à reação em cadeia da polimerase. Após a análise dos dados epidemiológicos, o consumo de vegetais foi a única variável associada à ocorrência da doença. A partir dos resultados demonstrou-se a importância dos vegetais como via de transmissão de
T. gondii e de outros patógenos, além disso, expõem alguns pontos críticos de contaminação, fornecendo suporte para capacitar os agricultores em boas práticas de gestão durante o processo de produção e ainda, oferecem as principais características epidemiológicas para o rápido reconhecimento de surto, notificação e investigação para que desta forma seja interrompida a cadeia de transmissão, impedindo a ocorrência de surtos.

Palavras-chave: Toxoplasmose. Investigação de surto. *Cryptosporidium* spp. *Giardia* spp. Horticultura orgânica.

FERREIRA, Fernanda Pinto. **Pathogen research in water, soil and raw vegetables.** 2019. 75 p. Thesis (/Doctor´s Degree in Animal Science) – Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

Diseases related to the ingestion of contaminated food affect one-third of the world's population, whether of viral, bacterial, fungal or parasitic origin. Besides being of great importance in public health, they are economically important due to treatment expenses. Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, which as well as giardiasis (*Giardia intestinalis*) and cryptosporidiosis (*Cryptosporidium* spp.) Are associated with water and food sources. Regarding to toxoplasmosis, analyzing its history through the systematic review, it was the cause of 35 outbreaks worldwide published in the last 50 years and the main transmission routes were: water, fruits, vegetables, raw or undercooked meat and milk of goat. The occurrence of outbreaks according to the biological form of the parasite was: sporozoite (oocyst) and bradyzoites (cyst) 45.7% (16/35) each; while for the tachyzoites 8.6% (3/35). In the 1970s and 1990s, outbreaks occurred mainly through the ingestion of cysts in meat and its derivatives; in the 1980s, milk contaminated with tachyzoites; in 2000, oocysts in water; as of 2010, there was an increase in the occurrence of outbreaks due to oocysts in raw vegetables. There has been a change in the epidemiology of outbreaks in the last 20 years. The plants had their consumption increased, these foods, when not properly sanitized, could contain parasite structures for the detection of parasites. Polymerase Chain Reaction, Hoffman, Willis and Faust were used, and contamination by *T. gondii* in 12.9% (8/62), *G. intestinalis* in 25.8% (16/62), *Cryptosporidium* spp. in 11.3% (7/62), and other parasites in 45.23% (19/42) of the analyzed vegetables. In addition, through the Colilert Commercial Kit of analysis of water contamination, it was observed that 81.0% (19/21) samples of irrigation water presented *Escherichia coli*. The use of river water in the irrigation showed an important role in the contamination of the vegetables, in contrast the supplementation of the soil with limestone contributed positively to the maintenance of safer vegetables. In 2015, there was an outbreak of toxoplasmosis at a research institution in Londrina, Paraná. A total of 20 individuals presented symptoms and serology compatible with acute infection. For the analysis of risk factors a case and control study was structured in the ratio 1: 2.25. All the participants of the investigation answered an epidemiological questionnaire. A total of 29 environmental samples were collected, all of them negative to the polymerase chain reaction. After analyzing the epidemiological data, vegetable consumption was the only variable associated with the occurrence of the disease. From the results, the importance of the vegetables as a transmission route of *T. gondii* and other pathogens has been demonstrated. In addition, they present some critical contamination points, providing support to train farmers on good management practices during the production process and also offer the main epidemiological characteristics for the rapid recognition of the outbreak, notification and investigation in order to interrupt the transmission chain, thus preventing the occurrence of outbreaks.

Keywords: Toxoplasmosis. Outbreak investigation. *Cryptosporidium* spp. *Giardia* spp. Organic horticulture.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

PCR	Polymerase Chain Reaction
OMS	Organização Mundial da Saúde
IBGE	Instituto Brasileiro de Geografia e Estatística
Scielo	Scientific Electronic Library Online (Scielo)
DOI	Digital Object Identifier
NMP	Número mais provável
CTT	Coliforme termo-tolerantes
CT	Coliformes Totais
CONAMA	Conselho Nacional do Meio Ambiente

SUMÁRIO

1 INTRODUÇÃO

A área ocupada com a horticultura no Brasil é de 4,4 milhões de hectares com produção de 71 milhões de toneladas de hortaliças (HORTIBRASIL, 2016) cultivadas, principalmente no sistema convencional. Nos últimos anos, observou-se um crescimento significativo no sistema de cultivo orgânico (MELO; VILELA, 2007), relacionado, principalmente, com a busca por um alimento mais saudável, de melhor sabor e que vise a preservação do meio ambiente (ARCHANJO; BRITO; SAUERBECK, 2001). A principal diferença entre esses dois tipos de cultivo é que o sistema de cultivo convencional pode utilizar insumos agrícolas (inseticidas, fertilizantes, herbicidas e pesticidas) e organismos geneticamente modificados (WILKINS; HILLERS, 1994).

As enteroparasitoses são doenças cosmopolitas e endêmicas nos países do terceiro mundo, representam um importante problema de saúde pública (CACCIÒ *et al.*, 2018). São adquiridas pela ingestão das formas infectantes, tanto de helmintos quanto de protozoários, contidas em alimentos ou água contaminados. As hortaliças consumidas cruas podem servir como importantes vias de transmissão (VOLLKOPF; LOPES; NAVARRO, 2006). As condições de cultivo, envolvendo a qualidade da água para irrigação, o tipo de adubo empregado, os meios de armazenamento, transporte e o manuseio da colheita estão diretamente relacionados a esta contaminação (PACHECO *et al.*, 2002).

Estima-se que um terço da população mundial sofra de doenças relacionadas à ingestão de alimentos contaminados (FAO, 2012). Entre as doenças transmitidas por alimentos, a toxoplasmose, está comumente associada à ocorrência de surtos, representando um importante problema de saúde pública e um impacto econômico significativo (CHOI *et al.*, 1997).

Toxoplasmose é uma zoonose causada pelo protozoário *Toxoplasma gondii* e que devido à sua importância médico e veterinária é um dos parasitas mais estudado no mundo, pode infectar todos os animais homeotérmicos, os felinos são os hospedeiros definitivos (HILL; DUBEY, 2002).

O ciclo evolutivo do *T. gondii* é heteroxeno facultativo, complexo e envolve três formas infectantes: taquizoítos, bradizoítos e esporozoítos (DUBEY; MILLER; FRENKEL, 1970).

Quando a ocorre a primo-infecção nos hospedeiros definitivos, por uma determinada cepa, há produção de milhares de oocistos, eliminados nas fezes, por um período

de até 21 dias. Sob condições ótimas de oxigenação, temperatura e umidade, sofrem a esporulação entre 1 e 5 dias, desenvolvendo oocistos infectantes. Devido a presença de parede dupla resistente na estrutura, podem permanecer no solo, viáveis por longo tempo (DUBEY; MILLER; FRENKEL, 1970; TENTER; HECKEROTH; WEISS, 2000) contaminando a água e os vegetais.

A via de transmissão do agente é variável, contudo, hábitos alimentares, culturais e fatores ambientais são determinantes na epidemiologia da toxoplasmose (KOLBEKOVA *et al.*, 2007). Nos últimos 50 anos, mais de 30 relatos de surtos foram publicados no mundo, sendo a maioria no Brasil. As principais vias de transmissão envolvidas foram: água, hortaliças, frutas por oocistos, carnes mal cozidas ou cruas por bradizoítas e leite de cabra não pasteurizado por taquizoítas (CHIARI *et al.*, 1984; DUTRA *et al.*, 2012; EKMAN *et al.*, 2012; MASUR *et al.*, 1978; DE MOURA, *et al.*, 2006; POMARES *et al.*, 2011).

A fácil contaminação durante o processo de produção de hortaliças reforça a necessidade de identificação dos principais fatores de riscos e implantação de boas práticas de produção que garantam segurança alimentar no consumo desses produtos (NASCIMENTO; ALENCAR, 2014).

REFERÊNCIAS

ARCHANJO, L. R.; BRITO, K. F. W. De; SAUERBECK, S. Alimentos Orgânicos em Curitiba: consumo e significado. **Revista Cadernos de Debate**, 2001. v. VIII, p. 1–6.

CACCIÒ, S. M. *et al.* Foodborne parasites: Outbreaks and outbreak investigations. A meeting report from the European network for foodborne parasites (Euro-FBP). **Food and Waterborne Parasitology**, 2018. v. 10, p. 1–5.

CHIARI, C. De A. *et al.* Toxoplasmose humana adquirida através da ingestão de leite de cabra. **Memórias do Instituto Oswaldo Cruz**, 1984. v. 79, n. 3, p. 337–340.

CHOI, W. Y. *et al.* Foodborne outbreaks of human toxoplasmosis. United States: **The Journal of infectious diseases**, 1997. v. 175, n. 5, p. 1280–1282.

DE MOURA, L. *et al.* Waterborne toxoplasmosis, Brazil, from field to gene. United States: **Emerging Infectious Diseases**, 2006. v. 12, n. 2, p. 326–329.

DUBEY, J. P.; MILLER, N. L.; FRENKEL, J. K. Characterization of the new fecal form of *Toxoplasma gondii*. **The Journal of parasitology**, 1970. v.56, n. 3, p. 447–456.

DUTRA, L. H. *et al.* Investigação de surto de toxoplasmose transmitido pelo consumo de açaí em Rondônia, Brasil, 2011. Brasília - Distrito Federal: Ministério da Saúde, 2012. p. 108–109.

EKMAN, C. C. J. *et al.* Case-control study of an outbreak of acute toxoplasmosis in an industrial plant in the state of São Paulo, Brazil. **Revista do Instituto de Medicina Tropical de São Paulo**, 2012. v. 54, n. 5, p. 239–244.

FAO. Rapport de la FAO sur l'ins_ecurit_e alimentaire dans le monde. 2012. Disponível em: <http://www.fao.org/docrep/017/i3027f/i3027f.pdf.>.

HILL, D.; DUBEY, J. P. *Toxoplasma gondii*: transmission, diagnosis and prevention. England: **Clinical Microbiology and Infectious Diseases**, out. 2002. v. 8, n. 10, p. 634–640.

HORTIBRASIL. Tecnologia no hortinegócio. [S.l.], 2016. Disponível em: <https://www.hortibrasil.org.br/154-artigos/1298-tecnologia-no-hortinegocio.html>. Acesso em: 6 mar. 2019.

KOLBEKOVA, P. *et al.* New and old risk-factors for *Toxoplasma gondii* infection: prospective cross-sectional study among military personnel in the Czech Republic. England: **Clinical Microbiology and Infectious Diseases**, 2007. v. 13, n. 10, p. 1012–1017.

MASUR, H. *et al.* Outbreak of toxoplasmosis in a family and documentation of acquired retinochoroiditis. United States: **The American journal of medicine**, 1978. v. 64, n. 3, p. 396–402.

MELO, P. C. T. De; VILELA, N. J. Importância da cadeia produtiva brasileira de hortaliças. **Associação Brasileira de Horticultura (ABH)**, 2007. n. 1, p. 11.

NASCIMENTO, E. D.; ALENCAR, F. L. S. Eficiência antimicrobiana e antiparasitária de desinfetantes na higienização de hortaliças na cidade de Natal - RN. **Ciência e Natura**, 2014. v. 36, n. 2, p. 92–106.

PACHECO, M. A. S. R. *et al.* **Monitoramento das condições higiênico-sanitárias de alfaces produzidas no município de Ibiúna, SP**. Revista Eletrônica de Epidemiologia das Doenças Transmitidas por Alimentos - Edição de divulgação dos resumos do II Simpósio de Segurança Alimentar e Saúde. Disponível em: <ftp://ftp.cve.saude.sp.gov.br/doc_tec/hidrica/revp03_vol3n2.pdf>.

POMARES, C. *et al.* Toxoplasmosis and horse meat, France. United States: **Emerging infectious diseases**, jul. 2011. v. 17, n. 7, p. 1327–1328.

TENTER, A.; HECKEROTH, A.; WEISS, L. *Toxoplasma gondii*: from animals to humans. England: **International Journal for Parasitology**, 2000. v. 30, n. 12–13, p. 1217–58.

VOLLKOPF, P. C. P.; LOPES, F. M. R.; NAVARRO, I. T. Ocorência de enteroparasitas em amostrasde alface (*Lactuca sativa*) comercializadas em Porto Murtinho - MS. **Arquivos de Ciências Veterinárias e Zoologia da Unipar**, 2006. v. 9, p. 37–40.

WILKINS, J. L.; HILLERS, V. N. Influences of pesticide residue and environmental concerns on organic food preference among food cooperative members and non-members in Washington State. **Journal of Nutrition Education**, 1994. v. 26, n. 1, p. 26–33.

2 OBJETIVOS

2.1 Objetivo Geral

Avaliar hortaliças como uma via de transmissão de *Toxoplasma gondii* e outros patógenos, com base na literatura e nos principais riscos durante o cultivo.

2.2 Objetivos Específicos

- Realizar revisão sistemática da literatura sobre a evolução temporal dos surtos de toxoplasmose.
- Observar por meio de coleta de amostras ambientais e dados epidemiológicos os principais pontos críticos para contaminação por *T. gondii* e outros patógenos em hortaliças de horta orgânica.
- Avaliar fatores ambientais e alimentares associados a surtos de toxoplasmose.

3 REVISÃO DE LITERATURA

A revisão sistemática de literatura (ARTIGO A) foi submetida à Revista *Emerging Infectious Diseases* em 2 de outubro de 2018, o status atual é *Awaiting Final Decision.*

3.1 Artigo A

EMERGING INFECTIOUS DISEASES®

Temporal evolution in the patterns of the transmission routes and sources of infection in global outbreaks of human toxoplasmosis - A systematic review

Journal:	*Emerging Infectious Diseases*
Manuscript ID	Draft
Manuscript Type:	Synopsis
Date Submitted by the Author:	n/a
Complete List of Authors:	Ferreira, FERNANDA; Universidade Estadual de Londrina Centro de Ciencias Agrarias, Department of Preventive Veterinary Medicine Caldart, Eloiza; Universidade Estadual de Londrina Centro de Ciencias Agrarias, Department of Preventive Veterinary Medicine Pasquali, Aline; Unoesc Mitsuka-Breganó, Regina; Universidade Estadual de Londrina Centro de Ciencias Agrarias, Department of Preventive Veterinary Medicine Freire, Roberta; Universidade Estadual de Londrina Centro de Ciencias Agrarias, Department of Preventive Veterinary Medicine Navarro, Italmar; State University of Londrina - UEL, Department of Veterinary Clinics
Keywords:	Toxoplasma gondii, oocysts, water, vegetables, human
Abstract:	The objective of the current study was to evaluate the temporal progression of the probable sources of infection and transmission routes described in articles on human toxoplasmosis outbreaks throughout the world. This systematic review was carried out with keyword searches of the Scielo, Web of Science, Pubmed and Scopus databases. A total of 35 reports of acute toxoplasmosis outbreaks were selected. The incidence of outbreaks due to the parasitic forms of the oocyst and bradyzoite was 45.7% (16/35) for each, whereas for tachyzoites the incidence was 8.6% (3/35). In the 1960s and 1990s, outbreaks mainly occurred through ingestion of cysts in meat and their derivatives, in the 1980s, occurred through milk contaminated with tachyzoites, and 2000 outbreaks mainly occurred due to the presence of oocysts in water and contact with feline feces. As of 2010, there has been an increase in the incidence of outbreaks due to oocysts in vegetables.

Temporal evolution in the patterns of the transmission routes and sources of

infection in reported outbreaks of human toxoplasmosis - A systematic review

*Fernanda Pinto-Ferreira; Eloiza Teles Caldart; Aline Kuhn Sbruzzi Pasquali; Regina Mitsuka-Breganó, Roberta Lemos Freire, Italmar Teodorico Navarro

Abstract

The objective of the current study was to report a temporal progression of the probable sources of infection and/or transmission routes described in articles on human toxoplasmosis outbreaks in the world. To that, the keywords "Toxoplasma AND outbreak OR toxoplasmosis AND outbreak" were searched on the Scielo, Web of Science, Pubmed and Scopus databases. In 1960s and 1990s, outbreaks mainly occurred through ingestion of cysts in meat and derivatives; in 1980s, through milk contaminated with tachyzoites; in 2000 due to the presence of oocysts in water, sand and land and in 2010, due to oocysts in raw vegetables. Based on the results obtained in this study, it is possible that there has been a change in the epidemiology of the reported toxoplasmosis outbreaks over the last 20 years. We suggest that greater attention must be paid to the sanitation of the vegetables, to the quality of drinking and irrigation water.

Keywords: *Toxoplasma gondii*, oocysts, water, vegetables, human

Introduction

Toxoplasmosis is a zoonosis caused by the protozoan *Toxoplasma gondii* of the phylum Apicomplexa. *T. gondii* is an obligate intracellular parasite with a worldwide

distribution and affects mammals and birds (1). While its intermediate hosts are all warm-blooded animals, its definitive hosts are only felids, which are responsible for the elimination of oocysts and resulting environmental contamination (2).

Because of a high exposure to *T. gondii* parasite in the world, a high serological prevalence is observed in humans, with a variability between 10.0 and 97.4% in the adult population; however, cases of clinical disease are less frequent (3). The environmental conditions, cultural habits and fauna are factors that may explain the variability of the prevalence of this infection in different geographic areas (4). Transmission mainly occurs through the ingestion of water, vegetables or soil contaminated with oocysts (sporozoites); raw or undercooked meat containing viable tissue cysts (bradyzoites), which characterize this disease as a food zoonosis (3).

When the pattern of occurrence of toxoplasmosis outbreaks is known, more effective and targeted prevention and control measures can be applied. The objective of the current study was to report a possible temporal progression of the probable sources of infection and transmission routes described in articles on human toxoplasmosis outbreaks throughout the world.

Material and methods

This systematic review was performed by searching the Scientific Electronic Library Online (Scielo), Web of Science, Pubmed and Scopus databases. The keywords used were: "Toxoplasma AND outbreak OR toxoplasmosis AND outbreak" starting from the year 1967, when the first relevant article was published (5). Data were collected between February and March 2018 by two reviewers. The primary question was: based on the published articles, has there been a change in the pattern of transmission routes and sources of infection of human toxoplasmosis outbreaks in the world?

The inclusion criteria adopted in the research were: articles with at least the abstract in English or Portuguese language and whose contents are reports of outbreaks of toxoplasmosis in humans. Exclusion criteria were articles of outbreaks of toxoplasmosis in other animal species, and studies without information about the transmission route.

For each outbreak report, information was extracted, including the year, country of outbreak occurrence, probable route of transmission, number of affected and affected group. Based on the probable source of infection and transmission route defined by the authors of the selected papers, the parasitic form involved in each case or outbreak report was inferred. The organization, exclusion and selection of references were performed using software Mendeley (Elsevier, Amsterdam, Netherlands). The software EpiInfo 3.5.4.4 (6) was used to tabulate the variables obtained from information extracted from the selected articles. Statistical analyzes using Chi-square tests or Fisher's exact tests were performed using the R 3.4.1 program (7).

Multiple correspondence analysis was performed in R 3.4.1 with the FactoMineR package. This technique is an exploratory technique that does not rely on statistical tests, but allows visualization of the most important relationships in a large set of variables (8). This technique also helps to visualize the multivariate relation between the categories of different variables as the geometric proximity of the variables in the graph suggests a possible association between them.

Results

A total of 573 articles were found, of which 10 were from Scielo, 224 from the Web of Science, 83 Pubmed and 256 Scopus. Duplicate, incomplete (untitled, authors, abstract) articles or those that did not contain the likely route of transmission were excluded.

An article, even eligible for review, was excluded because it did not have access possible via the Internet or via commute. Thirty-three articles were selected, with 34 reports of outbreaks of acute toxoplasmosis (one article with two outbreak reports). The details are shown in Figure 1 and Table 1.

The geographic distribution of the selected outbreaks is plotted on the map in Figure 2. The highest concentration was in the Americas, with 73.5% (25/34). Of this total, Brazil represented 35.3% (12/34) of the published outbreaks selected by the present study.

The incidence of cyst related outbreaks was 47.1% (16/34), while oocysts were implicated in 44.1% (15/34) of the outbreaks and tachyzoites in 8.8% (3/34). Ingestion of contaminated meat and its derivatives was the suspected cause of 47.1% (16/34) of toxoplasmosis outbreaks. Transmission through the intake of oocysts in water occurred with a frequency of 20.6% (7/34), sand and land contact with a frequency of 17.6% (6/34), the intake of oocysts in vegetables with 5.9% (2/34) of frequency. Regarding reported outbreaks because of tachyzoites in raw milk, the frequency was of 8.8% (3/34). A total of approximately 1,416 individuals were affected in the 15 outbreaks of toxoplasmosis caused by oocysts (bradyzoites), while 290 out of 16 outbreaks caused by tissue cysts (bradyzoites) and 15 out of 3 outbreaks by tachyzoites.

No statistical significance was observed in the variables extracted from the articles. An evaluation of the multiple correspondence analysis results (Figure 3) presents that in the 1960s and 1990s, outbreaks mainly occurred through the ingestion of cysts in meat and its derivatives. In the 1980s, by milk contaminated with tachyzoites. In 2000, by oocysts in water and contact with feline feces. As of 2010, there has been an increase in the occurrence of outbreaks due to oocyst intake from raw vegetables.

Discussion

The limitations encountered in this study were: 1. many outbreaks published only in the gray literature (non-indexed journals, unofficial websites, conference abstracts); 2. the search was limited to articles with, at least, the abstract written in Portuguese or English; 3. a long lag time between the occurrence of an outbreak and its publication, with an average of three years and up to seven years; and 4. lack of defined transmission routes, which can be measured only by means of epidemiological investigation (9,10). Although this review was performed based on reports from the world literature, it demonstrates much of the Brazilian reality, perhaps because it is the country with the largest number of reports and affected.

