UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
DEPARTAMENTO DE MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIA ANIMAL

ANÁLISE COMPARATIVA ENTRE DIFERENTES TÉCNICAS PARA DETECÇÃO DE PARASITOS ZOONÓTICOS EM AMOSTRAS VEGETAIS E DE SOLO

INGRID CARLA DO NASCIMENTO RAMOS

RECIFE – PE

2023

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
DEPARTAMENTO DE MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIA ANIMAL

ANÁLISE COMPARATIVA ENTRE DIFERENTES TÉCNICAS PARA DETECÇÃO DE PARASITOS ZOONÓTICOS EM AMOSTRAS VEGETAIS E DE SOLO

INGRID CARLA DO NASCIMENTO RAMOS

Tese apresentada ao Programa de Pós-Graduação em Biociência Animal da Universidade Federal Rural de Pernambuco como parte dos requisitos para obtenção do grau de Doutor em Biociência Animal.

Orientador: Prof. Dr. Leucio Câmara Alves

RECIFE – PE

2023

FICHA CATALOGRÁFICA

Dados Internacionais de Catalogação na Publicação
Universidade Federal Rural de Pernambuco
Sistema Integrado de Bibliotecas
Gerada automaticamente, mediante os dados fornecidos pelo(a) autor(a)

R 175a Ramos, Ingrid Carla do Nascimento
Análise comparativa entre diferentes técnicas para detecção de parasitos zoonóticos em amostras vegetais e de solo / Ingrid Carla do Nascimento Ramos. - 2023.
61 f.

Orientador: Leucio Camara Alves.
Inclui referências e anexo(s).

Tese (Doutorado) - Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Biociência Animal, Recife, 2023.

1. FLOTAC. 2. saúde pública. 3. diagnóstico. 4. parasito zoonótico. I. Alves, Leucio Camara, orient. II. Título

CDD 636.089

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

DEPARTAMENTO DE MEDICINA VETERINÁRIA

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIA ANIMAL

ANÁLISE COMPARATIVA ENTRE DIFERENTES TÉCNICAS PARA DETECÇÃO DE PARASITOS ZOONÓTICOS EM AMOSTRAS VEGETAIS E DE SOLO

INGRID CARLA DO NASCIMENTO RAMOS

Aprovada em________________de 2023

BANCA EXAMINADORA:

____

Profº. Drº. Leucio Câmara Alves

Departamento de Medicina Veterinária-

UFRPE Orientador

____

Profª. Drª. Renata Pimentel Bandeira de Melo

Departamento de Medicina Veterinária-UFRPE

____

Profº. Drº. José Pompeo dos Santos Filho

Departamento de Biologia-UFRPE

____

Drª. Maria Fernanda Melo Monteiro

Bióloga

____

Profª. Drª. Carina Lucena Mendes Marques

Departamento de Medicina Veterinária -UFRPE

AGRADECIMENTOS

Agradeço primeiramente A Deus, pois está escrito "... *sem Mim, nada podeis fazer*" (João15:5).

Aos meus pais Maria Isabel do Nascimento Ramos e Everaldo da Silva Ramos querenunciaram tantas coisas na vida com o próposito de nos permitir aprender.

Aos meus irmãos Carlos e Rafael, dois grandes tesouros para mim, valiosos e especiais.

Ao meu esposo, Geraldo, por estar sempre ao meu lado, compartilhando todos os momentos e representar tanto na minha vida.

Ao professor Leucio, por toda sua atenção e ter permitido minha presença no seu grupo de pesquisa. Acreditando e apostando que eu poderia aprender sobre coisas tão distintas da minha formação e contribuir de alguma forma. Sua paciência é generosa e caridosa, muito, muito, muito obrigada.

Agradeço a professora Gílcia por ter me estendido a mão tantas vezes e caminhado comigo durante a admissão no programa de pós-graduação.

Muitíssimo obrigada, de todo coração aos professores Pompeo e Milena por toda ajuda na minha qualificação. Gostaria de tê-los conhecido durante a execução do trabalho, suas contribuições seriam técnicas, brilhantes e valiosas.

E a todos que contribuíram direta ou indiretamente para a realização deste trabalho.

SUMÁRIO

LISTA DE FIGURAS

CAPÍTULO 3

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

RESUMO

Áreas públicas como parques, praias e feiras são de grande importância para dinâmica das cidades, pois agregam valores econômico, ambiental e social. Nesses locais é comum encontrarcães, gatos e outros animais frequentando livremente os espaços, o que pode muitas vezes representar um problema de saúde pública. Nesse contexto, o conhecimento do grau de contaminação do solo e de alimentos é tão útil aos propósitos epidemiológicos. Podendo-se determinar, dessa forma, o risco de exposição de frequentadores desses locais e fornecer dados para o estabelecimento