Three major clones of *T. gondii* are described in the literature (genotypes I, II and III), they differ from one another due to virulence and epidemiology (11–14). America, there is no clear domain of any genotype, although some have relatively higher frequencies (13). In Brazil, the prevalence of toxoplasmosis in humans ranges from 21.5 to 97.4%(15) and the occurrence of atypical genotypes (different from clonal genotypes I, II and III) is more frequent, which may justify the most severe form of the disease (16) and the bigger number of publications in this country. Proof of this is that the two largest outbreaks of human toxoplasmosis occurred in Brazil: 1- Santa Isabel do Ivaí city, Paraná state(17), that occurred in 2001 which involved more than 400 people due to contamination of the municipal water supply network and 2- Santa Maria city, Rio Grande do Sul state, which occurred in 2018 and affected more than 900 people; the cause of that outbreak has not yet been determined (data not yet published).

Oocyst and cyst intake are the most important transmission via of *T. gondii* (9). According to our results, until 1990, cysts were the main biological form of infection. However, it is possible that with the prevention program for complex teniasis-cysticercosis, a large number of people have begun to avoid consumption of raw or poorly cooked meats in

South America, as tissue cysts are sensitive to heat, cooked meat is considered safe for the avoidance of *T. gondii* infection (18). Another important point is that the technology of livestock farming has promoted improved management and reduction of infection of animals by pathogens, such as *T. gondii*, making the meat safer. In Brazil, to prevent food-borne diseases, the Ministry of Agriculture, Livestock and Supply, through Administrative Rule No. 46, of February 10, 1998, adopted the Hazard Analysis and Critical Control Point System (HACCP), a pre-requisite to export meat, in order to improve Good Production Practices (GPP) (19). After the improvements in the system, a reduction in seropositivity to *T. gondii* could be observed over the years (9). Another important point was the popularization of the use of freezer, the freezing of the meats either -10°C degrees for 3 days or -20°C degrees for 2 days, is sufficient for the inactivation of cysts (20).

Beef was the suspected route of transmission in at least three outbreaks that occurred in the United States and Brazil, affecting five and six and 99 people respectively (39–41). However, beef has less epidemiological value since these animals have a low ability to form tissue cysts (42).

Feeding habits can often facilitate infection by *T. gondii* in the form of bradyzoites and tachyzoites (4). For example, consumption of raw kibbe made from lamb meat, which is an Arabian tradition, was the cause of five outbreaks between 1975 and 2006; two from Brazil(21,22), England (23), United States (24) and Australia (25) with one outbreak each.

Consumption of undercooked hunting animals, such as reindeer, tapir, deer, wild boar, armadillo and wild pig, was an important factor associated with toxoplasmic infection in some of the previously described outbreaks in the world (26–29). Some studies have also determined that the unusual abundance of atypical strains, which are commonly found in the wild, can cause toxoplasmosis in its most severe form, even in immunocompetent individuals (16,26,30–32). This greater severity results from poor host adaptation to the

circulating *T. gondii* zoonotic neotropical strains (33). In 2009, Pino et al. (31) described severe cardiac involvement in a military man who, during an operation in the jungle, consumed untreated water. Previously, Carme et al., 2002 (26) reported in French Guiana 16 cases of severe toxoplasmosis in immunocompetent patients hospitalized with nonspecific infectious diseases with at least one visceral alteration, mainly severe pulmonary involvement (87.5%). The parasite was isolated from three patients and, in all cases, the genotype was characterized as atypical. Hunting animals were the source of infection in 31.25% (5/16) of outbreaks whose likely route of transmission were tissue cysts.

Infection through milk consumption was described in three of the articles selected (1975-1988), all of which were intra-familial, involved raw goat's milk and affected a small number of individuals (34–36). The ability of goats to eliminate tachyzoites in milk (37,38) is well-known, and these tachyzoites are resistant to the processing of fresh cheese (39). Proper hygiene during milking and heat treatment are the main preventive measures against milk contamination, regardless of the species of the producing animal or agent (Listeria monocytogenes, *Brucell*a spp. and *Mycobacterium* spp.). It is possible that the good practices applied in the prevention of these agents, such as pasteurization and brucellosis and tuberculosis prevention programs, have influenced the reduction of *T. gondii* infection and, consequently, the outbreaks by contaminated milk have decreased in recent decades.

The previous cited Santa Isabel do Ivaí outbreak (17) was a milestone in the history of toxoplasmosis, as well as having a larger number of affected individuals, also had the agent isolated from the transmission via. From this milestone, the outbreaks were investigated with more attention to this biological form and this factor should not be discarded as justification for the increase of outbreaks of water origin.

One of the main forms of transmission of toxoplasmosis is fecal-oral, the felines, definitive hosts, eliminate the oocysts in the environment and these can remain viable

for several months in appropriate conditions of temperature and humidity, allowing the infection(2). The habit of cats defecating in land and sand, contacts these places an important risk factor. Land and sand contact were the route of transmission in 17.6% (6/34) of outbreaks reported, in 67.0% the group of people affected was with children and / or adolescents, probably due to the habit children have play in these environments and indirectly perform geophagy.

In the last 20 years, there has been an increase in the consumption of healthy foods, such as vegetables, related to changing habits to combat obesity (40). Vegetables provide micronutrients and fibers as well as aid in body weight maintenance due to their low energy values (41). Considering the increased food safety attributed in recent years to food of animal origin and concomitant increase in the consumption of raw vegetables and fruits, it is likely that foods contaminated by oocysts will become the main routes of transmission of toxoplasmosis outbreaks.

Outbreaks caused by vegetables generally occur because of a poor hygiene of these foods, or contamination during the production stages that involve planting, harvesting, transport, distribution or even during processing and final consumption (42). In 2009, an outbreak occurred in a factory in São Paulo state, Brazil, with a total of 2,300 employees, of which 11 individuals had acute toxoplasmosis, vegetable ingestion was the suspected transmission via (43). In 2013, the municipality of Ponta de Pedras, Pará state, Brazil, was the scene of an outbreak of toxoplasmosis caused by açaí consumption, involving 73 cases with clinical and laboratorial profile compatible with the disease. Ponta das Pedras is one of the main producers of açaí in Brazil, however, the outbreak occurred during the period when local production of açaí was practically nil and to guarantee the population's demand, açaí vendors acquired the fruit from other locations (44). These events demonstrate inadequacy in the control of sanitation in industrial restaurants and also the need to improve quality control of commercial vegetables, common problems in developing countries.

Other important point observed in the current study was the number of affected individuals. Oocysts were responsible for statewide outbreaks that involved many people, such as those occurring in city districts or entire municipalities. Although outbreaks due to oocysts and cysts were reported to occur at a similar number, outbreaks due to oocysts affected 100 times more people than outbreaks due to cysts. Consumption of water was already responsible for the largest outbreak of toxoplasmosis described (17), and yet, when used in irrigation, serve as a contamination route for vegetables and fruits (17,43,45). Domestic and wild felids, when infected, can eliminate thousands of oocysts per gram of feces for approximately two weeks, causing wide water dispersion and environmental contamination (46). Cysts infection is more related to intra-familial or party restricted outbreaks, limiting the number of affected individuals (47,48).

Health actions regarding toxoplasmosis have been focused, frequently, on congenital infections due to its importance. However, with the occurrence of outbreaks in healthy humans, it is suggested that control of infection and health education should also be directed to the rest of the population. According to World Health Organization estimates, toxoplasmosis is among the leading foodborne parasitic diseases and in recent years has affected more than 10.3 million people worldwide (49). The disease is not of compulsory notification, so it is necessary that at least toxoplasmosis outbreaks are reported in the literature, in order to know more deeply the chain of transmission and, thus, to reduce the outbreaks occurrence.

Conclusion

Based on the results obtained in this study, it is possible that there has been a change in the epidemiology of the reported toxoplasmosis outbreaks over the last 20 years.

Thus, we suggest that greater attention must be paid to the production and sanitation of the vegetables, to the quality of drinking and irrigation water, and to the adoption of legislation for the traceability with the aim to eliminate transmission routes, avoiding exposure or inactivating the parasite before consumption.

Figure 1 - Schematic demonstration of the selection of articles for the systematic review of outbreaks of human toxoplasmosis throughout the world.

Figure 2 - Geographic distribution of 34 outbreaks of human toxoplasmosis worldwide and its probable route of transmission - works published until March 2018 selected through systematic review.
The size of the circle is proportional to the number of outbreaks.

Figure 3 - Multiple correspondence analysis of the variables extracted from articles that published human toxoplasmosis outbreaks until March 2018 selected through systematic review.
The multiple correspondence technique helps to visualize the multivariate relation between the categories of different variables as the geometric proximity of the variables in the graph suggests a possible association between them.

Table 1 - Complete articles of human toxoplasmosis outbreaks included in the systematic review published until March 2018.

References

1. Jacobs L, Lunde F. The interrelation of toxoplasmosis in swine, cattle, dogs, and man. Public health reports. 1957;72(10):872–82.
2. Dubey JP, Miller NL, Frenkel JK, Frenkel AJK. The Toxoplasma gondii oocyst from cat feces. The Journal of experimental medicine [Internet]. 1970 Oct 1 [cited 2017 Aug 18];132(4):636–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4927658%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2138867
3. Tenter AMM, Heckeroth ARR, Weiss LMM. Toxoplasma gondii: from animals to humans. International journal for parasitology [Internet]. 2000 Nov [cited 2017 May 2];30(12–13):1217–58. Available from: http://www.sciencedirect.com/science/article/pii/S0020751900001247
4. Dubey JP. Toxoplasma, Hammondia, Besnoitia, Sarcocystis and others tissue cyst-forming coccidia of man and animals. In: Kreier JP, editor. Parasitic Protozoa. 3rd ed. New York: Academic Press; 1977. p. 101–237.
5. Magaldi C, Elkis H, Pattoli D, Queiróz JC, Coscina AL, Ferreira JM. Surto de toxoplasmose em um seminário de Bragança Paulista (Estado de São Paulo): aspectos clínicos, sorológicos e epidemiológicos. Revista de Saúde Pública. 1967;1(1):141–71.
6. Dean AG, Dean JA, Burton AH, Discker RC. Epi-Info, Version 5: a word processing, database, and statistics programme for epidemiology on microcomputers. Atlanta, GA: USD Inc. 1990;
7. R Core T. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Online: http://www R-project org. 2013;
8. Lebart L, Morineau A, Warwick KM. Multivariate descriptive statistical analysis; correspondence analysis and related techniques for large matrices. 1984;
9. Dubey JP, Lago EG, Gennari SM, Su C, Jones JL. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitology [Internet]. 2012 Sep 10;139(11):1375–424. Available from: http://www.journals.cambridge.org/abstract_S0031182012000765
10. Petersen E, Vesco G, Villari S, Buffolano W. What do we know about risk factors for infection in humans with Toxoplasma gondii and how can we prevent infections? Zoonoses and public health. 2010 Feb;57(1):8–17.
11. Sibley LD, Boothroyd JC. Virulent strains of Toxoplasma gondii comprise a single clonal lineage. Nature. 1992 Sep;359(6390):82–5.
12. Howe DK, Sibley LD. Toxoplasma gondii Comprises Three Clonal Lineages : Correlation of Parasite Genotype with Human Disease Function p30 Surface Antigen p22 Surface Antigen Rhoptry protein Unknown cDNA Unknown Unknown cDNA. The Journal of Infectious Diseases. 1995;1561–6.
13. Shwab EK, Zhu X-Q, Majumdar D, Pena HFJ, Gennari SM, Dubey JP, et al. Geographical patterns of Toxoplasma gondii genetic diversity revealed by multilocus PCR-RFLP genotyping. Parasitology. 2014;141(4):453–61.
14. Su C, Khan A, Zhou P, Majumdar D, Ajzenberg D, Darde M-L, et al. Globally diverse Toxoplasma gondii isolates comprise six major clades originating from a small number of distinct ancestral lineages. Proceedings of the National Academy of Sciences [Internet]. 2012 Apr 10 [cited 2018 Feb 5];109(15):5844–9. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1203190109
15. Dubey JP, Lago EG, Gennari SM, Su C, Jones JL. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitology. 2012 Sep 10;139(11):1375–424.

16. Salvador-Guillouet F, Ajzenberg D, Chaillou-Opitz S, Saint-Paul MC, Dunais B, Dellamonica P, et al. Severe pneumonia during primary infection with an atypical strain of Toxoplasma gondii in an immunocompetent young man. Journal of Infection. 2006 Aug;53(2):e47–50.
17. de Moura L, Bahia-Oliveira LMG, Wada MY, Jones JL, Tuboi SH, Carmo EH, et al. Waterborne toxoplasmosis, Brazil, from field to gene. Emerging Infectious Diseases [Internet]. 2006 Feb [cited 2017 Aug 31];12(2):326–9. Available from: http://wwwnc.cdc.gov/eid/article/12/2/04-1115_article.htm
18. Dubey JP, Kotula AW, Sharar A, Andrews CD, Lindsay DS. Effect of high temperature on infectivity of Toxoplasma gondii tissue cysts in pork. The Journal of parasitology. 1990 Apr;76(2):201–4.
19. Rossi GAM, Hoppe EGL, Martins AMCV, Prata LF. Zoonoses parasitárias veiculadas por alimentos de origem animal: revisão sobre a situação no Brasil. Arquivos do Instituto Biológico [Internet]. 2014;81(3):290–8. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1808-16572014000300290&lng=pt&nrm=iso&tlng=en
20. El-Nawawi FA, Tawfik MA, Shaapan RM. Methods for inactivation of Toxoplasma gondii cysts in meat and tissues of experimentally infected sheep. Foodborne pathogens and disease. 2008 Oct;5(5):687–90.
21. Bonametti AM, Passos JN, da Silva EM, Bortoliero AL. Outbreak of acute toxoplasmosis transmitted thru the ingestion of ovine raw meat [Surto de toxoplasmose aguda transmitida através da ingestão de carne crua de gado ovino.]. Revista da Sociedade Brasileira de Medicina Tropical [Internet]. 1996;30(1):21–5. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-0029678025&partnerID=40&md5=2f3270e436499fd19e437e519178be9a
22. Renoiner EIM, Siqueira AA, Garcia MH, Alves RM, Cardoso ME, Ferreira ABPL, et al. Surto de toxoplasmose adquirida, Anápolis -GO, fevereiro de 2006. Boletim Eletrônico Epidemiológico. 2007;(8):1–6.
23. Fertig A, Selwyn S, Tibble MJ. Tetracycline treatment in a food-borne outbreak of toxoplasmosis. British medical journal [Internet]. 1977 Apr [cited 2017 May 2];1(6068):1064. Available from: https://www.cabdirect.org/cabdirect/abstract/19772702762
24. Masur H, Jones TC, Lempert JA, Cherubini TD. Outbreak of toxoplasmosis in a family and documentation of acquired retinochoroiditis. The American journal of medicine. 1978 Mar;64(3):396–402.
25. Silva LM, Mulcahy DL, Kamath KR. A family outbreak of toxoplasmosis: a serendipitous finding. Journal of Infection. 1984;8:163–7.
26. Carme B, Bissuel F, Ajzenberg D, Bouyne R, Aznar C, Demar M, et al. Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. Journal of Clinical Microbiology [Internet]. 2002;40(11):4037–44. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-0036840027&doi=10.1128%2FJCM.40.11.4037-4044.2002&partnerID=40&md5=7a50ab19413c642336c4ac82dbde8a84
27. Choi WY, Nam HW, Kwak NH, Huh W, Kim YR, Kang MW, et al. Foodborne outbreaks of human toxoplasmosis. The Journal of infectious diseases. 1997 May;175(5):1280–2.
28. Sacks JJ, Delgado DG, Lobel HO, Parker RL. Toxoplasmosis infection associated with eating undercooked venison. American journal of epidemiology. 1983 Dec;118(6):832–8.
29. McDonald JC, Gyorkos TW, Alberton B, MacLean JD, Richer G, Juranek D. An outbreak of toxoplasmosis in pregnant women in northern Quebec. The Journal of infectious diseases [Internet]. 1990 Apr;161(4):769–74. Available from:

https://www.scopus.com/inward/record.uri?eid=2-s2.0-0025332258&doi=10.1093%2Finfdis%2F161.4.769&partnerID=40&md5=69313171e2848b52e876d08c716cec54
30. Sobanski V, Ajzenberg D, Delhaes L, Bautin N, Just N. Severe toxoplasmosis in immunocompetent hosts: Be aware of atypical strains. American Journal of Respiratory and Critical Care Medicine. 2013;187(10):1143–5.
31. Pino LE, Salinas JE, López MC. Descripción de un brote epidémico de toxoplasmosis aguda en pacientes inmunocompetentes miembros de las fuerzas militares de Colombia durante operaciones de selva. Infectio [Internet]. 2009;13(2):83–91. Available from: http://www.sciencedirect.com/science/article/pii/S0123939209707295
32. Demar M, Ajzenberg D, Maubon D, Djossou F, Panchoe D, Punwasi W, et al. Fatal outbreak of human toxoplasmosis along the Maroni River: Epidemiological, clinical, and parasitological aspects. Clinical Infectious Diseases [Internet]. 2007;45(7):e88–95. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-34848812882&doi=10.1086%2F521246&partnerID=40&md5=fcf729bbe14417616031253c986f55ce
33. Carme B, Demar M, Ajzenberg D, Darde ML. Severe acquired toxoplasmosis caused by wild cycle of Toxoplasma gondii, French Guiana. Emerging infectious diseases. 2009 Apr;15(4):656–8.
34. Skinner LJ, Timperley AC, Wightman D, Chatterton JMW, Ho-Yen DO. Simultaneous diagnosis of toxoplasmosis in goats and goatowner's family. Scandinavian journal of infectious diseases [Internet]. 1990 [cited 2017 Sep 4];22(3):359–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2371548
35. Sacks JJ, Roberto RR, Brooks NF. Toxoplasmosis infection associated with raw goat's milk. JAMA [Internet]. 1982 Oct [cited 2017 May 2];248(14):1728–32. Available from: http://jama.jamanetwork.com/article.aspx?articleid=378479
36. Chiari CA, Neves DP. Human toxoplasmosis acquired by ingestion of goat's milk [Toxoplasmose humana adquirida através da ingestão de leite de cabra.]. Memorias do Instituto Oswaldo Cruz [Internet]. 1984;79(3):337–40. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-0021465104&partnerID=40&md5=506a8f61904cdd20d7c1a2d0b40cb62a
37. Bezerra MJG, Kim PCP, Moraes ÉPBX, Sá SG, Albuquerque PPF, Silva JG, et al. Detection of Toxoplasma gondii in the milk of naturally infected goats in the Northeast of Brazil. Transboundary and emerging diseases. 2015 Aug 1;62(4):421–4.
38. Spišák F, Turčeková Ľ, Reiterová K, Špilovská S, Dubinský P. Prevalence estimation and genotypization of Toxoplasma gondii in goats. Biologia. 2010 Jan 1;65(4):670–4.
39. Dubey JP, Verma SK, Ferreira LR, Oliveira S, Cassineli AB, Ying Y, et al. Detection and survival of Toxoplasma gondii in milk and cheese from experimentally infected goats. Journal of food protection. 2014 Oct;77(10):1747–53.
40. Brasil, Saúde M da. Vigitel Brasil - Vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico [Internet]. 2016. 157 p. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/vigitel_brasil_2016_saude suplementar.pdf
41. Brasil. Obesidade cresce 60% em dez anos no Brasil [Internet]. Ministério da Saúde do Brasil. 2017 [cited 2017 Nov 13]. p. 1. Available from: http://www.brasil.gov.br/saude/2017/04/obesidade-cresce-60-em-dez-anos-no-brasil
42. Ferreira FP, Caldart ET, Freire RL, Freitas FM De, Miura AC, Mareze M, et al. The effect of water source and soil supplementation on parasite contamination in organic vegetable gardens. Brazilian Journal of Veterinary Parasitology. 2018;2961:1–11.
43. Ekman CCJ, Chiossi MF do V, Meireles, Luciana Regina Andrade Júnior HF de, Figueiredo WM, Marciano MAM, Luna EJ de A, et al. Case-control study of an outbreak of

acute toxoplasmosis in an industrial plant in the state of São Paulo, Brazil. Revista do Instituto de Medicina Tropical de São Paulo [Internet]. 2012 [cited 2017 May 2];54(5):239–44. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0036-46652012000500001&lang=pt

44. Morais R dos APB, Freire ABC, Barbosa DRL, Silva L de CT da, Pinheiro AF, Costa SS da, et al. Surto de toxoplasmose aguda no Município de Ponta de Pedras, Arquipélago do Marajó, Estado do Pará, Brasil: características clínicas, laboratoriais e epidemiológicas. Revista Pan-Amazônica de Saúde. 2016 Dec;7:143–52.

45. Dutra LH, Saad E, Alves RM de S, Cabral CM, Batista FS, Lima R de, et al. Investigação de surto de toxoplasmose transmitido pelo consumo de açaí em Rondônia, Brasil, 2011. In: Anais da 12° EXPOEPI. Brasília - Distrito Federal: Ministério da Saúde; 2012. p. 108–9.

46. Silva JCR, Ogassawara S, Adania CH, Ferreira F, Gennari SM, Dubey JP, et al. Seroprevalence of Toxoplasma gondii in captive neotropical felids from Brazil. Veterinary Parasitology [Internet]. 2001;102(3):217–24. Available from: http://www.sciencedirect.com/science/article/pii/S0304401701005234

47. Almeida MABA, Alencar Júnio LR, Carmo GMI, Araújo WNA, Garcia MHO, Reis AK V, et al. Surto intra familiar de toxoplasmose, Santa Vitória do Palmar-RS, julho de 2005. Boletim eletrônico epidemiológico da secretária de vigilância em saúde. 2006;18(3):1–7.

48. Eduardo MB de P, Katsuya EM, Ramos SRTS, Pavanello EI, Paiva OR, Brito S do N, et al. Investigação do surto de toxoplasmose associado ao consumo de prato à base de carne crua ("steak tartar"), nos municípios de São Paulo e Guarujá, SP – Novembro de 2006. Boletim Epidemiológico Paulista. 2007;4(41):1–6.

49. Torgerson PR, Devleesschauwer B, Praet N, Speybroeck N, Willingham AL, Kasuga F, et al. World Health Organization Estimates of the Global and Regional Disease Burden of 11 Foodborne Parasitic Diseases, 2010: A Data Synthesis. PLOS MEDICINE. 2015;12(12).

ID	Year	Cases (n)	Transmission via	Parasitic form	Countries	Affected group
1a	1965	9	Beef*	Cyst	Brazil	Seminar
2a	1966	99	Beef	Cyst	Brazil	Institutional
3a	1968	5	Beef	Cyst	United States	Snack bar
4a	1975	6	Ovine raw meat	Cyst	United States	Intrafamily
5a	1976	3	Ovine raw meat	Cyst	England	Intrafamily
6a	1976	21	Pork	Cyst	Brazil	Intrafamily
7a	1979	4	Ovine raw meat	Cyst	Australia	Intrafamily
8a	1980	3	Venision (hunting animals)	Cyst	United States	Hunters
9a	1987	22	Raw reindeer meat (hunting animals)	Cyst	Canada	Pregnant womens
10a	1993	17	Ovine raw meat	Cyst	Brazil	Party participants
11a	1994	3	Wild pig viscera (hunting animals)	Cyst	South Korea	Merchants
12a	1994	5	Pig viscera (hunting animals)	Cyst	South Korea	Soldiers
13a	1995	16	Game meat (hunting animals)	Cyst	French Guiana	Hospitalized patients
14a	2005	10	Pork sausage	Cyst	Brazil	Intrafamily
15[a]	2006	6	Beef	Cyst	Brazil	Party participants
16[a]	2006	61	Ovine raw meat	Cyst	Brazil	Party participants
1b	1978	10	Goat milk	Tachyzoite	United States	Intrafamily
2b	1983	3	Goat milk	Tachyzoite	Brazil	Intrafamily
3b	1988	2	Goat milk	Tachyzoite	Scotland	Intrafamily
1c	1976	10	Sand and land contact	Oocyst	United States	Intrafamily
2c	1977	37	Sand and land contact	Oocyst	United States	Horse riding
3c	1979	35	Water	Oocyst	Panama	Soldiers

4c	1982	9	Sand and land contact	Oocyst	United States	Intrafamily
5c	1990	4	Sand and land contact	Oocyst	Iraq	Intrafamily
6c	1995	110	Water	Oocyst	Canada	Municipal outbreak
7c	2001	426	Water	Oocyst	Brazil	Municipal outbreak
8c	2002	171	Sand and land contact	Oocyst	Turkey	Boarding school
9c	2003	11	Water	Oocyst	Suriname	Outbreak in neighborhood
10c	2004	248	Water	Oocyst	India	Municipal outbreak
11c	2004	213	Water	Oocyst	India	Municipal outbreak
12c	2004	40	Sand and land contact	Oocyst	Brazil	Municipal outbreak
13c	2008	18	Water	Oocyst	Colombia	Soldiers
14c	2009	11	Raw vegetables	Oocyst	Brazil	Restaurant factory
15[a]	2013	73	Açaí	Oocyst	Brazil	Municipal outbreak

*Transmission route deduced through the epidemiological evaluation of the article, at the time the transmission chain of toxoplasmosis had not yet been fully elucidated, and the authors suspected ticks and dogs as sources of infection.

1a-Magaldi et al. Surto de toxoplasmose em um seminário de Bragança Paulista (Estado de São Paulo): aspectos clínicos, sorológicos e epidemiológicos. Revista de Saúde Pública. 1967;1(1):141–71.

2a-Magaldi et al. Epidemic of toxoplasmosis at a university in Sao José dos Campos, SP, Brazil. Revista Latinoamericana de Microbiologia. 1969;11(1):05–13.

3a-Kean BH et al. An epidemic of acute toxoplasmosis. JAMA. 1969;208(6):1002–4.

4a-Masur et al. Outbreak of toxoplasmosis in a family and documentation of acquired retinochoroiditis. The American journal of medicine. 1978;64(3):396–402.

5a-Fertig et al. Tetracycline treatment in a food-borne outbreak of toxoplasmosis. British medical journal. 1977;1(6068):1064.

6a-Coutinho et al. Outbreak of human toxoplasmosis in a rural area. A three year serologic follow-up study. Memorias do Instituto Oswaldo Cruz. 1982;77(1):29–36.

7a-Silva et al. A family outbreak of toxoplasmosis: A serendipitous finding. Journal of Infection. 1984;8(2):163–7.

8a-Sacks et al. Toxoplasmosis infection associated with eating undercooked venison. American journal of epidemiology. 1983;118(6):832–8.

9a-McDonald et al. An outbreak of toxoplasmosis in pregnant women in northern Quebec. The Journal of infectious diseases. 1990 Apr;161(4):769–74.

10a-Bonametti et al. Surto de toxoplasmose aguda transmitida através da ingestão de carne crua de gado ovino. Revista da Sociedade Brasileira de Medicina Tropical. 1997; 30(1):21–5.

11a e 12a-Choi et al. Foodborne outbreaks of human toxoplasmosis. The Journal of infectious diseases. 1997 May;175(5):1280–2.

13a-Carme et al. Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. Journal of Clinical Microbiology.2002;40(11):4037–44.

14a-Almeida et al. Surto intra familiar de toxoplasmose, Santa Vitória do Palmar-RS. Boletim eletrônico epidemiológico da secretária de vigilância em saúde. 2006;18(3):1–7.

15a-Eduardo et al. Investigação do surto de toxoplasmose associado ao consumo de prato à base de carne crua ("steak tartar"), nos municípios de São Paulo e Guarujá, SP – Novembro de 2006. Boletim Epidemiológico Paulista. 2007;4(41):1–6.

16a-Renoiner et al. Surto de toxoplasmose adquirida, Anápolis -GO. Boletim Eletrônico Epidemiológico. 2007;(8):1–6.

1b-Sacks et al.Toxoplasmosis infection associated with raw goat's milk. JAMA. 1982;248(14):1728–32.

2b-Chiari & Neves. Toxoplasmose humana adquirida através da ingestão de leite de cabra. Memórias do Instituto Oswaldo Cruz. 1984 Sep;79(3):337–40.

3b-Skinner et al. Simultaneous diagnosis of toxoplasmosis in goats and goatowner's family. Scandinavian journal of infectious diseases. 1990;22(3):359–61.

1c-Stagno et al. An outbreak of toxoplasmosis linked to cats. Pediatrics. 1980;65(4):706–12.

2c-Teutsch et al. Epidemic toxoplasmosis associated with infected cats. The New England journal of medicine. 1979;300(13):695–9.

3c-Benenson et al. Oocyst-transmited toxoplasmosis associated with ingeston of contaminated water. The New England Journal of Medicine. 1982;307(11):666–9.

4c-Shenep et al. An outbreak of toxoplasmosis on an Illinois farm. Pediatric infectious disease . 1984; 3(6):518–22.

5c-Abood AA. A small outbreak of spontaneous abortion in four patients from two households. Infection control and hospital epidemiology. 1992 Aug;13(8):451.

6c-Bell et al. Outbreak of toxoplasmosis associated with municipal drinking water — British Columbia. Canada Communicable Disease Report. 1995;21–18:161–164.

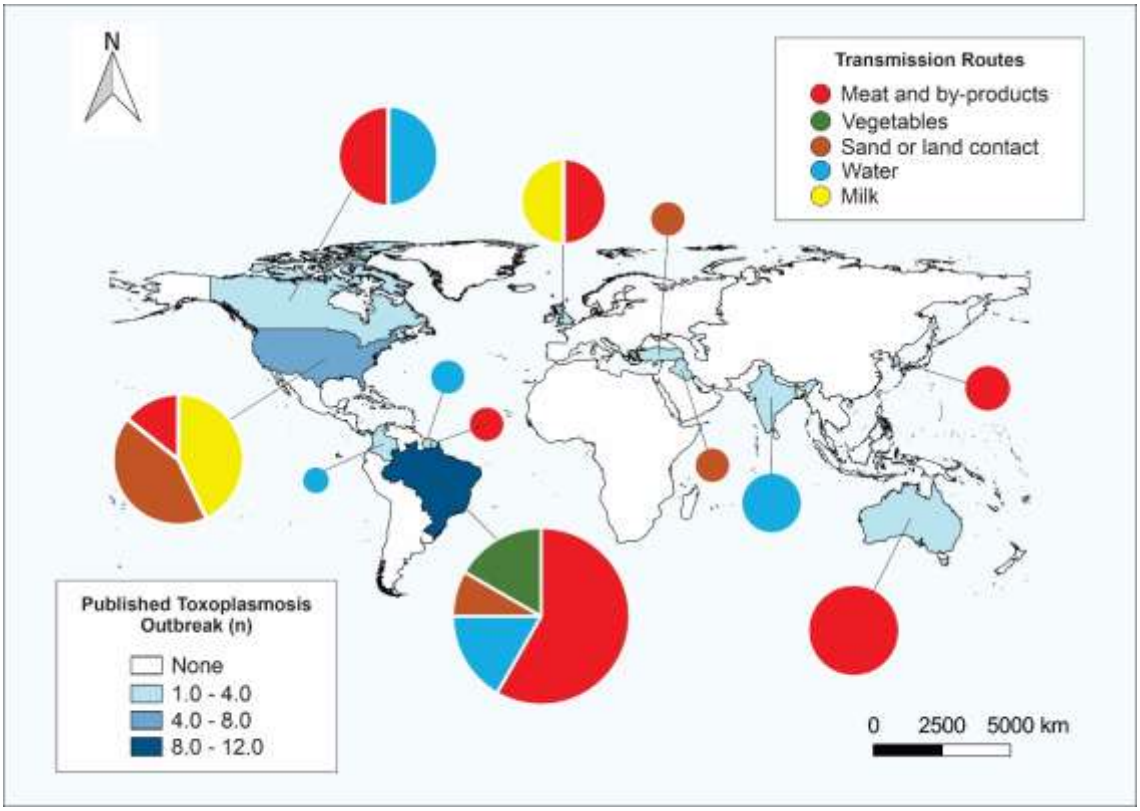
7c-de Moura L, Bahia-Oliveira LMG, Wada MY, Jones JL, Tuboi SH, Carmo EH, et al. Waterborne toxoplasmosis, Brazil, from field to gene. Emerging Infectious Diseases . 2006;12(2):326–9.

8c-Doganci L, Tanyuksel M, Araz ER, Besirbellioglu BA, Erdem U, Ozoguz CA, et al. A probable outbreak of toxoplasmosis among boarding school students in Turkey. Clinical Microbiology Infection. 2006 Jul;12(7):672–94.

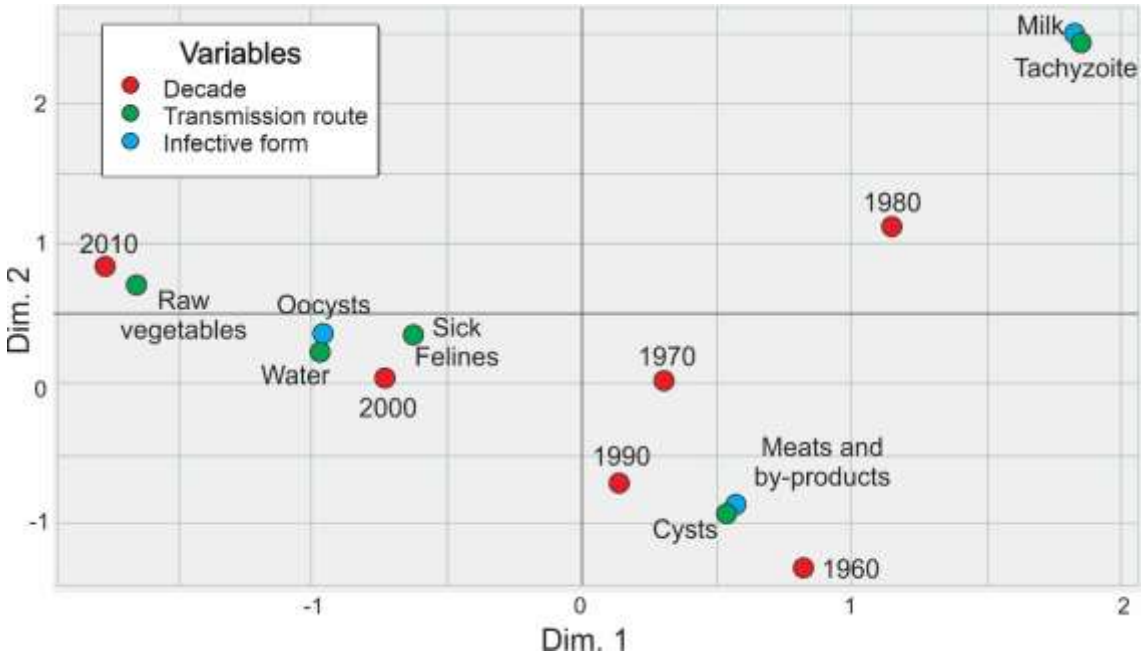
9c-Demar et al. Fatal outbreak of human toxoplasmosis along the Maroni River: Epidemiological, clinical, and parasitological aspects. Clinical Infectious Diseases. 2007;45(7):e88–95.

10c-Balasundaram & Andavar. Outbreak of acquired ocular toxoplasmosis involving 248 patients. Archives of Ophthalmology. 2010; 128(1): 28-34.

11c-Palanisamy et al. Outbreak of ocular toxoplasmosis in Coimbatore, India. Indian Journal of Ophthalmology. 2006;54(2):129–31.
12c-Carmo et al. Surto de toxoplasmose humana no Distrito de Monte Dourado, Município de Almeirim, Pará, Brasil. Rev Pan-Amazônica Saúde.2010;1(1):61–6.
13c-Pino et al. Descripción de un brote epidémico de toxoplasmosis aguda en pacientes inmunocompetentes miembros de las fuerzas militares de Colombia durante operaciones de selva. Infectio. 2009;13(2):83–91.
14c-Ekman et al. Case-control study of an outbreak of acute toxoplasmosis in an industrial plant in the state of São Paulo, Brazil. Revista do Instituto de Medicina Tropical de São Paulo. 2012;54(5):239–44.
15c-Morais et al. Surto de toxoplasmose aguda no Município de Ponta de Pedras, Arquipélago do Marajó, Estado do Pará, Brasil: características clínicas, laboratoriais e epidemiológicas. Revista Pan-Amazônica de Saúde. 2016;7:143–52.


N
Transmission Routes
Meat and by-products
Vegetables
Sand or land contact
Water
Milk
Published Toxoplasmosis Outbreak (n)
None
1.0 - 4.0
4.0 - 8.0
8.0 - 12.0
0 2500 5000 km

1


Variables
Decade
Transmission route
Infective form
Milk
Tachyzoite
1980
2010
Raw vegetables
Oocysts
Sick Felines
Water
2000
1970
1990
Meats and by-products
Cysts
1960
Dim. 2
Dim. 1
2
1
0
-1
-1
0
1
2

2

3

4

5

6

4 ARTIGO B

O artigo a seguir foi publicado na *Revista Brasileira de Parasitologia Veterinária*, em julho de 2018, sob DOI: https://doi.org/10.1590/S1984-296120180050

Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, v. 27, n. 3, p. 327-337, july.-sept. 2018
Doi: https://doi.org/10.1590/S1984-296120180050

Original Article

The effect of water source and soil supplementation on parasite contamination in organic vegetable gardens

O efeito da fonte de água e suplementação de solo na contaminação parasitária em hortas orgânicas

Fernanda Pinto Ferreira[1]*; Eloiza Teles Caldart[1]; Roberta Lemos Freire[1]; Regina Mitsuka-Breganó[1]; Felipe Machado de Freitas[2]; Ana Carolina Miura[1]; Marcelle Mareze[1]; Felippe Danyel Cardoso Martins[1]; Mariana Ragassi Urbano[3]; Adilson Luiz Seifert[2]; Italmar Teodorico Navarro[1]

[1] Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina – UEL, Londrina, PR, Brasil

[2] Departamento de Agronomia, Universidade Estadual de Londrina – UEL, Londrina, PR, Brasil

[3] Departamento de Estatística, Universidade Estadual de Londrina – UEL, Londrina, PR, Brasil

Received March 21, 2018
Accepted June 12, 2018

Abstract

The objective of this study was to determine factors associated with vegetable contamination with zoonotic protozoan. Samples of water, soil and vegetables were collected from July/2014 to May/2016, totaling 83 samples, 21 properties of Londrina region, Paraná, Brazil. DNA amplification of *Toxoplasma gondii*, *Cryptosporidium* spp. and *Giardia intestinalis* in the samples was conducted using polymerase chain reaction (PCR). The PCR results were positive for *T. gondii* in 12.9% (8/62), *Cryptosporidium* spp. in 11.3% (7/62) and *G. intestinalis* in 25.8% (16/62) of the samples. DNA sequencing identified *C. parvum* in five samples and *G. intestinalis* Assemblage E in three. The statistical associations demonstrated greater probability of positive samples for *T. gondii* and for at least one of the three protozoa when the source of irrigation water was the river; a greater chance of positive samples for *Cryptosporidium* spp. when deer were present on the property; and a smaller chance of positive samples for at least one of the three etiologic agents when soil was supplemented with limestone. The results expose some critical contamination points, providing support for training farmers on good management practices during the production process.

Keywords: Vegetables, water, environmental contamination, *Cryptosporidium* spp., *Toxoplasma gondii*, *Giardia intestinalis*.

Resumo

O trabalho teve como objetivo determinar os fatores associados à contaminação de vegetais por protozoários zoonóticos. Amostras de água, solo e vegetais foram coletadas de julho/2014 a maio/2016, totalizando 83 amostras de 21 propriedades da região de Londrina, Paraná, Brasil. A amplificação de fragmentos de DNA de *T. gondii*, *Cryptosporidium* spp. e *Giardia intestinalis* foi realizada por meio da reação em cadeia da polimerase (PCR). Os resultados da PCR foram positivos para *T. gondii* em 12,9% (8/62), *Cryptosporidium* spp. em 11,3% (7/62) e *G. intestinalis*. em 25,8% (16/62) das amostras. O sequenciamento de DNA identificou *C. parvum* em cinco amostras e *G. intestinalis*, *Assemblage* E em três amostras. As associações estatísticas evidenciaram maior probabilidade de amostras serem positivas para *T. gondii* ou para pelo menos um dos três protozoários quando a fonte de água de irrigação era o rio; uma maior chance de amostras positivas para *Cryptosporidium* spp. quando havia cervos na propriedade; e uma menor chance das amostras serem positivas para pelo menos um dos três agentes etiológicos quando o solo era suplementado com calcário. Os resultados expõem alguns pontos críticos de contaminação, fornecendo suporte para capacitar os agricultores em boas práticas de gestão durante o processo de produção.

Palavras-chave: Legumes, água, contaminação ambiental, *Cryptosporidium* spp., *Toxoplasma gondii*, *Giardia intestinalis*.

Introduction

Vegetables, including all vegetables grown in gardens, are foods rich in vitamins, carbohydrates, fiber and minerals; are easy to digest; and offer high satiety, a high antioxidant content and a low caloric content. Due to their nutritional importance, vegetables are essential in human nutrition (SINGH et al., 2001; WORTHINGTON, 2001).

In Brazil, vegetables are produced commonly by the conventional farming system; however, in recent years there was an important growth in organic cultivation. It is mainly related to the search for healthier food, with better quality and flavor, environmental preservation and pesticides' free (ARCHANJO et al., 2001). The main differences between organic and conventional cultivation are the use, in the latter, of agricultural pesticides, chemical fertilizers and genetically modified organisms. Organic agriculture excludes these elements from the production process, using natural implements and predators (BOURN & PRESCOTT, 2002; GARCIA & TEIXEIRA, 2017; GOMIERO et al., 2011; WILKINS & HILLERS, 1994).

Contamination of vegetables can occur during production, transportation, storage and commercialization; however, cultivation conditions involving the quality of irrigation water, fertilizer type, the presence of animals in the property and direct contamination from farm workers are the main risk points (ALMEIDA et al., 2013; AMAHMID et al., 1999; CHAIDEZ et al., 2005; DIXON et al., 2013; DIXON, 2016).

Toxoplasmosis is one of the most common parasitic zoonoses in the world, and it can affect all warm-blooded animals, presenting a wide variability in clinical outcomes (DUBEY, 2010). Cryptosporidiosis and giardiasis are zoonoses and are described as important causes of diarrhea (FAYER, 1997; PEDROSO & AMARANTE, 2006). In an immunocompetent individual, these diseases may assume a self-limiting characteristic with clinical manifestations of low expression, but in an immunocompromised patient they may manifest in a severe and prolonged form (MITSUKA-BREGANÓ et al., 2010; PEDROSO & AMARANTE, 2006; SHIKANI & WEISS, 2014). The description of these protozoa in food and waterborne outbreaks (BERGER et al., 2010; CHEUN et al., 2013; MAC KENZIE et al., 1994; SILVA et al., 2005; VAUDAUX et al., 2010; YEUNG et al., 2013) highlights the importance of leafy vegetables, which are consumed *in natura* and facilitate the transmission of these protozoa and other pathogens.

Considering the health benefits of consuming vegetables, their greater inclusion in the diet of people worldwide and their risk as a source of zoonotic protozoa such as *T. gondii*, *Cryptosporidium* spp. and *G. intestinalis*, this work aimed to evaluate the contamination and hygienic-sanitary conditions of their production in organic gardens.

Materials and Methods

The study was carried out from July 2014 to May 2016 on 21 horticultural properties of the municipalities of Apucarana, Marilândia do Sul, Ortigueira, Rolândia and Londrina (District of Guaravera), in the state of Paraná (Figure 1). As an inclusion criterion, small commercial vegetable-producing organic properties were assisted by the Organic Certification Project of the State University of Londrina. On eleven properties, water and vegetable samples were collected, and soil samples were included on the other properties.

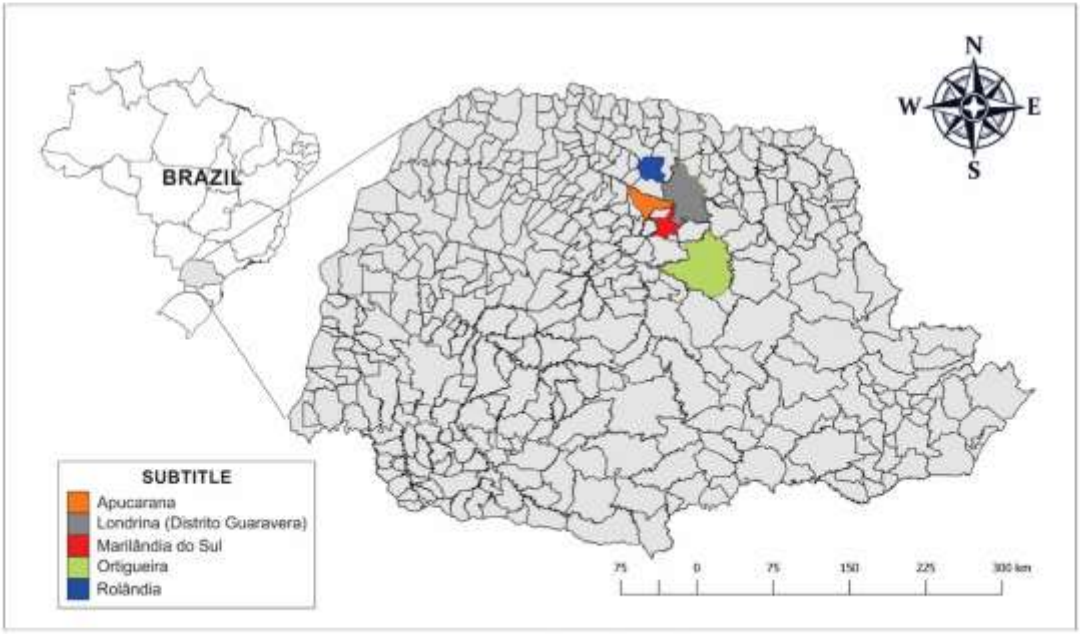


Figure 1. Map of Paraná highlighting the municipalities where samples of vegetables, water and soil were collected and submitted for parasitological and microbiological research from 2014 to 2016, Paraná, Brazil.

Obtaining and analyzing samples of vegetables

Forty-two clumps of leafy vegetables were randomly collected, two per property (from property 1 to 21), packed in plastic bags and kept in refrigeration. For *T. gondii*, *Cryptosporidium* spp. and *G. intestinalis* detection, 50 g of the leaves were washed in 300 mL of 1% Tween 80 extraction solution in a plastic bag under manual shaking for 10 minutes and then filtered into 500 mL glass beakers through two layers of gauze. The wash was divided into conical tubes and subjected twice to centrifugation at 2100 x g for 10 minutes. The pellet was aliquoted into microtubes and stored at -20 °C until DNA extraction.

Obtaining and analyzing soil samples

For *T. gondii*, *Cryptosporidium* spp. and *G. intestinalis* detection, a total of 10 samples of approximately 10 g of soil, collected from the surface, one per property (from property 12 to 21) and stored in 50 mL conical tubes with 30 mL of 1M glycine, were homogenized with the aid of a stirrer for 30 minutes. They were then kept at rest for five minutes for sedimentation. After the pellet was discarded, the supernatant was centrifuged at 1500 x g for 15 minutes. The final concentrate was aliquoted into microtubes and stored at -20 °C until DNA extraction.

Obtaining and analyzing water samples

For the microbiological analysis by means of the chromogenic substrate technique (APHA, 2005), 21 samples of water, one per property (from property 1 to 21), were collected from the irrigation tap of the vegetable gardens, as recommended by the protocol in Brazil (BRASIL, 2013).

For *T. gondii*, *Cryptosporidium* spp. and *G. intestinalis* detection, a total of 10 samples (from property 12 to 21) of 10 mL were collected in clean plastic bottles from irrigation tap. The water was filtered through a cellulose ester membrane with a 47 mm-diameter and 1.2 µm porosity (Millipore®, Billerica, Massachusetts, USA) in a filter holder system using a vacuum pump (4 L/min). After filtration, the material was eluted in 0.1% Tween 80 with the aid of flexible plastic loops (Thermo Fisher Scientific, Massachusetts, USA) (BRANCO et al., 2012). The obtained material was concentrated by centrifugation twice at 1050 x g for 15 min at 4°C. The obtained pellet was stored in microtubes at -20 °C until DNA extraction.

Molecular analyses

The samples were previously submitted to freeze-thaw (5 cycles of freezing at -80 °C and thawing at 56 °C), then DNA extraction was performed using a commercial kit (NucleoSpin Tissue®, Macherey-Nagel, Düren, Germany) in accordance with the manufacturer's instructions and DNA was collected in a final volume of 100 µL. DNA extracted were stored at -20°C until polymerase chain reaction (PCR) processing.

PCR assays for *T. gondii* were performed as previously described by Homan et al. (2000), to amplify a fragment of 529 bp. *Cryptosporidium* spp. were detected using a nested-PCR reaction with primers described by Xiao et al. (1999), which target a fragment of 18S rRNA gene between 826 and 840 bp. For *G. intestinalis* DNA detection, the samples were subjected to nested-PCR to amplify an approximately 300-bp fragment of the 18S rRNA gene from *G. intestinalis* (COKLIN et al., 2007), in addition to 530 bp of the TPI (triose-phosphate isomerase) gene with primers described by Sulaiman et al. (2003) and 432 bp of the GDH (glutamate dehydrogenase) gene with primers described by Read et al. (2004) in a semi-nested PCR.

PCR products were visualized by 1.5% agarose gel electrophoresis stained with SYBR Safe DNA (Invitrogen®, California, USA). In addition, when positive for *Cryptosporidium* spp. and for *G. intestinalis*, the PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany), and the DNA was quantified by a Picodrop (Thermo Fisher Scientific, Wilmington, USA). DNA sequencing was performed with the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, USA) with the corresponding forward or reverse primers on a 3500 genetic analyzer (Applied Biosystems, Carlsbad, USA), according to the instructions of the manufacturer. The sequences obtained were examined with PHRED software (EWING & GREEN, 1998) and inspected with CHROMAS software for quality analysis of chromatograms. Consensus sequences were determined by the CAP3 software (http://asparagin.cenargen.embrapa.br/cgi-bin/phph/cap3.pl), and their identities were compared to the sequences deposited in GenBank using BLAST software (CAMACHO et al., 2009). The nucleotide sequence data reported in this article were deposited in GenBank under the following accession numbers: *Cryptosporidium parvum* (MF353924 to MF353928) and *Giardia intestinalis* (MF425817 to MF425819). The phylogenetic relationships between *G. intestinalis* sequences of the present study and GenBank standard sequences were characterized by the alignment of 390 nucleotides of the GDH coding gene using the maximum likelihood method with 250 bootstraps in the MEGA 4.0 program. The tree was rooted with *Giardia ardeae* (AF069060) and the GenBank sequences used in this tree are standards of assemblages A1 (AY178735), A2 (AY178737), B (AY826193), D (U60986), E (AY178741) and F (AF069057).

Statistical Analysis

A semi-structured questionnaire was applied to all horticulturists participating in the study, who were questioned about the type of planting and fertilization, soil supplementation, presence of animals on the property, irrigation system, toilet and sewage characteristics. The program EpiInfo 3.5.4 (DEAN et al., 1990) was used to tabulate the variables together with the microbiological and molecular results found. Statistical analysis was performed using the EpiInfo programs 3.5.4 and R 3.3.2 (R CORE TEAM, 2013) by means of the Chi-square test or Fisher's exact test, where appropriate. The analysis of these associations with a control of the confounding variables was performed applying simple and multiple logistic regression analysis (HOSMER et al., 2013). Only the variables whose p-values were less than 0.10 in the

screening analysis and had some biological meaning were included in the logistic regression models. The association measurement was obtained by the Odds Ratio (OR) calculation with a 95% confidence interval (CI). The multiple correspondence analysis was performed with program R 3.3.3 (R CORE TEAM, 2013) through the FactoMineR (HAIR et al., 2009) package, an exploratory technique that does not rely on statistical tests but allows the visualization of the most important relationships of a large set of variables among each other (HOFFMAN & FRANKE, 1986). In addition, it can help in visualizing the multivariate relationship between the categories of the different variables, and their geometric proximity in the graph, suggesting the possibility of associations between them.

Results

In total, 83 samples were collected, being 42 of vegetables, 31 of water (21 for microbiological analysis and 10 for parasitological analysis) and 10 of soil; from 21 properties of the municipalities of Apucarana (6/21), Marilândia do Sul (7/21), Ortigueira (6/21), Rolândia (1/21) and Londrina (District of Guaravera) (1/21). Among the leafy vegetables, 17 samples were of crisp lettuce (*Lactuca sativa*), seven of arugula (*Eruca sativa*), nine of chicory (*Cichorium intybus* and *Cichorium endivia*), five of chives (*Allium fistulosum*), two of purple lettuce, one spinach (*Spinacia oleracea*) and one chard (*Beta vulgaris* subsp. *Vulgaris*). On the first eleven properties, water was not submitted for molecular analysis, and no soil samples were collected.

Eighteen properties were organic cultivation certified, and three (14.3%) were in the certification phase. The organic fertilizer source was variable among the properties: 23.8% (5/21) used chicken manure, 14.3% (3/21) used mixed manure, 14.3% (3/21) used chicken litter, 14.3% (3/21) used commercial compounds and 33.3% (7/21) used bovine manure. Eleven (52.3%) of the properties underwent mineral supplementation on a regular basis, six with limestone (54.5%), three with a potassium supplement (27.2%) and one with mineral present in the fertilizer.

Wild birds and domestic animals, such as dogs, cats, horses, and cattle, were present on all the properties studied, and in five (23.8%) of them, the animals had access to the gardens. Wild animals, such as cervids, hare, armadillos, capybara, fox and cougar were present on 13 of the studied properties (61.9%). All the properties were washing their vegetables: 52.4% (11/21) of the properties in treated tap water and 47.6% (10/21) in rinsing tanks. Irrigation in 57.1% (13/21) was automated. The source of irrigation water on 57.1% (12/21) of the properties was spring, 14.3% (3/21) of the properties used untreated river water, and 28.6% (6/21) of the properties used artesian wells.

Of the water samples, 95.2% (20/21) presented total coliforms, with a variation of 1 to > 2419.6 CTNMP/100 mL; for CTT and *Escherichia coli*, 76.2% (16/21) of the samples were positive, ranging from 1 to 218.7 CTTNMP/100 mL.

Regarding the PCR results, 12.9% (8/62) of the samples were positive for *T. gondii*, 11.3% (7/62) for *Cryptosporidium* spp., and 25.8% (16/62) for *G. intestinalis* (two in TPI, seven in GDH, five in 18S rRNA and two in TPI and GDH) (Table 1). It was possible to identify the parasite species by DNA sequencing in

Table 1. Vegetable, water and soil samples positive for the protozoa *Toxoplasma gondii*, *Cryptosporidium* spp. and *Giardia intestinalis* from organic crop properties, from 2014 to 2016 in Paraná, Brazil. Results are based on molecular analyses.

Property	Municipality	*Toxoplasma gondii*			*Cryptosporidium* spp.			*G. intestinalis*		
		Vegetable	Soil	Water	Vegetable	Soil	Water	Vegetable	Soil	Water
1	Marilândia do Sul	-	NT	NT	-	NT	NT	+ Let	NT	NT
2	Marilândia do Sul	+ Let	NT	NT	-	NT	NT	+ Aru	NT	NT
3	Marilândia do Sul	-	NT	NT	-	NT	NT	-	NT	NT
4	Marilândia do Sul	+ Let	NT	NT	+ Aru*	NT	NT	+Let**+Aru**	NT	NT
5	Guaravera	+ Let Aru	NT	NT	-	NT	NT	+ Let**	NT	NT
6	Ortigueira	-	NT	NT	-	NT	NT	-	NT	NT
7	Ortigueira	-	NT	NT	-	NT	NT	-	NT	NT
8	Ortigueira	-	NT	NT	-	NT	NT	-	NT	NT
9	Ortigueira	-	NT	NT	-	NT	NT	-	NT	NT
10	Ortigueira	-	NT	NT	-	NT	NT	-	NT	NT
11	Ortigueira	-	NT	NT	-	NT	NT	+ Chic	NT	NT
12	Rolândia	-	+	-	-	-	-	+ Chic	-	-
13	Apucarana	-	-	+	-	-	-	+ Chic	+	-
14	Apucarana	-	-	-	-	-	+*	-	-	+
15	Apucarana	-	-	-	-	-	-	+ Let	-	-
16	Apucarana	-	+	+	-	+*	+*	+ Chiv	+	+
17	Apucarana	-	-	-	-	-	+*	-	-	+
18	Marilândia do Sul	-	-	-	-	-	-	+ Cha	-	-
19	Marilândia do Sul	-	-	-	-	-	-	-	-	-
20	Marilândia do Sul	-	-	-	+ Let	-	-	-	-	-
21	Apucarana	-	-	-	-	+	-	-	-	-

NT: Not tested; + positive sample; - negative sample; Let: Lettuce; Aru: Arugula; Cha: Chard; Chic: Chicory; Chiv: Chive. **C. parvum* or ***G. intestinalis* Assemblage E confirmed by the DNA results from Sanger sequencing.

seven of the positive samples. *C. parvum* was the species present in three water samples and one soil sample sample showing 100% of similarity with other sequences from GenBank. *G. intestinalis* Assemblage E was present in two vegetable samples showing 99% of similarity with other sequences from GenBank. In order to confirm the *G. intestinalis* Assemblage, the phylogenetic analysis with the partial sequences of the GDH gene of *G. intestinalis* obtained was performed, a clustering of samples with standard sequences of Assemblage E (Figure 2), commonly associated with giardiasis in cattle, was observed. In other PCR-positive samples, there was no success in sequencing due to the low amount of DNA.

Factors associated with the presence of DNA of zoonotic protozoa such as *T. gondii*, *Cryptosporidium* spp. and *G. intestinalis* in vegetables, soil and water are listed in Tables 2, 3 and 4.

The graph generated by the multiple correspondence analysis (Figure 3) confirmed the main findings of this study related to the risk factors for the presence of zoonotic protozoa in vegetables, water and soil of properties with organic production systems. It is shown in dimension one of the graph (x-axis) that the categories of negative variables for *T. gondii*, *Cryptosporidium* spp. and *G. intestinalis* and at least one of the three zoonotic protozoa in question are on the left along with the categories of limestone supplementation, spring water source and absence of cervids. On the other hand, on the right side of the graph we have the categories of positive variables for *T. gondii*, *G. intestinalis*, *Cryptosporidium* spp. and at least one of the three zoonotic protozoa together with the categories of potassium and fertilizer supplementation, river water source and the presence of cervids. The interpretation of

Table 2. Characteristics of the organic vegetable producing properties of northern Paraná, Brazil, significantly associated with the occurrence of *Toxoplasma gondii* and *Giardia intestinalis*.

Toxoplasma gondii	**Positive/Total (%)**	**Univariate Logistic Regression**	
		p-Value	**O.R. Gross (CI 95%)**
Water Source		< 0.05	
Spring	1/34 (2.9)		1.00
Well	4/22 (18.2)		7.11 (0.74 - 68.57)
River	3/6 (50.0)		32.00 (2.49 - 411.45)
G. intestinalis	**Positive/Total (%)**	**p-Value**	**O.R. Gross (CI 95%)**
Cryptosporidium spp. positive		< 0.05	
No	11/55 (20.0)		1.00
Yes	5/7 (71.4)		10.00 (1.71 - 58.59)

Table 3. Characteristics of the organic vegetable producing properties of northern Paraná, Brazil, significantly associated with the occurrence of *Cryptosporidium* spp.

	Positive/Total (%)	**Univariate Logistic Regression**		**Multiple Logistic Regression**	
		p-Value	**O.R. Gross (CI 95%)**	**p-Value**	**O.R. Adjusted (CI 95%)**
Presence of cervids		< 0.05		< 0.05	
No	3/52 (5.7)		1.00		1.00
Yes	4/10 (40.0)		10.89 (1.95 - 60.83)		7.87 (1.22 - 50.64)
***Giardia intestinalis* positive**		< 0.05		< 0.05	
No	2/46 (4.3)		1.00		1.00
Yes	5/16 (31.2)		10.00 (1.71 - 58.59)		7.51 (1.20 - 51.44)

Table 4. Characteristics of the organic vegetable producing properties of northern Paraná, Brazil, significantly associated with the occurrence of at least one of the protozoa (*Toxoplasma gondii*, *Cryptosporidium* spp. or *G. intestinalis*).

	Positive/Total (%)	**Univariate Logistic Regression**	
		p-Value	**O.R. Gross (CI 95%)**
Type of supplement		< 0.05	
Not supplemented	15/38 (39.5)		1.00
Limestone	1/16 (6.2)		0.10 (0.01 - 0.85)
Potassium Base	4/6 (66.7)		3.07 (0.50 - 18.89)
Water source		< 0.05	
Spring	7/34 (20.6)		1.00
Well	10/22 (45.4)		3.21 (0.98 - 10.47)
River	5/6 (83.3)		19.28 (1.92 - 192.82)

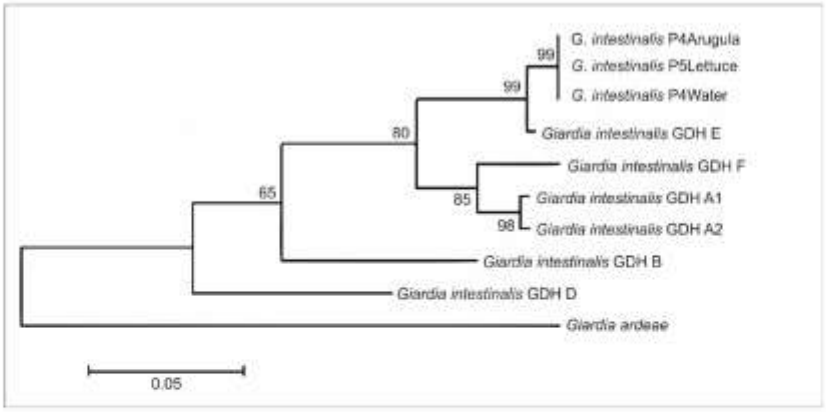


Figure 2. Phylogenetic relationships among *G. intestinalis* isolates characterized by the alignment of 390 nucleotides of the GDH coding gene using the maximum likelihood method with 250 bootstraps in the MEGA 4.0 program. The tree is rooted with *Giardia ardeae* (AF069060), and the GenBank sequences used in this tree are standards of the assemblies A1 (AY178735), A2 (AY178737), B (AY826193), D (U60986), E (AY178741) and F (AF069057).

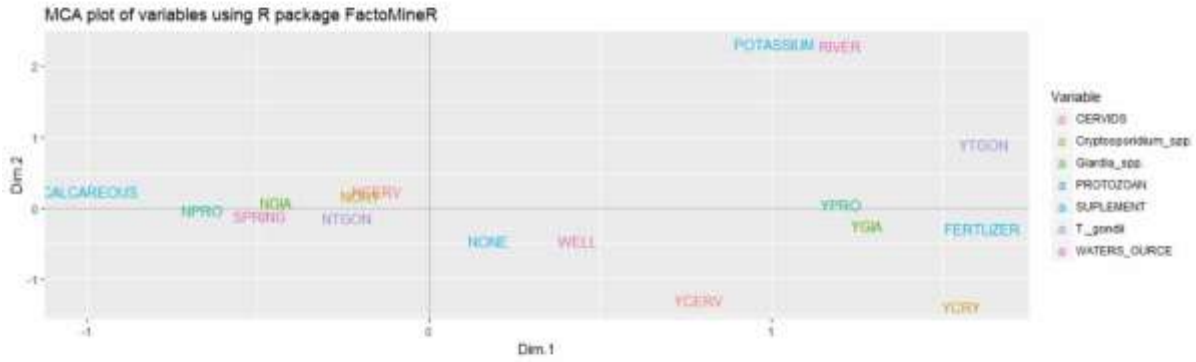


Figure 3. Graph resulting from the multiple correspondence analysis. Presence of cervids (NCERV, YCERV); positive results of *T. gondii* (NTGON, YTGON), *Cryptosporidium* spp. (NCRY, YCRY), *Giardia* spp. (NGIA, YGIA), and one of the three protozoa (NPRO, YPRO); water source (SPRING, WELL, RIVER) and supplement used in growing (LIMESTONE, NONE, POTASSIUM and FERTILIZER).

dimension two (y-axis) of the graph was not possible due to the lack of biological meaning.

Discussion

Resolution 357/05 of CONAMA (National Council for the Environment) (BRASIL, 2005) recommends that for the irrigation of vegetables and fruits with development on the ground, the Thermotolerant coliforms (CTT) count should not exceed 200/100 mL; *Escherichia coli* is the main species of the CTT group, whose exclusive habitat is the intestine (BRASIL, 2005). *E. coli* was present in 76.2% (16/21) of the analyzed water samples, and of these, 18.7% (3/16), one of spring (209.8 CTNMP/100mL), one of river (328.2 CTNMP/100mL) and another of artesian well (201.2 CTNMP/100mL), with higher counts than that allowed by the resolution. The use of faecal indicators, particularly, *E. coli*, is a better practical approach to identify contaminated water sources or food (ALLENDE & MONAGHAN, 2015; NATARO & KAPER, 1998). Recent studies associate the presence of parasites such as *G. intestinalis* and *Cryptosporidium* spp. to the presence of CTT (TOLEDO et al., 2017) in the water, indicating the necessity of monitoring parasite presence in vegetable gardens for the production of innocuous vegetables.

Regarding the PCR results, 12.9% (8/62) of the samples had DNA fragments compatible with *T. gondii*, and of these, 50.0% (4/8) were vegetables, 25.0% (2/8) were water and 25.0% (2/8) were soil. Lass et al. (2012) studied *T. gondii* in 216 fruits and vegetables in Poland and observed a frequency of 9.7% positive samples. The eight positive samples came from six properties, of which 50.0% (3/6) had cats, all with access to the cultivation area, suggesting direct contamination of vegetables by definitive hosts on these properties (FRENKEL et al., 1970). In vegetable gardens that did not have cats (3/6), the source of irrigation water from the river may have influenced the contamination due to the flow during rains, when the river receives several fecal pathogens

domestic animals to gardens, and contaminated irrigation water (BERALDO & FARACHE, 2011; CHAIDEZ et al., 2005), the latter was a factor associated with the presence on the samples of at least one of the agents. River water, in turn, had a greater number of positive samples than expected; the use of this type of water for irrigation is a big problem in Brazil (MAROUELLI & SILVA, 1998), because a significant number of rivers are polluted, probably due to the extensive area of agriculture and livestock around the surface water sources and the contamination by untreated municipal effluents, mainly sewage, constituting another important vehicle of transmission and dissemination of pathogens (AMORÓS et al., 2010; BERALDO & FARACHE, 2011; DIXON et al., 2013; MAROUELLI & SILVA, 1998; SILVA et al., 2005). Toledo et al. (2017) in a study in dairy farms in Paraná, Brazil, observed that the absence of vegetation and protective structures around the springs was associated with the frequency of positive water samples to *Cryptosporidium* spp. and *G. intestinalis*. The vegetation around the springs acts as a physical barrier, thus reducing the drainage of animal waste and other contaminants (TOLEDO et al., 2017). Most of the springs visited in this study were protected, which may have been an important factor for a smaller number of positive samples than expected in this source.

Of the 12 properties with at least one positive sample for one of the three agents, 66.7% (8/12) produced their own fertilizer with bovine manure or chicken litter, and of these, 87.5% (7/8) did not respect the period of composting. The maturity of the compost is related to the complete microbiological decomposition and the transformation of the organic matter into humus, and this process occurs after approximately 90 days of tanning; correct composting can promote the inactivation of some pathogens (DÉPORTES et al., 1998; KIEHL, 1998; VINNERÅS et al., 2003). Inadequate fertilizer production, in addition to causing contamination of vegetables and water, prevents the inactivation of pathogens present in the raw material, making the fertilizer unfit for use in edible vegetables.

Conclusion

The control of parasitological contamination of raw vegetables is a major challenge, since contamination can occur in all the stages that precede arrival to the consumers' table. The results indicate that inadequate hygienic-sanitary conditions in organic gardens, as well as the risk of infection by protozoa are of public health importance. In addition, the results expose some of the points of contamination risk, such as the water source, soil supplementation and presence of animals on properties. Thus, there is a need to raise awareness among producers and consumers about the implementation of good handling practices during the cultivation process and before the time of consumption.

Acknowledgements

We would like to acknowledge the CNPQ (National Research Council) for granting the doctoral scholarship.

References

Albuquerque JA, Bayer C, Ernani PR, Mafra AL, Fontana EC. Aplicação de calcário e fósforo e estabilidade da estrutura de um solo ácido. *Rev Bras Ciênc Solo* 2003; 27(5): 799-806. http://dx.doi.org/10.1590/S0100-06832003000500004.

Allende A, Monaghan J. Irrigation water quality for leafy crops: a perspective of risks and potential solutions. *Int J Environ Res Public Health* 2015; 12(7): 7457-7477. http://dx.doi.org/10.3390/ijerph120707457. PMid:26151764.

Al Mawly J, Grinberg A, Velathanthiri N, French N. Cross sectional study of prevalence, genetic diversity and zoonotic potential of *Cryptosporidium parvum* cycling in New Zealand dairy farms. *Parasit Vectors* 2015; 8(240): 1-7. http://dx.doi.org/10.1186/s13071-015-0855-9. PMid:25896433.

Almeida A, Pozio E, Cacciò SM. Genotyping of *Giardia duodenalis* cysts by new real-time PCR assays for detection of mixed infections in human samples. *Appl Environ Microbiol* 2010; 76(6): 1895-1901. http://dx.doi.org/10.1128/AEM.02305-09. PMid:20080999.

Almeida AJ, Lima VS, Rodrigues ABF, Di Filippo PA. Contamination with *Cryptosporidium* spp. in manure used as fertilizer in urban gardens. *Rev Port Ciênc Vet* 2013; 108(585-586): 23-27.

Amahmid O, Asmama S, Bouhoum K. The effect of waste water reuse in irrigation on the contamination level of food crops by *Giardia* cysts and *Ascaris* eggs. *Int J Food Microbiol* 1999; 49(1-2): 19-26. http://dx.doi.org/10.1016/S0168-1605(99)00058-6. PMid:10477066.

Amorós I, Alonso JL, Cuesta G. *Cryptosporidium* oocysts and *Giardia* cysts on salad products irrigated with contaminated water. *J Food Prot* 2010; 73(6): 1138-1140. http://dx.doi.org/10.4315/0362-028X-73.6.1138. PMid:20537274.

APHA. *Standard methods for the examination of water and wastewater.* Washington, DC; 2005.

Archanjo LR, Brito KFW, Sauerbeck S. Alimentos orgânicos em Curitiba: consumo e significado. *Rev Cad Debate* 2001; 8: 1-6.

Benenson MW ,Takafuji ET ,Lemon SM ,Greenup RL ,Sulzer AJ .Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. *N Engl J Med* 1982; 307(11): 666-669. http://dx.doi.org/10.1056/NEJM198209093071107. PMid:7110216.

Beraldo RM, Farache A Fo. Bacteriological quality of irrigation water from vegetable gardens in the Municipalities of Araraquara, Boa Esperança do Sul and Ibitinga, SP. *Aliment Nutr* 2011; 22(3): 345-350.

Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, Hand P, et al. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ Microbiol* 2010; 12(9): 2385-2397. http://dx.doi.org/10.1111/j.1462-2920.2010.02297.x. PMid:20636374.

Bowie WR, King AS, Werker DH, Isaac-Renton JL, Bell A, Eng SB, et al. Outbreak of toxoplasmosis associated with municipal drinking water. *Lancet* 1997; 350(9072): 173-177. http://dx.doi.org/10.1016/S0140-6736(96)11105-3. PMid:9250185.

Bourn D, Prescott J. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Crit Rev Food Sci Nutr* 2002; 42(1): 1-34. http://dx.doi.org/10.1080/10408690290825439. PMid:11833635.

Branco N, Leal DAG, Franco RMB. A parasitological survey of natural water springs and inhabitants of a tourist city in southeastern Brazil. *Vector*

1

Borne Zoonotic Dis 2012; 12(5): 410-417. http://dx.doi.org/10.1089/vbz.2011.0679. PMid:22217166.

Brasil. Ministério da Saúde, Fundação Nacional de Saúde, Departamento de Saúde Ambiental, Coordenação de Controle da Qualidade da Água. *Manual prático de análise de água*. [Online]. 4. ed. Brasília: FUNASA; 2013. [cited 2018 Jun 13]. Available from: http://www.funasa.gov.br/site/wp-content/files_mf/manual_pratico_de_analise_de_agua_2.pdf

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. *BMC Bioinformatics* 2009; 10(1): 421. http://dx.doi.org/10.1186/1471-2105-10-421. PMid:20003500.

Capizzi-Banas S, Deloge M, Remy M, Schwartzbrod J. Liming as an advanced treatment for sludge sanitisation: helminth eggs elimination - *Ascaris* eggs as model. *Water Res* 2004; 38(14-15): 3251-3258. http://dx.doi.org/10.1016/j.watres.2004.04.015. PMid:15276741.

Chaidez C, Soto M, Gortares P, Mena K. Occurrence of *Cryptosporidium* and *Giardia* in irrigation water and its impact on the fresh produce industry. *Int J Environ Health Res* 2005; 15(5): 339-345. http://dx.doi.org/10.1080/09603120500289010. PMid:16416751.

Cheun H-I, Kim C-H, Cho S-H, Ma D-W, Goo B-L, Na MS, et al. The first outbreak of giardiasis with drinking water in Korea. *Osong Public Health Res Perspect* 2013; 4(2): 89-92. http://dx.doi.org/10.1016/j.phrp.2013.03.003. PMid:24159537.

Coklin T, Farber J, Parrington L, Dixon B. Prevalence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in dairy cattle in Ontario, Canada. *Vet Parasitol* 2007; 150(4): 297-305. http://dx.doi.org/10.1016/j.vetpar.2007.09.014. PMid:17964724.

Brasil. Conselho Nacional do Meio Ambiente - CONAMA. Resolução nº 357 de 18 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. [online]. *Diário Oficial da República Federativa do Brasil*, Brasília, 2005 [cited 2018 Feb 10]. Available from: http://www.mma.gov.br/port/conama/res/res05/res35705.pdf

Dean AG, Dean JA, Burton AH, Discker RC. Epi-Info, Version 5: a word processing, database, and statistics programme for epidemiology on microcomputers. Atlanta, GA USD Inc.; 1990.

Déportes I, Benoit-Guyod JL, Zmirou D, Bouvier MC. Microbial disinfection capacity of municipal solid waste (MSW) composting. *J Appl Microbiol* 1998; 85(2): 238-246. http://dx.doi.org/10.1046/j.1365-2672.1998.00484.x. PMid:9750296.

Dixon BR. Parasitic illnesses associated with the consumption of fresh produce - an emerging issue in developed countries. *Curr Opin Food Sci* 2016; 8: 104-109. http://dx.doi.org/10.1016/j.cofs.2016.04.009.

Dixon B, Parrington L, Cook A, Pollari F, Farber J. Detection of *Cyclospora*, *Cryptosporidium*, and *Giardia* in Ready-to-Eat Packaged Leafy Greens in Ontario, Canada. *J Food Prot* 2013; 76(2): 307-313. http://dx.doi.org/10.4315/0362-028X.JFP-12-282. PMid:23433379.

Dubey JP, Sharma SP, Juranek DD, Sulzer AJ, Teutsch SM. Characterization of *Toxoplasma gondii* isolates from an outbreak of toxoplasmosis in Atlanta, Georgia. *Am J Vet Res* 1981; 42(6): 1007-1010. PMid:7283229.

Dubey JP. *Toxoplasmosis of Animals and Humans*. Maryland: CRC Press; 2010.

Erdogrul O, Sener H. The contamination of various fruit and vegetable with *Enterobius vermicularis*, *Ascaris* eggs, *Entamoeba histolyca* cysts and *Giardia* cysts. *Food Control* 2005; 16(6): 559-562.

Ernani PR, Bayer C, Maestri L. Corn yield as affected by liming and tillage system on an acid Brazilian Oxisol. *Agron J* 2002 ;94(2 :)305-309. http://dx.doi.org/10.2134/agronj2002.0305.

Ewald MPC, Martins FDC, Caldart ET, Vieira FEG, Yamamura MH, Sasse JP, et al. The first study of molecular prevalence and species characterization of *Cryptosporidium* in free-range chicken (*Gallus gallus domesticus*) from Brazil. *Rev Bras Parasitol Vet* 2017; 26(4): 472-478. http://dx.doi.org/10.1590/s1984-29612017068. PMid:29185602.

Ewing B, Green P. Base-calling of automated sequencer traces using phred. II Error probabilities. *Genome Res* 1998; 8(3): 186-194. http://dx.doi.org/10.1101/gr.8.3.186. PMid:9521922.

Ey PL, Mansouri M, Kulda J, Nohýnková E, Monis PT, Andrews RH, et al. Genetic analysis of *Giardia* from hoofed farm animals reveals artiodactyl-specific and potentially zoonotic genotypes. *J Eukaryot Microbiol* 1997; 44(6): 626-635. http://dx.doi.org/10.1111/j.1550-7408.1997.tb05970.x. PMid:9435134.

Guilherme AL, Araújo SM, Falavigna DL, Pupulim AR, Dias ML, Oliveira HS, et al. Prevalência de enteroparasitas em horticultores e hortaliças da Feira do Produtor de Maringá, Paraná. *Rev Soc Bras Med Trop* 1999; 32(4): 405-411. http://dx.doi.org/10.1590/S0037-86821999000400012. PMid:10495671.

Falavigna LM, Freitas CBR, Melo GC, Nishi L, Araújo SM, Falavigna-Guilherme AL. Qualidade de hortaliças comercializadas no noroeste do Paraná, Brasil. *Parasitol Latinoam* 2005; 60(3-4): 144-149.

Fayer R. *Cryptosporidium and Cryptosporidiosis*. Maryland: CRC Press; 1997.

Franco RMB, Branco N, Leal DAG. Parasitologia Ambiental: Métodos de concentração e detecção de *Cryptosporidium* spp. e *Giardia* spp. em amostras de água. *Rev Patol Trop* 2012; 41(2): 119-135. http://dx.doi.org/10.5216/rpt.v41i2.19320.

Frenkel JK, Dubey JP, Miller NL. *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. *Science* 1970; 167(3919): 893-896. http://dx.doi.org/10.1126/science.167.3919.893. PMid:4903651.

Furtado C, Adak GK, Stuart JM, Wall PG, Evans HS, Casemore DP. Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992-5. *Epidemiol Infect* 1998; 121(1): 109-119. http://dx.doi.org/10.1017/S0950268898001083. PMid:9747762.

Garcia JM, Teixeira P. Organic versus conventional food: A comparison regarding food safety. *Food Rev Int* 2017; 33(4): 424-446. http://dx.doi.org/10.1080/87559129.2016.1196490.

Geurden T, Vercruysse J, Claerebout E. Is *Giardia* a significant pathogen in production animals? *Exp Parasitol* 2010; 124(1): 98-106. http://dx.doi.org/10.1016/j.exppara.2009.03.001. PMid:19285075.

Gomiero T, Pimentel D, Paoletti MG. Environmental Impact of Different Agricultural Management Practices: Conventional vs. Organic Agriculture. *Crit Rev Plant Sci* 2011; 30(1-2): 95-124. http://dx.doi.org/10.1080/07352689.2011.554355.

Hair JF, Black WC, Babin BJ, Anderson RE, Tatham RL. *Análise multivariada de dados*. Porto Alegre: Bookman Editora; 2009.

Hamnes IS, Gjerde B, Robertson L, Vikøren T, Handeland K. Prevalence of *Cryptosporidium* and *Giardia* in free-ranging wild cervids in Norway. *Vet Parasitol* 2006; 141(1-2): 30-41. http://dx.doi.org/10.1016/j.vetpar.2006.05.004. PMid:16797126.

Helmy YA, Krücken J, Abdelwhab ESM, von Samson-Himmelstjerna G, Hafez HM. Molecular diagnosis and characterization of *Cryptosporidium* spp. in turkeys and chickens in Germany reveals evidence for previously

1

undetected parasite species. *PLoS One* 2017; 12(6): e0177150. http://dx.doi.org/10.1371/journal.pone.0177150. PMid:28575116.

Hoffman DL, Franke GR. Correspondence analysis: graphical representation of categorical data in marketing research. *J Mark Res* 1986; 23(3): 213-227. http://dx.doi.org/10.2307/3151480.

Homan WL, Vercammen M, De Braekeleer J, Verschueren H. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. *Int J Parasitol* 2000; 30(1): 69-75. http://dx.doi.org/10.1016/S0020-7519(99)00170-8. PMid:10675747.

Hosmer DW Jr, Lemeshow S, Sturdivant RX. *Applied logistic regression*. New Jersey: John Wiley & Sons; 2013. http://dx.doi.org/10.1002/9781118548387.

Hunter PR, Thompson RCA. The zoonotic transmission of *Giardia* and *Cryptosporidium*. *Int J Parasitol* 2005; 35(11-12): 1181-1190. http://dx.doi.org/10.1016/j.ijpara.2005.07.009. PMid:16159658.

Kiehl EJM. *Manual de compostagem: maturação e qualidade do composto*. Piracicaba; 1998.

Lass A, Pietkiewicz H, Szostakowska B, Myjak P. The first detection of *Toxoplasma gondii* DNA in environmental fruits and vegetables samples. *Eur J Clin Microbiol Infect Dis* 2012; 31(6): 1101-1108. http://dx.doi.org/10.1007/s10096-011-1414-8. PMid:21948336.

Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, et al. Massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N Engl J Med* 1994; 331(3): 161-167. http://dx.doi.org/10.1056/NEJM199407213310304. PMid:7818640.

Marouelli WA, Silva HR. *Aspectos sanitários da água para fins de irrigação*. [online]. Brasília: EMBRAPA; 1998. (Comunicado Técnico; vol. 5). [cited 2017 Dec 10]. Available from: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/107307/1/Aspectos-sanitarios-da-agua-para-fins.pdf.

Mitsuka-Breganó R, Lopes-Mori FMR, Navarro IT. *Toxoplasmose adquirida na gestação e congênita: vigilância em saúde, diagnóstico, tratamento e condutas*. Londrina: Eduel; 2010. http://dx.doi.org/10.7476/9788572166768.

Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998; 11(1): 142-201. PMid:9457432.

Pedroso RF, Amarante MK. Giardíase: aspectos parasitológicos e imunológicos. *Biosaúde* 2006; 8(1): 61-71.

Plutzer J, Ongerth J, Karanis P. Giardia taxonomy, phylogeny and epidemiology: facts and open questions. *Int J Hyg Environ Health* 2010; 213(5): 321-333. http://dx.doi.org/10.1016/j.ijheh.2010.06.005. PMid:20619729.

Poulton M, Colbourne J, Dennis PJ. Thames water's experiences with *Cryptosporidium*. *Water Sci Technol* 1991; 24(2): 21-26. http://dx.doi.org/10.2166/wst.1991.0023.

Prado RM, Fernandes FM. Escória de siderurgia e calcário na correção da acidez do solo cultivado com cana-de-açúcar em vaso. *Sci Agric* 2000; 57(4): 739-744. http://dx.doi.org/10.1590/S0103-90162000000400023.

R Core Team. *A language and environment for statistical computing* [online]. 2013. [cited 2017 Dec 10] Available from: http//www.R-project.org

Ranjbar-Bahadori S, Mostoophi A, Shemshadi B. Study on *Cryptosporidium* contamination in vegetable farms around Tehran. *Trop Biomed* 2013; 30(2): 193-198. PMid:23959484.

Read C, Monis P, Andrewthompson R. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. *Infect Genet Evol* 2004; 4(2): 125-130. http://dx.doi.org/10.1016/j.meegid.2004.02.001. PMid:15157630.

Ryan U, Fayer R, Xiao L. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology* 2014; 141(13): 1667-1685. http://dx.doi.org/10.1017/S0031182014001085. PMid:25111501.

Santín M. Clinical and subclinical infections with *Cryptosporidium* in animals. *N Z Vet J* 2013; 61(1): 1-10. http://dx.doi.org/10.1080/00480169.2012.731681. PMid:23134088.

Shikani H, Weiss LM. Human cryptosporidiosis: a clinical perspective. In: Cacció SM, Widmer G. *Cryptosporidium: parasite and disease*. 1st ed. New York: Springer; 2014. p. 383-421. http://dx.doi.org/10.1007/978-3-7091-1562-6_9.

Singh G, Kawatra A, Sehgal S. Nutritional composition of selected green leafy vegetables, herbs and carrots. *Plant Foods Hum Nutr* 2001; 56(4): 359-364. http://dx.doi.org/10.1023/A:1011873119620. PMid:11678441.

Silva CGM, Andrade SAC, Stamford TLM. Occurrence of *Cryptosporidium* spp. and others parasites in vegetables consumed in natura, Recife, Brazil. *Cien Saude Colet* 2005; 10(Suppl): 63-69. http://dx.doi.org/10.1590/S1413-81232005000500009.

Slifko TR, Smith HV, Rose JB. Emerging parasite zoonoses associated with water and food. *Int J Parasitol* 2000; 30(12-13): 1379-1393. http://dx.doi.org/10.1016/S0020-7519(00)00128-4. PMid:11113263.

Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, et al. Triosephosphate Isomerase Gene Characterization and Potential Zoonotic Transmission of *Giardia duodenalis*. *Emerg Infect Dis* 2003; 9(11): 1444-1452. http://dx.doi.org/10.3201/eid0911.030084. PMid:14718089.

Toledo RDS, Martins FDC, Ferreira FP, de Almeida JC, Ogawa L, Dos Santos HL, et al. *Cryptosporidium* spp. and *Giardia* spp. in feces and water and the associated exposure factors on dairy farms. *PLoS One* 2017; 12(4): e0175311. http://dx.doi.org/10.1371/journal.pone.0175311. PMid:28403147.

Vaudaux JD, Muccioli C, James ER, Silveira C, Magargal SL, Jung C, et al. Identification of an atypical strain of *Toxoplasma gondii* as the cause of a waterborne outbreak of toxoplasmosis in Santa Isabel do Ivai, Brazil. *J Infect Dis* 2010; 202(8): 1226-1233. http://dx.doi.org/10.1086/656397. PMid:20836703.

Vinnerås B ,Björklund A ,Jönsson H .Thermal composting of faecal matter as treatment and possible disinfection method - Laboratory-scale and pilot-scale studies. *Bioresour Technol* 2003; 88(1): 47-54. http://dx.doi.org/10.1016/S0960-8524(02)00268-7. PMid:12573563.

Wells B, Shaw H, Hotchkiss E, Gilray J, Ayton R, Green J, et al. Prevalence, species identification and genotyping *Cryptosporidium* from livestock and deer in a catchment in the Cairngorms with a history of a contaminated public water supply. *Parasit Vectors* 2015; 8(1): 66. http://dx.doi.org/10.1186/s13071-015-0684-x. PMid:25650114.

Wilkins JL, Hillers VN. Influences of pesticide residue and environmental concerns on organic food preference among food cooperative members and non-members in Washington State. *J Nutr Educ* 1994; 26(1): 26-33. http://dx.doi.org/10.1016/S0022-3182(12)80831-4.

Worthington V. Nutritional quality of organic versus conventional fruits, vegetables, and grains. *J Altern Complement Med* 2001; 7(2): 161-173. http://dx.doi.org/10.1089/107555301750164244. PMid:11327522.

5 ARTIGO C

Biotemas, 32 (3): 43-50, setembro de 2019
ISSNe 2175-7925
http://dx.doi.org/10.5007/2175-7925.2019v32n3p43

Organic horticulture: a current demand, whose proper management is the only guarantee of safe food

Fernanda Pinto-Ferreira *
Roberta Lemos Freire
Eloiza Teles Caldart
Aline Ticiani Pereira Paschoal
Gabriela Bahr Arias
Winni Alves Ladeia
Regina Mitsuka-Breganó
Italmar Teodorico Navarro

Universidade Estadual de Londrina
Rodovia Celso Garcia Cid, PR 445- Km 388
Caixa Postal 10.011-000, CEP 86057-970, Londrina – PR, Brasil
* Autor para correspondência
nandaferreiravet@gmail.com

Submetido em 23/11/2018
Aceito para publicação em 06/05/2019

Resumo

Horticultura orgânica: uma demanda atual, cujo manejo adequado é a única garantia de alimento seguro. O estudo foi realizado entre julho de 2014 e maio de 2016, em 21 propriedades hortícolas do estado do Paraná, Brasil. Foram coletadas duas amostras de vegetais folhosos e uma amostra de água de irrigação por propriedade. As amostras de água foram analisadas pela técnica do substrato cromogênico para avaliar a contaminação por coliformes totais e *Escherichia coli*, e os vegetais foram avaliados pelas técnicas de Willis (1921), Hoffman et al. (1934), Faust et al. (1939) e quanto à contaminação por parasitas. Observou-se presença de *E. coli* em 80,95% (17/21) das amostras de água; com relação aos vegetais, 19 (45,23%) continham pelo menos uma espécie de parasita, tais como: ancilostomatideos, *Chilomatix* spp., *Dipillidium* spp., *Entamoeba* spp., *Strongyloides* spp., *Trichuris* spp., larva de vida livre, larva de nematódeo, oocisto não-esporulado. Houve associação estatística entre o destino do esgoto (fossa seca) e a positividade aos parasitas. Os dados mostram contaminação fecal em número significativo de amostras e confirmam a necessidade de maiores exigências sanitárias durante o cultivo de hortaliças folhosas, que, na maioria das vezes, são consumidas cruas.

Palavras-chave: *Escherichia coli*; Helminto; Parasita; Protozoário

Abstract

The objective of this study was to evaluate the contamination by *Escherichia coli* in irrigation water and parasites in leafy vegetables cultivated in small organic horticultural properties and to investigate the critical points

Abstract

The study was carried out between July 2014 and May 2016 in 21 horticultural properties in the state of Paraná, Brazil. Two samples of leafy vegetables and one sample of irrigation water were collected per property. Water samples were analyzed by the chromogenic substrate technique to evaluate contamination by total coliforms and *Escherichia coli*, and the vegetables were evaluated by the techniques of Willis (1921), Hoffman et al. (1934) and Faust et al. (1939) for parasite contamination. The presence of *E. coli* was observed in 80.95% (17/21) of the water samples; with respect to vegetables, 19 (45.23%) contained at least one parasite species, such as: hookworms, *Chilomatix* spp., *Dipillidium* spp., *Entamoeba* spp., *Strongyloides* spp., *Trichuris* spp., free-nematode larva, non-sporulated oocyst. There was a statistical association between the fate of the sewage (dry sewage) and the positivity to the parasites. The data show fecal contamination in a significant number of samples and confirm the need for greater sanitary requirements during the cultivation of leafy vegetables, which are mostly consumed raw.

Key words: *Escherichia coli*; Helminth; Parasite; Protozoan

Título resumido: Safe food: points of risk in organic agriculture

Introduction

Organic products have been preferred by consumers because these products are associated with a production system that avoids or excludes the use of industrialized chemicals such as synthetic fertilizers, growth regulators and pesticides or agrochemicals (SANTANA et al., 2006; ABREU et al., 2016). On the other hand, there is growing concern about the risk of infection by enteroparasites, since many vegetables are served raw for human consumption, thereby contributing to the oral transmission of these parasites (ESTEVES; FIGUEIRÔA, 2012).

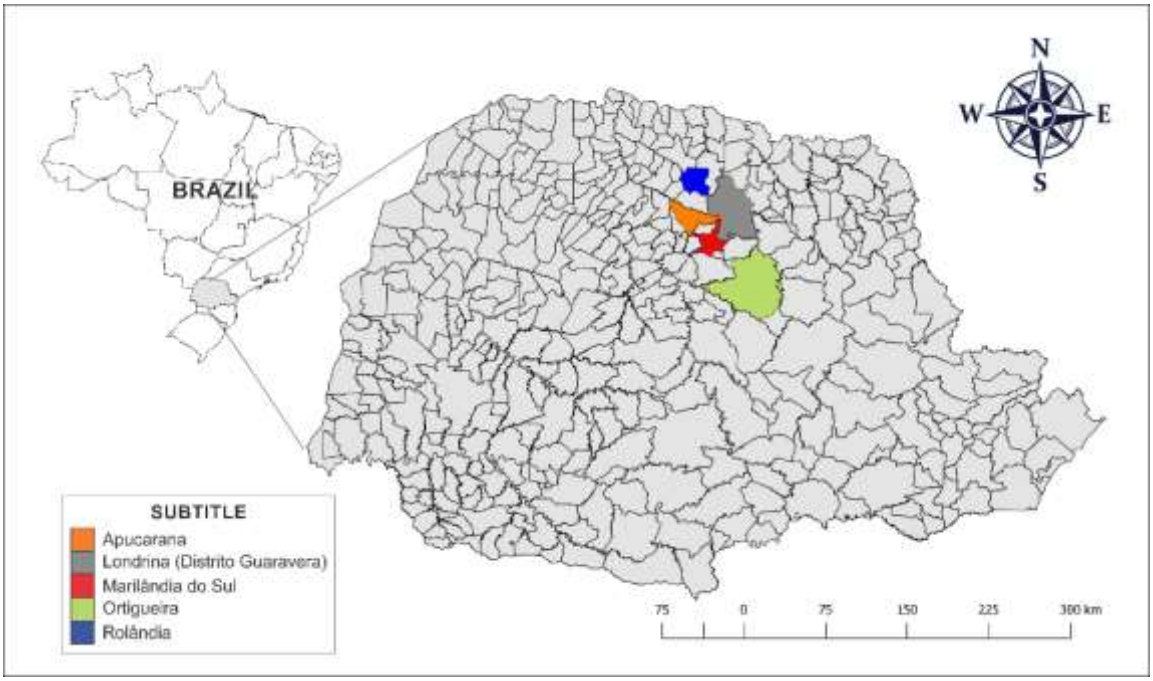
Endoparasites are cosmopolitan and endemic in third-world countries and represent an important problem in public health. They are acquired by ingestion of infective forms contained in contaminated food or water (CACCIÒ et al., 2018). When consumed raw, vegetables can serve as a transmission via (VOLLKOPF et al., 2006; FERREIRA et al., 2018). The cultivation conditions, including quality of irrigation water, the type of fertilizer used, the storage conditions, transport and handling of the crop by the producer are directly related to this contamination (PACHECO et al., 2003).

The objective of this study was to evaluate the contamination by *Escherichia coli* in irrigation water and parasites in leafy vegetables cultivated in small organic horticultural properties and to investigate the critical points during cultivation.

Material and Methods

The study was carried out from July 2014 to May 2016 at horticultural properties in the municipalities of Apucarana, Marilândia do Sul, Ortigueira, Rolândia and Londrina (District Guaravera), in the state of Paraná (Figure 1). Small commercial organic leafy vegetable production properties assisted by the Organic Certification Project of the State University of Londrina were included in analyses.

FIGURA 1: Map of the state of Paraná highlighting the municipalities of Apucarana, Londrina, Marilândia do Sul, Ortigueira and Rolândia, sampling sites of vegetables and water subjected to parasitological and microbiological evaluation, from 2014 to 2016, Paraná, Brazil.



Source: authors

Forty-two clumps of leafy vegetables were collected (two per property), packed in plastic bags and kept under refrigeration. For detection of helminths, 500 mL of Glycine 1 M and 100 g of the vegetables were homogenized in a plastic bag, manually shaken for 10 minutes, filtered through double gauze, collected in a conical chalice, and allowed to settle for 12 h. Then, 20 µL of the

sediment was observed under an objective microscope at 40x (HOFFMAN et al., 1934). The supernatant was removed from the chalice with a pipette, the pellet was centrifuged at 1120 x g for 5 minutes. With the final sediments (approximately 0.5 mL) slides stained with Lugol's iodine (MATOSINHOS, 2012) were mounted to facilitate the detection and identification of eggs and larvae under an optical microscope.

For detection of protozoa, 50 g of the vegetables and 300 mL of Tween 80 (1%) solution were placed in a plastic bag and homogenized with a stirrer for three minutes. Subsequently, the solution resulting from the first step was filtered through double gauze into a 500-mL glass beaker. The filtered extract was divided into tubes and centrifuged at 2100 x g for 10 minutes each time (CARELI, 2009). Approximately 5 mL of the extract was processed and observed by the technique of Willis (1921) e Faust et al. (1939).

The microbiological analysis was performed using the chromogenic substrate technique, according to the manufacturer's recommendation (Colilert, Idexx, Westbrook, Maine, EUA), the results are expressed as NMP/100 mL (most probable number of total coliforms or *Escherichia coli* per 100 mL of water), 21 water samples (one per property) were collected directly from the irrigation tap of the gardens vegetables, as recommended by Brazilian protocol (BRASIL, 2013).

A semi-structured questionnaire was applied to all horticulturists participating in the study, with questions regarding the type of planting and fertilization, soil supplementation, use of pesticides, presence of animals on the property, irrigation system and characteristics of the bathroom and sewage. This work was approved by the Research Ethics Committee Involving Human Beings of the State University of Londrina under number 2,481,228.

The program EpiInfo 3.5.4 (DEAN, 1990) was used to tabulate the variables together with the microbiological and molecular results. Statistical analyzes were performed using the EpiInfo 3.5.4 and R 3.4.1 programs (R CORE, 2003) using the Chi-square test or Fisher's exact test, when appropriate. The data were compared using odds ratios (ORs) with confidence intervals (CIs) of 95%, significance level of 5%.

Results

A total of 42 samples of vegetables and 21 water samples were collected from 21 properties in the municipalities of Apucarana (5/21), Marilândia do Sul (8/21), Ortigueira (6/21), Rolândia (1/21) and Londrina (District Guaravera) (1/21). Among the vegetables, 19 samples were of lettuce (*Lactuca sativa*), nine were chicory samples (*Cichorium endivia*), seven were arugula samples

(*Eruca sativa*), five were chives samples (*Allium fistulosum*), one was a spinach samples (*Spinacia oleracea*) and one was a chard sample (*Beta vulgaris* subsp. *vulgaris*).

Eighteen properties were certified for organic cultivation, and three (14.28%) were in the certification phase. The organic fertilizer source was variable among the properties: eight (38.09%) used cow manure, seven (33.33%) used chicken manure, three (14.28%) used mixed manure (chicken and cow) and three (14.28%) used a commercial compound. Nine properties were regularly supplemented with mineral soil, six with limestone (66.67%), and three with potassium supplement (33.33%). Domestic animals, such as dogs, cats, horses, cattle, were present in all properties studied and, in four (19.04%) of them, the animals had access to the gardens.

Wild animals (deer, hares, skunks, felines and birds) were present in all properties. All the properties performed vegetable washing – 11 (52.38%) in treated tap water and 10 (47.61%) in rinsing tanks. The irrigation was manual in nine (42.85%) and automated in 12 (57.14%) properties; the source of water for irrigation in 12 (57.14%) properties was from mines, three (14.28%) from rivers and six (28.57%) from artesian wells.

Water analysis showed that 18 (85.71%) had total coliforms (CT) ranging from 1 to > 2419,6 CTNMP/100 mL, among these, 10 were mine water, five artesian well and three of river samples. Regarding the presence of *Escherichia coli*, 17 (80.95%) samples were positive, varying from 1 to 218.7 NMP/100 mL, among them, nine were from mine water, five from artesian wells and three from rivers.

Among the properties visited, 71.4% (15/21) had at least one sample contaminated by parasites. In relation to the methods used for parasitological research, 13 (30.95%) vegetables samples were positive in the Hoffman et al. (1934) technique, 5 (11.90%) in the Faust et al. (1939) technique, 5 (11.90%) in the Willis (1921) technique and 19 (45.23%) were positive in at least one of the three parasitological techniques used in the present study. The results are detailed by property, vegetable and parasite found in Table 1. When considering positivity in at least one of the parasitological methods, a statistically significant difference was observed in the fate of the sewer variable ($p = 0.045$), with the dry sewage being the most positive sewage destination.

TABLE 1: Results of the Faust et al. (1939), Hoffman et al. (1934) and Willis (1921) parasitological techniques from samples of vegetables from organic farm properties in Paraná, Brazil, from 2014 to 2016.

Property	Municipality	Faust et al. (1939)	Parasite	Hoffman et al. (1934)	Parasite	Wiilis (1921)	Parasite
1	Mar. Sul	-		-		-	
2	Mar. Sul	-		+ Let + Aru	Chil, Ent, MiE	-	
3	Mar. Sul	-		+ Aru	OoN	+ Let + Aru	OoN, Ent, NeL
4	Mar. Sul	-		-		+ Let	NeL
5	Guaravera	+ Let	Ent	+ Aru	MiE	+ Aru	MiE
6	Ortigueira	-		+ Chi	Ent	-	
7	Ortigueira	-		-		-	
8	Ortigueira	+ Let	Str, Ent	+ Let	OoN	-	
9	Ortigueira	-		-		-	
10	Ortigueira	-		-		-	
11	Ortigueira	-		+ Let	Ent	-	
12	Rolândia	-		+ Chi	Dipil	-	
13	Apucarana	+ Chi	Hoo	-		-	
14	Apucarana	-		-		+ Let	Trich
15	Apucarana	-		+ Let	Str	-	
16	Apucarana	-		-		-	
17	Apucarana	-		-		-	
18	Mar. Sul	+ Chi	Ent	+ Chd	MiE	-	
19	Mar. Sul	+ Chi	Ent	+ Chi	NeL	-	
20	Mar. Sul	-		+ Let	Lar	-	
21	Mar. Sul	-		+ Chv	NeL	-	

* + positive sample; - negative sample. Chd: Chard; Let: Lettuce; Chv: Chive; Chi: Chicory; Aru: Arugula. MiE: Mite egg; Hoo: hookworms; Chil: *Chilomatix* spp.; Dipil: *Dipillidium* spp.; Ent: *Entamoeba* spp.; Str: *Strongyloides* spp.; Lar: free-living Larva; NeL: nematode larva; OoN: non-sporulated oocyst; Trich: *Trichuris* spp.

Discussion

In the study, the presence of *E. coli* was observed in 17 (80.95%) of the analyzed water samples, of which 3 (17.65%) levels higher allowed by CONAMA (National Environment Council) (CONAMA, 2005). Resolution 357/05 recommends that, for the irrigation of raw vegetables and fruit that have yet to fully develop, a CTT count of less than 200/100 mL is appropriate. *Escherichia coli* is a facultative, anaerobic gram-negative bacteria, commensal in the intestinal microbiota; besides being the main indicator of fecal contamination (CONAMA, 2005), it can often become an opportunistic pathogen (NATARO; KAPER, 1998; FRANCO; LANDGRAF, 2003). It is known that

the contamination of vegetables by pathogenic microorganisms is directly related to the quality of irrigation water (SCHERER et al., 2016), so it is imperative that this type of water be of high quality, passing through prior treatment, when necessary. The *E. coli* contamination has also been previously described in irrigation water in conventional cultive (SCHERER et al., 2016; SILVA et al., 2016a), which confirms that regardless of the type of cultive, water can be an important pathway for the transmission of pathogens, and chlorination is an efficient technique for the killing of bacteria and viruses.

Among the protozoa found, *Chilomatix* spp. and some species of *Entamoeba* spp. are commensal protozoans, even if not pathogenic, they indicate fecal contamination of human origin (OLIVEIRA; GERMANO, 1992). *Entamoeba histolytica* is important to public health due to its high pathogenicity and cyst resistance to conventional water treatment (MELO et al., 2004; MONTANHER et al., 2007). Arbos et al. (2010) suggested that the presence of parasitic structures in vegetables is due to improper cultivation, with respect to hygiene, either through irrigation water, access of wild or domestic animals to gardens and/or, the use of fertilizers with insufficient composting time.

Samples with non-sporulated oocysts were observed. It was not possible to confirm the species identity through sporulation; however, it is important to consider that several parasites pathogenic to humans present this biological form in their life cycles, such as *Cryptosporidium* spp. (TYZZER, 1907), *T. gondii* (DUBEY et al., 1970), *Cyclospora* spp. (ORTEGA et al., 1993). One of the main difficulties when working with environmental samples is the fact that many samples go through adverse conditions such as intense sun and low humidity, favoring alterations in the morphologies and metabolism of the microorganisms, hindering the diagnosis by conventional microscopy and sporulation, respectively.

Mites were found in three samples and are the most important aeroallergens in tropical climate regions; they are associated with respiratory allergies, such as allergic asthma and rhinitis. In addition, they can contaminate food, especially if kept in hot and humid environments, facilitating their proliferation. When ingested, they can trigger allergic reactions and anaphylaxis in atopic individuals, so their presence in vegetables presents a potential risk to human health (GELLER et al., 1995; 2009).

A total of 45.23% (19/42) of the vegetables were contaminated by helminths. Cultivation in direct contact with the soil can facilitate this contamination, since these parasites are commonly found in the soil (SILVA et al., 2016b) and animal manure used for fertilization (FERNANDES et al., 2015). *Strongyloides* spp. are opportunistic parasites of animals. The presence of *Strongyloides*

spp. deserves special attention because *Strongyloides stercoralis* have the capacity to infect humans, mainly immunocompromised individuals (MAIA et al., 2006; SILVA et al., 2016b).

Among the techniques used, a greater percentage of positive results were observed when using the Hoffman et al. (1934) technique. This may have occurred because the test was more sensitive to the parasites found relative to the other methods. A greater sensitivity by the test was also observed by Mesquita et al. (2015) in a study carried out in community gardens in the state of Piauí, Brazil, when they used both the Hoffman et al. (1934) and Willis (1921) methods.

All the properties included in the study had domestic animals; in five of them, the animals had access to the vegetable gardens. Domestic and wild animals are important sources of contamination of soil and water (CASSENOTE et al., 2010; WELLS et al., 2015). The most significant elimination pathway of endoparasites is fecal (NEWELL et al., 2010), which justifies the need to prevent the access of animals to cultivated areas.

A statistical association between sewage destination and positive results was observed in at least one of the parasitological methods used. The form of sewage destination is of extreme importance so that feces do not contaminate the soil and the source of irrigation water (NEWELL et al., 2010; TIYO et al., 2015). The dry sump is the least sanitary type compared to the septic or septic tank biodigester. This is because the dry sewage allows slow degradation of pathogens due to anaerobic digestion, and due to the possibility of diffusion of fecal material into the soil, to depths of three meters and a radius of one meter and to groundwater, in which dispersion varies according to the flow level. To avoid risks to the health safety recommendations must be followed, such as the construction of a dry sewage on the lowest ground with a minimum distance of 15 m from any source of water and as far away as possible from cultivation sites (BRASIL, 2007). Concludes, the presence of parasites, even if apathogenic, in the vegetables may indicate fecal contamination; the main points associated with this contamination were the presence of domestic animals with access to the cultivation area, the use of contaminated water for irrigation and the types of sewage destination; the latter may be responsible for soil and water contamination.

Acknowledgment

To CNPq to granting of scholarship

References

ABREU, E. S.; LIMA, M. B. A.; MACHADO, A. D.; PERSOLI, L. B. L. Análise da qualidade parasitológica de alfaces orgânicas comercializadas em uma rede de supermercados do município de São Paulo-SP. **Revista da Universidade Vale do Rio Verde**, Três Corações, v. 14, n. 2, p. 516-521, 2016.

ARBOS, K. A.; FREITAS, R. J. S.; STERTZ, S. C.; CARVALHO, L. A. Segurança alimentar de hortaliças orgânicas: aspectos sanitários e nutricionais. **Ciência e Tecnologia de Alimentos**, Campinas, v. 30, n. 1, p. 215-220, 2010.

BRASIL. **Manual de saneamento:** orientações técnicas. 2007. Disponível em <http://bvsms.saude.gov.br/bvs/publicacoes/manual_saneamento_3ed_rev_p1.pdf>.

BRASIL. **Manual prático de análise de água.** 2013. Disponível em <http://www.funasa.gov.br/site/wp-content/files_mf/manual_pratico_de_analise_de_agua_2.pdf>.

CARELI, R. T. **Ocorrência de *Cryptosporidium* spp. na cadeia produtiva de alface (*Lactuca sativa* L.) sua captura e retenção por biofilmes de *Pseudomonas fluorescens* e seu controle por surfactantes.** 2009. 154 f. Tese (Doutorado em Ciência e Tecnologia de alimentos) - Universidade Federal de Viçosa, Viçosa. 2009.

CACCIÒ, S. M.; CHALMERS, R. M.; DORNY, P.; ROBERTSON, L. J. Foodborne parasites: outbreaks and outbreak investigations. A meeting report from the European network for foodborne parasites (Euro-FBP). **Food and Waterborne Parasitology**, Amsterdam, v. 10, p. 1-5, 2018.

CASSENOTE, A. J. F.; NETO, J. M. P.; CATELANI, A. R. A. L.; FERREIRA, A. W. Soil contamination by eggs of soil-transmitted helminths with zoonotic potential in the town of Fernandópolis, State of São Paulo, Brazil, between 2007 and 2008. **Revista da Sociedade Brasileira de Medicina Tropical**, Uberaba, v. 44, n. 3, p. 11-14, 2010.

CONAMA. Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente. **Resolução n. 357, de 17 de março de 2005**. Disponível em: <http://www.mma.gov.br/port/CONAMA/res/res5/res35705.pdf>.

DEAN, A. G. **Epi-Info, Version 5:** a word processing, database, and statistics programme for epidemiology on microcomputers. Atlanta: Centers for Disease Control and Prevention, 1990. 391 p.

DUBEY, J. P.; MILLER, N. L.; FRENKEL, J. K. Characterization of the new fecal form of *Toxoplasma gondii*. **Journal of Parasitology**, Lawrence, v. 56, n. 3, p. 447-456, 1970.

ESTEVES, F.; FIGUEIRÔA, E. Detecção de enteroparasitas em hortaliças comercializadas em feiras livres do município de Caruaru (PE). **Revista Baiana de Saúde Pública**, Salvador, v. 33, n. 2, p. 38-47, 2012.

FAUST, E. C.; SAWITZ, W.; TOBIE, J.; ODOM, V.; PERES, C.; LINCICOME, D. R. Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces. **Journal of Parasitology**, Lawrence, v. 25, n. 3, p. 241-262, 1939.

FERNANDES, N. D. S.; GUIMARÃES, H. R.; AMORIM, A. C. S.; REIS, M. B. Avaliação parasitológica de hortaliças: da horta ao consumidor final. **Saúde e Pesquisa**, Maringá, v. 8, n. 2, p. 255-265, 2015.

FERREIRA, F. P.; CALDART, E. T.; FREIRE, R. L.; MITSUKA-BREGANÓ, R.; FREITAS, F. P.; MIURA, A. C.; MAREZE, M.; MARTINS, F. D. C.; URBANO, M. R.; SEIFERT, A. L.; NAVARRO, I. T. The effect of water source and soil supplementation on parasite contamination in organic vegetable gardens. **Revista Brasileira de Parasitologia Veterinária**, Jaboticabal, v. 27, n. 2, p. 327-337, 2018.

FRANCO, B. D. G. de M.; LANDGRAF, M. **Microbiologia dos alimentos.** São Paulo: Atheneu, 2003. 182 p.

GELLER, M.; ESCH, R. E.; FERNÁNDEZ CALDAS, E. Respiratory atopy and domestic mite sensitization in Rio de Janeiro. **Revista Brasileira de Alergia e Imunopatologia,** São Paulo, v. 18, n. 1, p. 13-18, 1995.

GELLER, M.; HAHNSTADT, R. L.; REGO, R. M.; CALDAS, E. F. Anafilaxia induzida por farinha de trigo contaminada por ácaros Dust mite-contaminated wheat flour induced anaphylaxis. **Revista Brasileira de Alergia e Imunopatologia**, São Paulo, v. 32, n. 5, p. 199-201, 2009.

HOFFMAN, W. A.; PONS, J. A.; JANER, J. L. Sedimentation concentration method in schistosomiasis, Puerto Rico. **Journal of Public Health**, Oxford, v. 9, n. 1, p. 283-298, 1934.

MAIA, T. M. C.; VASCONCELOS, P. R. L.; FAUTH, S.; MOTTA NETO, R. *Strongyloides stercoralis* over infestation. **Revista Brasileira em Promoção da Saúde**, Fortaleza, v. 19, n. 2, p. 118-121, 2006.

MATOSINHOS, F. C. L. **Padronização de metodologia para detecção de ovos e larvas de helmintos em alface.** 2012. 98 f. Dissertação (Mestre em Parasitologia) - Universidade Federal de Minas Gerais, Belo Horizonte. 2012.

MELO, M. C. B.; KLEM, V. G. Q.; MOTA, J. A. C.; PENNA, F. J. Parasitoses Intestinais. **Revista Médica de Minas Gerais**, Belo Horizonte, v.14, p. 3-12, 2004.

MESQUITA, D. R. de; SILVA, J. P.; MONTE, N. D. P.; SOUSA, R. L. T.; SILVA, R. V. S.; OLIVEIRA, S. S.; LEAL, A. R. S.; FREIRE, S. M. Ocorrência de parasitos em alface-crespa (*Lactuca sativa* L.) em hortas comunitárias de Teresina, Piauí, Brasil. **Revista de Patologia Tropical**, Goiânia, v. 44, n. 1, p. 67-76, 2015.

MONTANHER, C. C.; CORADIN, D. D. C.; FONTOURA-DA-SILVA, S. E. Parasitologic evaluation of eggs, cysts and larval forms of enteroparasites found in lettuces (*Lactuca sativa*) served in buffet restaurants in Curitiba City, State of Paraná, Brazil. **Estudos de Biologia: Ambiente e Diversidade**, Curitiba, v. 29, n. 66, p. 63-71, 2007.

NATARO, J. P.; KAPER, J. B. Diarrheagenic *Escherichia coli*. **Clinical Microbiology Reviews**, Washington, v. 11, n. 1, p. 142-201, 1998.

NEWELL, D. G.; KOOPMANS, M.; VERHOEF, L.; DUIZER, E.; AIDARA-KANE, A.; SPRONG, H.; OPSTEEGH, M.; LANGELAAR, M.; THREFALL, J.; SCHEUTZ, F.; GIESSEN, J. van der; KRUSE, H. Food-borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge. **International Journal of Food Microbiology**, Summit-Argo, v. 139, Suppl. 1, p. S3-S15, 2010.

OLIVEIRA, C. A. F. de; GERMANO, P. M. L. Estudo da ocorrência de enteroparasitas em hortaliças comercializadas na região metropolitana de São Paulo – SP , Brasil. **Revista Saúde Pública**, São Paulo, v. 26, n. 5, p. 332-335, 1992.

ORTEGA, Y. R.; STERLING, C. R.; GILMAN, R. H.; CARNA, V. A.; DIAZ, F. *Cyclospora* species - a new protozoan pathogen of humans. **New England Journal of Medicine**, Boston, v. 328, n. 18, p. 1308-1312, 1993.

PACHECO, M. A. S. R.; SOTO, F. R. M.; FONSECA, S. K.; GOMES, A. H. S.; GIANCOLI, M.; LEITE JÚNIOR, A. P.; DIAS, H. G. G.; CANDIDO, V. L. P.; ARMELIN, I. M. Monitoramento das condições higiênico-sanitárias de alfaces produzidas no município de Ibiúna, SP. **Revista Eletrônica de Epidemiologia das Doenças Transmitidas por Alimentos**, São Paulo, v. 3, n. 2, p. 52-53, 2003.

R CORE, T. **R:** a language and environment for statistical computing. 2003. Disponível em <http://www.R-project.org>. Acesso em: 17 dezembro 2018.

SANTANA, L. R. R.; CARVALHO, R. D. S.; LEITE, C. C.; ALCÂNTARA, L. M.; OLIVEIRA, T. W. S.; RODRIGUES, B. M. Qualidade física, microbiológica e parasitológica de alfaces (*Lactuca sativa*) de diferentes sistemas de cultivo. **Ciência e Tecnologia de Alimentos**, Campinas, v. 26, n. 2, p. 264-269, 2006.

SCHERER, K.; GRANADA, C. E.; STULP, S.; SPEROTTO. R. A. Avaliação bacteriológica e físico-química de águas de irrigação, solo e alface (*Lactuca sativa L.*). **Revista Ambiente e Água**, Taubaté, v. 11, n. 3, p. 665-676, 2016.

SILVA, A. F. S.; LIMA, C. A.; QUEIROZ, J. J. F.; JACOMÉ, P. R. L. A.; JÁCOME-JÚNIOR, A. T. Análise bacteriológica das águas de irrigação de horticulturas. **Revista Ambiente e Água**, Taubaté, v. 11, n. 2, p. 428-438, 2016a.

SILVA, A. S.; REBOUÇAS, L. T.; ALMEIDA, J. S.; ROCHA, E. V. S.; AMOR, A. L. M. Parasitological and microbiological analyis of vegetables sold in Santo Antônio de Jesus, Bahia (Brazil). **Vigilância Sanitária em Debate**, Rio de Janeiro, v. 4, n. 3, p. 77-85, 2016b.

TIYO, R.; SOUZA, C. Z.; NISHI, L.; BRUSTOLIN, C. F.; RATTI, B. A.; FALAVIGNA GUILHERME, A. L. Water from different sources used for the irrigation of vegetables to be marketed: research on *Cryptosporidium* spp., *Giardia* spp., and coliforms in Paraná, Brazil. **Revista do Instituto de Medicina Tropical de São Paulo**, São Paulo, v. 57, n. 4, p. 333-336, 2015.

TYZZER, E. E. A protozoan found in the peptic glands of the common mouse. **Proceedings of the Society for Experimental Biology and Medicine**, Malden, v. 5, n. 1, p. 12-13, 1907.

VOLLKOPF, P. C. P.; LOPES, F. M. R.; NAVARRO, I. T. Ocorência de enteroparasitas em amostrasde alface (*Lactuca sativa*) comercializadas em Porto Murtinho – MS. **Arquivos de Ciências Veterinárias e Zoologia da Unipar**, Umuarama, v. 9, n. 1, p. 37-40, 2006.

WELLS, B.; SHAW, H.; HOTCHKISS, E.; GILRAY, J.; AYTON, R.; GREEN, J.; KATZER, F.; WELLS, A.; INNES, E. Prevalence, species identification and genotyping *Cryptosporidium* from livestock and deer in a catchment in the Cairngorms with a history of a contaminated public water supply. **Parasites & Vectors**, London, v. 8, n. 1, p. 1-13, 2015.

WILLIS, H. H. A simple levitation method for the detection of hookworm ova. **Medical Journal of Australia**, Sydney, v. 2, n. 18, p. 375-376, 1921.

6 ARTIGO D

Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, v. 28, n. 3, p. 518-521, july.-sept. 2019
Doi: https://doi.org/10.1590/S1984-29612019044

Short Communication

Investigation and environmental analysis of samples from outbreak of toxoplasmosis at research institution in Londrina, Paraná, Brazil, 2016

Investigação e análise ambiental de amostras oriundas de surto de toxoplasmose em uma instituição de pesquisa em Londrina, Paraná, Brasil, 2016

Fernanda Pinto-Ferreira[1]*; Regina Mitsuka-Breganó[2]; Thais Cabral Monica[1]; Felippe Danyel Cardoso Martins[1]; Ricardo Luís Nascimento de Matos[3]; Marcelle Mareze[1]; Beatriz de Souza Lima Nino[1]; Simone Garani Narciso[3]; Roberta Lemos Freire[1]; Italmar Teodorico Navarro[1]

[1] Departamento de Medicina Veterinária Preventiva, Laboratório de Zoonoses e Saúde Pública, Universidade Estadual de Londrina – UEL, Londrina, PR, Brasil

[2] Departamento de Medicina Veterinária Preventiva, Laboratório de Parasitologia Veterinária, Universidade Estadual de Londrina – UEL, Londrina, PR, Brasil

[3] Secretaria de Saúde Municipal de Londrina, Londrina, PR, Brasil

Received January 14, 2019
Accepted May 22, 2019

Abstract

The objective of this study was to report an outbreak of human toxoplasmosis at a research institution in Londrina, Paraná, from December 2015 to February 2016. Blood samples from 26 symptomatic individuals were collected and the microparticle chemiluminescence immunoassay was performed to detect IgM, IgG and specific IgG avidity test in the official laboratory. A total of 20 people with symptoms and serology compatible with acute toxoplasmosis (IgM positive and IgG with low avidity) were selected as cases, while 45 asymptomatic employees working in the same teams and during the same shifts were selected as controls. All the participants of the investigation answered an epidemiological questionnaire. Three samples of water and one sludge from the institution's supply cisterns, 10 soil samples, 11 plant samples, three cat fecal samples and one domestic feline cadaver were collected for analysis of the polymerase chain reaction (PCR) for *T. gondii*. After analyzing the epidemiological data, the consumption of vegetables in the restaurant of the institution was the only variable associated with the occurrence of the disease. In laboratory results, all the samples showed negative results to PCR. The rapid recognition of the outbreak, early notification and investigation could have broken the chain of transmission early, thus preventing the emergence of new cases. In addition, the adoption of good food handling practices could have prevented the occurrence of the outbreak.

Keywords: Epidemiology, *Toxoplasma gondii*, vegetables, foodborne, outbreak.

Resumo

O objetivo deste estudo foi relatar um surto de toxoplasmose humana em uma instituição de pesquisa em Londrina, Paraná, no período de dezembro de 2015 a fevereiro de 2016. Amostras de sangue de 26 indivíduos sintomáticos foram coletadas e o imunoensaio de quimioluminescência de micropartículas foi realizado para detectar IgM, IgG e teste de avidez de IgG específica em laboratório oficial. Um total de 20 pessoas com sintomas e sorologia compatíveis com toxoplasmose aguda (IgM positiva e IgG com baixa avidez) foi selecionado como casos, enquanto 45 funcionários assintomáticos que trabalhavam nas mesmas equipes e durante os mesmos turnos foram utilizados como controles. Todos os participantes da investigação responderam a um questionário epidemiológico. Foram coletadas três amostras de água e uma de lodo das cisternas de abastecimento da instituição, 10 de solo, 11 de vegetais, três amostras de fezes de gato e um cadáver de filhote felino doméstico para detecção de *T. gondii* pela reação em cadeia da polimerase (PCR). Após análise dos dados epidemiológicos, o consumo de hortaliças no restaurante da instituição foi a única variável associada à

*Corresponding author: Fernanda Pinto-Ferreira. Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina – UEL, Avenida Juvenal Pietraroia, 1125, CEP 86057-080, Londrina, PR, Brasil.
e-mail: nandaferreiravet@gmail.com

Resumo

O objetivo deste estudo foi relatar um surto de toxoplasmose humana em uma instituição de pesquisa em Londrina, Paraná, no período de dezembro de 2015 a fevereiro de 2016. Amostras de sangue foram coletadas de indivíduos com sintomatologia e o imunoensaio de quimioluminescência de micropartículas foi realizado. para detectar IgM, IgG e teste de avidez de IgG específica em laboratório oficial. Um total de 20 indivíduos com sintomas e sorologia compatíveis com toxoplasmose aguda (IgM positiva e IgG com baixa avidez) foram selecionados como casos, enquanto 45 funcionários assintomáticos que trabalhavam nas mesmas equipes e durante os mesmos turnos foram selecionados como controles. Todos os participantes da investigação responderam um questionário epidemiológico. Três amostras de água, uma de lodo, 10 de solo, 11 de vegetais, três de fezes de gato e um cadáver foram coletados para análise da reação em cadeia da polimerase. Após análise dos dados epidemiológicos, o consumo de hortaliças foi a única variável significativamente associada à ocorrência da doença. Em resultados laboratoriais, todas as amostras apresentaram resultados negativos. O rápido reconhecimento do surto, notificação e investigação prematura poderia ter quebrado a cadeia de transmissão precocemente, evitando assim o surgimento de novos casos. Além disso, a adoção de boas práticas de manipulação de alimentos poderia ter impedido a ocorrência do surto.

Palavras-chave: epidemiologia, *Toxoplasma gondii*, vegetais, origem alimentar, surto

Toxoplasmosis is a zoonosis caused by the protozoan *Toxoplasma gondii*; due to its public' health importance, it is one of the most studied parasites in the world and can infect all homeothermic animals with felines serving as the definitive hosts (JACOBS; MELTON, 1957). The transmission pathway of the agent is variable; however, eating habits as well as cultural and environmental factors are determinants of the epidemiology of toxoplasmosis (KOLBEKOVA; KOURBATOVA; NOVOTNA, 2007).

In Brazil, outbreak notification and epidemiological research is a mandatory activity in health surveillance (BRASIL, 2017). Over the past 50 years, more than 40 outbreak reports for toxoplasmosis have been published worldwide, with the majority being in Brazil (FERREIRA et al., 2018a). The main transmission routes involved were water (DE MOURA et al., 2006),

vegetables (EKMAN et al., 2012), fruits (DUTRA et al., 2012), undercooked meats or raw (POMARES et al., 2011) and unpasteurized goat's milk (CHIARI et al., 1984). The objective of this work was to describe an outbreak of toxoplasmosis that occurred at research institution located in the Municipality of Londrina, Paraná, Brazil.

On February 18, 2016, the Londrina Epidemiological Surveillance Coordination was informed of that three individuals had confirmed acute toxoplasmosis and five were presenting with clinical manifestations. All cases were reported from a research institution in the city of Londrina. Based on the reports, the hypothesis that there was an outbreak was proposed. Thus, an active search for acute toxoplasmosis cases was started, and all individuals who had been present at the institution's facilities from December 2015 to February 2016 were considered as potential cases, including those who had visited restaurants, consumed water collected by the company and had presented with at least one of the symptoms such as fever, myalgia, asthenia, headache, and lymphadenopathy.

A confirmed case was defined as individual who, in Microparticle Chemiluminescent Immunoassay (CMIA) (PETERSON et al., 2005), presented antibodies of the IgM class reagent and IgG with low avidity. The suspected patients that showed reagent IgM and IgG high-avidity or nonreactive IgM and reactive IgG were considered immune or with old exposure to the agent. These immune individuals, along with susceptible (nonreactive IgM and IgG) individuals were not considered as part of the outbreak.

During the visit to the institution, information was collected about the daily menu provided by the restaurant during the study period, including information about the types of raw and cooked foods served. A semistructured questionnaire was applied to all participants, and information about gender, age, work area, eating habits, water consumption and soil manipulation was collected.

A case-control epidemiological study was carried out to identify the probable source of the contamination of the studied population using the information collected in the questionnaire and the results of the tests. For the analysis of the variables studied and comparisons of proportions, the chi-square test or Fisher's Exact, with a significance level of 5% and confidence interval of 95% was used by software EpiInfo 3.5.4 (CDC, Atlanta, USA).

Three water samples (20 L of water from cisterns 1 and 2 and 10 L from cistern 3) were collected as well as a 500 mL sample from the sludge (cistern 1). Ten soil samples collected from the surface (eight from greenhouses and two from vegetable gardens, 100g each), 11

vegetables clumps (eight from the vegetable gardens and three from the restaurant), three samples of cat feces and a cadaver of kitten were collected.

The water samples were filtered using a filter membrane technique and processed as described by Franco et al. (2012). The sludge, in turn, was centrifuged in 50 mL Falcon tubes at 2,100 g/10 min, yielding a final supernatant volume of 50 mL. The vegetables were processed according to Ferreira et al. (2018b). The DNA extraction was performed using a commercial kit (NucleoSpin Tissue, Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions, and DNA was eluted in a final volume of 50 µl. The PCR assays were performed as previously described by Homan et al. (HOMAN et al., 2000) and amplified a 529 bp fragment.

A domestic feline, approximately six months old, was found dead during the investigation and referred for autopsy, sample collection (brain, heart, peritoneal exudate, liver, diaphragm and feces) were performed. The Sheater's technique (SHEATER, 1923) was used to detect *T. gondii* oocysts both in the feces of the dead cat and the samples found in the environment. The other samples were divided into aliquots and stored under refrigeration until DNA extraction.

To identify the factors associated with the occurrence of the outbreak and the possible routes of transmission, such as food and water, a case-control study was conducted. Twenty individuals with serological results and symptomatology compatible with a recent *T. gondii* infection were included as cases. As controls, 45 asymptomatic employees who worked in the same staff and in the same work shifts were selected. The study ratio was 1:2.25.

Based on the initial date of the reported symptoms, the outbreak period was from December 10, 2015 to February 3, 2016, and the dates of the probable exposure were from 12/1/15 to 01/21/16, considering the mean incubation period of 20 days (EKMAN et al., 2012) of all the cases identified. The period with the highest concentration of symptomatic cases was between 01/10/16 and 01/16/16 (2nd epidemiological week of 2016). The general distribution of the symptomatic cases is shown in Figure 1. The most frequently reported symptoms were nausea (100%), headache (85%) and lymphadenopathy (80%) (Table 1).

Of the 20 patients, 55% (11/20) were males, with ages ranging from 25 to 60 years and a median age of 45 years. Regarding workplace locality, 20% (4/20) of the cases came from Block 13 (the block in front of the restaurant), and 60.0% (12/20) worked in manipulating land and planting. About the food consumption at the institution, all the patients drank water from drinking fountains and consumed vegetables, beef and chicken at the institution's restaurant.

Significant events were analyzed based on the habits, environment and food consumption in the institution, using the patients (20 individuals) and controls (45 individuals). The consumption of vegetables ($p<0,05$) was the only variable significantly associated with the incidence of the disease (Table 2). It is known that even asymptomatic people can present serology compatible with acute disease (EKMAN et al., 2012), but in all cases commonly in outbreaks of toxoplasmosis, due to the amount of inoculum and strain, the presence of symptomatology is high frequency (DEMAR et al., 2007).

During the restaurant inspection, there were some irregularities noted, such as the presence of food without a record of origin and invoice, incorrect fractionation of portions with missing validity data, and presence dripping rain water under the grilled meats. In addition, the screens of two windows were destroyed, there were cats near the establishment, and inadequate washing of vegetables was observed.

Worldwide, outbreaks of toxoplasmosis are increasing in frequency, with water (BELL et al., 1995; DE MOURA et al., 2006), meat (EDUARDO et al., 2007), milk (SACKS; ROBERTO; BROOKS, 1982) and vegetables (DUTRA et al., 2012; EKMAN et al., 2012; MORAIS et al., 2016) constituting transmission routes being vegetables in particular responsible for a growing number of outbreaks.

The workforce was approximately 800 people among servers, statutory and outsourced. The outbreak at the Londrina research institution affected 20 individuals. The relatively small number of cases (20/800) reduces the chances that water served as the route of transmission for *T. gondii*. When contaminated with oocysts, water has a greater distribution, affecting large numbers of people and causing an increase in cases with time, as has been previously observed in other outbreaks such as those recorded in Santa Isabel do Ivaí, Brazil (DE MOURA et al., 2006) and British Columbia, Canada (BELL et al., 1995). In these outbreaks, unlike this case study, the appearance of the last case was less than 2 months after the notification of the first.

The number of cases reported in this study was superior to that of some outbreaks previously reported in Brazil (EDUARDO et al., 2007; EKMAN et al., 2012) and lower than that reported in the Santa Maria outbreak, Rio Grande do Sul, occurred in 2018 with a total of 746 cases being considered as the largest recorded outbreak in the world so far (data not yet published) and in Santa Isabel do Ivaí, Paraná, occurred between 2001 and 2002 with a total of 426 cases and is considered to be the largest recorded outbreak in the world so far (DE MOURA et al., 2006)

Like the other reported outbreaks (MASUR et al., 1978; MORAIS et al., 2016), the most frequent symptoms were nausea (100%), headache (85%), lymphadenopathy (80%) and myalgia (75%). Within the workplace, a greater frequency of patients was observed in Block 13. A likely explanation is that Block 13 is in front of the restaurant, and these employees were probably the first to have arrived and eaten from the same batch of food.

Due to inadequate hygienic conditions in the restaurant, it was not possible to reach the producers of the meat and vegetables. Traceability of these producers, even late screenings, would have been of great importance as they could have provided evidence of the primary sources of contamination and may have reduced the chances of new outbreaks (EDUARDO et al., 2007).

All the environmental and food samples yielded negative in PCR tests. However, these results did not rule out any of the possible transmission routes. The assessment of food and environmental samples in outbreaks of toxoplasmosis still presents a challenge, since the incubation period of the disease is long and consequently there are no stored leftovers, and water sources are renewed; these factors make it difficult to collect representative samples. There were few outbreaks where the responsible agent was successfully identified in samples, as was the case in the Santa Isabel do Ivaí outbreak (DE MOURA et al., 2006); identification was possible only since the school was closed and had stored water from the beginning of the outbreak period.

Based on the menus, incubation period, number of cases and epidemiological evidence, it is probable that the vegetables were the route of transmission of the outbreak. Fruits and vegetables have already been to be the suspected causes of various outbreaks of toxoplasmosis (DUTRA et al., 2012; EKMAN et al., 2012; MORAIS et al., 2016). In 2009, a similar outbreak occurred in a factory in São Paulo, with a total of 2,300 employees. These, 11 individuals presented with acute toxoplasmosis, a number that is lower than that reported in the present study; however, vegetable intake was also the main suspected cause (EKMAN et al., 2012).

The main points for contamination of plants by *T. gondii* and other parasites are during production. Horizontalization of agriculture makes it difficult to track microbes and assure food quality, since responsibility is decentralized and the goods are handled at a greater number of different establishments, increasing the chances of contamination during the entire process (washing, packaging, transport, storage, distribution and sale) (FERREIRA et al., 2018; PACHECO et al., 2002).

There was an outbreak of toxoplasmosis in Londrina, Paraná, involving 20 people. The likely route of transmission of the outbreak was raw vegetables consumed at the research institution's restaurant. The rapid recognition of the outbreak and notification could have broken the chain of the transmission early, preventing the appearance of new cases.

References

Bell A, Gill R, Isaac-Renton J, King A, Martinez L, Roscoe D, Werker D, Eng S, Johnstone T, Stanwick R. Outbreak of toxoplasmosis associated with municipal drinking water-British Columbia. *Can Commun Dis Rep* 1995; 21(18):161–164.

Brasil, Ministério da Saúde. Portaria n°4 de 28 de setembro de 2017. *Consolidação das normas sobre os sistemas e os subsistemas do Sistema Único de Saúde*, 2017.

Chiari C de A, Neves DP, Chiari Cléa de Andrade NDP, Chiari C de A, Neves DP. Toxoplasmose humana adquirida através da ingestão de leite de cabra. *Mem Inst Oswaldo Cruz* 1984; 79(3):337–340.

Demar M, Ajzenberg D, Maubon D, Djossou F, Panchoe D, Punwasi W, Valery N, Peneau C, Daigre J-L, Aznar C, Cottrelle B, Terzan L, Darde M-L, Carme B. Fatal Outbreak of Human Toxoplasmosis along the Maroni River: Epidemiological, Clinical, and Parasitological Aspects. *Clin infec dis* 2007; 45 (7): 88-95.

de Moura L, Bahia-Oliveira LMG, Wada MY, Jones JL, Tuboi SH, Carmo EH, Ramalho WM, Camargo NJ, Trevisan R, Graça RMT, Da Silva AJ, Moura I, Dubey JP, Garrett DO. Waterborne toxoplasmosis, Brazil, from field to gene. *Emerging Infect Dis* 2006; 12(2):326–329.

Dutra, LH, Saad E, Alves RMS, Cabral CM, Batista FS, Lima R, Bitencourt PA, Rodrigues JMB, Arouca ML, Navarro IT, Wada MY. Investigação de surto de toxoplasmose transmitido pelo consumo de açaí em Rondônia, Brasil, 2011. *Anais da 12° EXPOEPI.* 2012; 108-109.

Eduardo MBP, Katsuya EM, Ramos SRTS, Pavanello EI, Paiva OR, Brito S do N, Madalosso G. Investigação do surto de toxoplasmose associado ao consumo de prato à base de carne crua ("steak tartar"), nos municípios de São Paulo e Guarujá, SP. *Boletim Epidemiológico Paulista.* 2007; 4(41):1–6.

Ekman CCJ, Chiossi MF do V, Meireles, Luciana Regina Andrade Júnior HF de, Figueiredo WM, Marciano MAM, Luna EJ de A. Case-control study of an outbreak of acute toxoplasmosis in an industrial plant in the state of São Paulo, Brazil. *Rev Inst Med Trop São Paulo* 2012; 54(5):239–244.

Ferreira FP, Caldart ET, Pasquali AKS, Freire RL, Mistsuka-Breganó R, Navarro, IT. Temporal evolution in the patterns of transmission routes and sources of infection in global outbreaks of human toxoplasmosis - a systematic review. *Anais do 54°MEDTROP*. 2018a; 1717.

Ferreira FP, Caldart ET, Freire RL, Freitas FM De, Miura AC, Mareze M, Danyel F, Martins C, Urbano MR, Seifert AL, Navarro IT. The effect of water source and soil supplementation on parasite contamination in organic vegetable gardens. *Braz J Vet Parasitol* 2018b; 2961:1–11.

Franco RM, Branco N, Leal DAG. Parasitologia Ambiental: Métodos de concentração e detecção de *Cryptosporidium* spp. e *Giardia* spp. em amostras de água. *Rev Pat Trop* 2012; 41(2):119–135.

Homan WL, Vercammen M, De Braekeleer J, Verschueren H. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. *Int J Parasitol* 2000; 30(1):69–75.

Jacobs L, Melton ML. Studies on the resistance of pseudocysts of *Toxoplasma gondii*. *J Parasitol* 1957; 43(5):38.

Kolbekova P, Kourbatova E, Novotna M. New and old risk-factors for *Toxoplasma gondii* infection: prospective cross-sectional study among military personnel in the Czech Republic. *Clin Microbiol Infect* 2007; 13(10):1012-73.

Masur H, Jones TC, Lempert JA, Cherubini TD. Outbreak of toxoplasmosis in a family and documentation of acquired retinochoroiditis. *Am J Med* 1978; 64(3):396–402.

Morais RAFB, Freire ABC, Barbosa DRL, Silva LCT, Pinheiro AF, Costa SS, Ramos FLP, Bichara CNC, Lima CJB, Silva AV, Souza SRP, Piqueira Neto LP, Gonçalves NV, Póvoa MM, Carmo EL. Surto de toxoplasmose aguda no Município de Ponta de Pedras, Arquipélago do Marajó, Estado do Pará, Brasil: características clínicas, laboratoriais e epidemiológicas.

Rev Pan-Amaz Saude 2016; 7:143-152

Pacheco MA dos SR, Soto FRM, Fonseca YSK, Gomes AH de S, Giancoli M, Júnior APL, Dias HGG, Candido VLP, Armelin IM. Monitoramento das condições higiênico-sanitárias de alfaces produzidas no município de Ibiúna, SP. *REVNET* 2003; 3(2): 36.

Petersen E, Borobio MV, Guy E, Liesenfeld O, Meroni V, Naessens A, Spranzi E, Thulliez P. European multicenter study of the LIAISON automated diagnostic system for determination of *Toxoplasma gondii*-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. *J Clin Microbiol* 2005;43(4):1570-4.

Pomares C, Ajzenberg D, Bornard L, Bernardin G, Hasseine L, Darde M-L, Marty P, Dardé M-L, Marty P. Toxoplasmosis and horse meat, France. *Emerging Infect Dis* 2011; 17(7):1327–1328.

Sacks JJ, Roberto RR, Brooks NF. Toxoplasmosis infection associated with raw goat's milk. *JAMA* 1982; 248(14):1728–1732.

Sheater AL. The Detection of Intestinal Protozoa and Mange Parasites by a Floatation Technique. *J Pathol Therapy* 1923; 36(pt. 4):266–275

Figure 1 - Number of cases per epidemiological week (onset of symptoms) in the outbreak of toxoplasmosis at a research institution in Londrina, Paraná, Brazil, 2016.

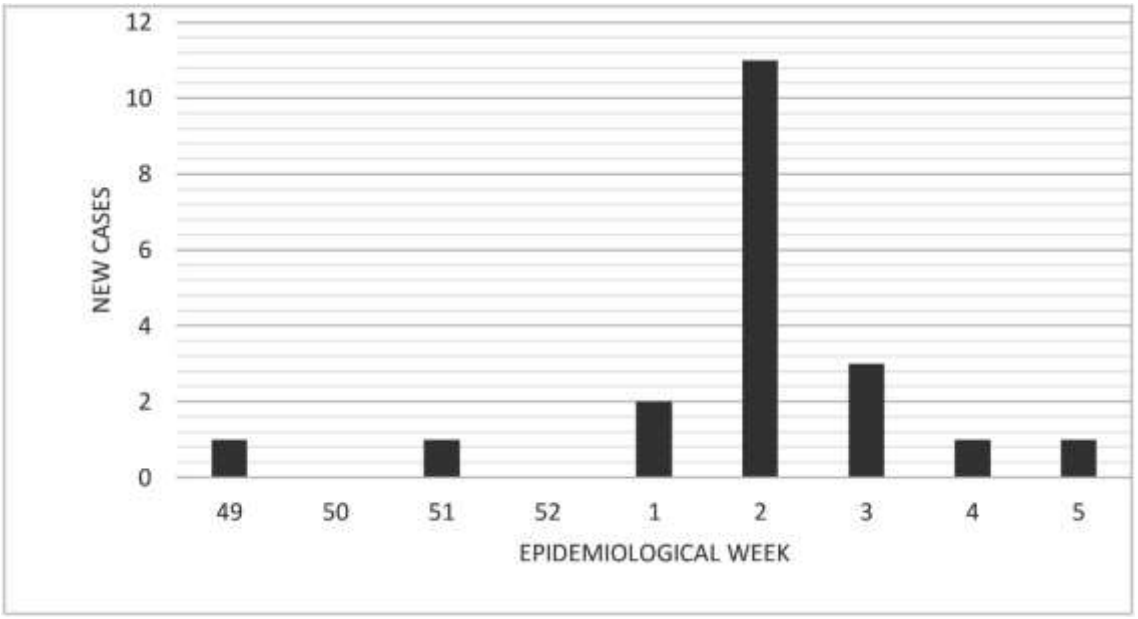


Table 1 - Frequency of clinical signs manifested by cases during the outbreak of toxoplasmosis at a research institution in Londrina, Paraná, Brazil, 2016.

SYMPTOMS	(N/TOTAL)	FREQUENCY
Nausea	(20/20)	100%
Headache	(17/20)	85%
Lymphadenopathy	(16/20)	80%
Myalgia	(15/20)	75%
Apathy	(15/20)	75%
Asthenia	(9/20)	45%
Cough	(6/20)	30%
Arthralgia	(6/20)	30%
Ophthalmic changes	(3/20)	15%

Table 2 - Variables related to the outbreak of toxoplasmosis at a research institution in Londrina, Paraná, Brazil, 2016.

Variables	Cases (+/total)	Controls (+/total)	P-value	OR (95%IC)
Consumption of vegetables	19/20	25/45	0.004	14.72 (2.36 – 333.70)

7 CONCLUSÃO

- Houve uma mudança na epidemiologia dos surtos de toxoplasmose nos últimos 20 anos. A forma biológica de oocisto (esporozoítos) passou a ter maior importância e incidência como via de transmissão.
- Fonte de água, a não suplementação do solo com calcário e a presença de animais nas propriedades são pontos de risco para a contaminação por *Toxoplasma gondii*, *Cryptosporidium* spp. e *Giardia intestinalis*.
- Foi observada a presença de parasitas apatogênicos nos vegetais orgânicos, sugerindo a contaminação fecal. Os principais pontos associados a essa contaminação foram a presença de animais domésticos com acesso à área de cultivo, o uso de água contaminada para irrigação e os tipos de esgoto de destino.
- O estudo epidemiológico de campo foram fundamentais para identificar a causa de um surto de toxoplasmose em Londrina, Paraná. A via provável de transmissão foi o consume de vegetais crus em restaurante de uma instituição de pesquisa.

1

APÊNDICE A

Universidade Estadual de Londrina

1) Data da colheita das amostras:

Data: _____/_____/_____

2) Identificação:

Município: _____ Código da Propriedade: _____

Entrevistado: _____ Função do entrevistado na horta: _____

Telefone para contato: (___) _____

Horta ☐ Comunitária ☐ Particular : _____ Área em metros: _____

3) Tipo de Cultivo:

☐ Orgânica ☐ convencional ☐ em transição

4) Hortaliças Cultivadas:

☐ Alface Crespa ☐ Alface americana ☐ Escarola ☐ Almeirão ☐ Cebolinha ☐ Salsinha ☐ Acelga

5) Tipo de Adubação:

5.1) ☐ Químico ☐ orgânico ☐ Mista

5.2) Base do adubo:

☐ esterco bovino, ovino ou caprino ☐ esterco suíno ☐ esterco de aves ☐ Cama de frango ☐ Restos de alimentos ☐ Outros _____

5.3) ☐ Comercial ☐ Produção Própria

5.3.1) Se produção própria:

☐ Compostagem ☐ Esterco fresco ☐ Esterco Curtido

5.3.1.1) Se o Esterco é curtido, qual o tempo de curtimento?

☐ Até 90 dias ☐ mais de 90 dias

5.4) Frequência de adubação:

☐ 1 mês – 6 meses ☐ 6 meses – 1 ano ☐ mais de 1 ano

6) Há suplementação mineral do solo regularmente? ☐ Não ☐ Sim – Qual? _____

7) Utiliza defensivos agrícolas?

☐ Não ☐ Sim Quais? _____

7.1) Se não, qual a forma de controle de parasitas? _____

8) Presença de animais na propriedade:

☐ Bovinos, ovinos ou caprinos ☐ Equídeos ☐ Suínos ☐ Aves ☐ Cão ☐ Gato ☐ Outros _____

8.1) Algum dos animais tem acesso à horta? ☐ Sim ☐ Não Qual? : _____

9) Espécies silvestres em vida livre na propriedade:

☐ Não tem ☐ Cervídeos ☐ Capivaras ☐ marsupiais (gambá) ☐ Ratos ☐ Pombos ☐ Outras _____

10) Tipo de Irrigação

☐ Manual ☐ Tecnificada

10.1) Se tecnificada:

☐ Aspersão ☐ Gotejamento

10.2) Fonte da água usada na irrigação:

☐ Sistema de Tratamento ☐ Mina ☐ Poço artesiano ☐ Rio ☐ Outras _____

11) Lavagem das hortaliças:

□ Água corrente □ Tanque de enxague □ não é lavado □ outros

Se tanque de enxgue, qual o tempo de permanência da água?_____

12) Banheiro mais próximo da horta:

12.1) □ Com vaso sanitário □ Sem vaso sanitário

12.2) □ Com torneira □ Sem torneira

12.3) □ Dentro da casa/ edificação □ Fora da casa / edificação

12.4) Distância aproximada da horta:

□ 0 – 20 metros □ 20 – 50 metros □ 50 – 100 metros □ Acima de 100 metros

13) Destino do esgoto:

□ Fossa séptica □ Fossa seca □ Outros

14) Direcionamento final da produção:

□ Consumo próprio □ Venda □ Distribuição para a comunidade

1

APÊNDICE B

Questionário Epidemiológico referente ao ARTIGO D

FICHA DE INVESTIGAÇÃO EPIDEMIOLÓGICA PARA TOXOPLASMOSE -

N^O da amostra _____ Data da coleta ___/___/___

1. IDENTIFICAÇÃO

Nome:_____ Data de nascimento

___/___/___ Sexo: ☐ M ☐ F . Se mulher: está grávida? ☐ sim ☐ não

De quantos meses? _____

Estado Civil: ☐ solteiro ☐ casado ☐outro_____

Naturalidade _____ Nacionalidade _____

Endereço residencial: _____

Telefone: _____ /_____ Município: _____ UF: ___

Local da residência: ☐ zona rural ☐ zona urbana => ☐Casa ☐Apto

Grau de instrução: ☐ 1^O grau incompleto ☐ 1^O grau completo ☐ 2^O grau incompleto ☐ 2^O grau completo ☐ superior incompleto ☐ superior completo

2. ATIVIDADE DESENVOLVIDA

local de trabalho: _____ função: _____

Tempo de atividade: _____ anos período: ☐ diurno ☐ noturno

3. HISTÓRICO DO PACIENTE

Participou de alguma festa nos últimos 15 a 20 dias? ☐ sim ☐ não

Tipo de festa? _____

Apresentou algum sintoma nos últimos dois meses? ☐ sim ☐ não

Data do início dos sintomas? _____

Quais os sintomas? _____

Tomou algum tipo de medicamento? ☐ sim ☐ não Qual? _____

Apresentou algum problema de visão nos últimos dois meses? ☐ sim ☐ não

Qual? _____

Fez exame de Fundo de Olho nos últimos dois meses? ☐ sim ☐ não

Qual resultado:_____

4. HÁBITOS

Toma leite cru: ☐ sim ☐ não freqüência semanal: _____

origem do leite: ☐ vaca ☐ cabra

Ingere queijo fresco sem inspeção: ☐ sim ☐ não freqüência semanal: _____

origem do queijo: ☐ vaca ☐ cabra

Come carne: ☐ sim ☐ não

3.1 Come carne crua ou mal passada? ☐ sim ☐ não

3.2 Qual? ☐ carppaccio ☐ kibe cru ☐ bife tártaro ☐ linguiça frescal

☐ carne mal passada ☐ churrasco mal passado ☐ outro _____

3.3 Qual tipo de carne crua ou mal passada? ☐ suíno ☐ ovino ☐ caprino ☐ bovino ☐ outros

Come hortaliças ou frutas cruas: ☐ sim ☐ não

4.1 Quantas vezes come salada crua na semana? _____

Água de consumo em casa: ☐ rede pública ☐ Outros. Qual? _____

Possui cão? ☐ sim ☐ não

Possui gatos? ☐ sim ☐ não

Se tem gatos tem com menos de um ano de idade? ☐ sim ☐ não

8.1 Gatos são alimentados com carne crua: ☐ sim ☐ não

Se sim, qual a freqüência:_____(por semana) tipo de carne:_____

8.2 Gatos têm contato com: ☐ terra ☐ areia

8.3 Gatos caçam? ☐ sim ☐ não

Presença de ratos: ☐ domicílio ☐ quintal ☐ instalações dos animais ☐ não
9.1 Previne roedores? ☐ sim ☐ não ☐ ratoeiras ☐ iscas ☐ raticidas ☐ outros
Mexe com terra ou areia? ☐ sim ☐ não
Qual a procedência do seu alimento no trabalho: ☐ come no refeitório ☐ traz de casa ☐ compra marmitex ☐ outro
Se come no refeitório assinale os alimentos que costuma ingerir: ☐ saladas cruas ☐ carne mal passada ☐ carne bem passada ☐ legumes cozidos
Ingere água da instituição: ☐ sim ☐ não
Você fez o exame para toxoplasmose? ☐ sim ☐ não
14.1 Qual o resultado? _____
14.2 Fez tratamento? ☐ sim ☐ não Qual ?
_____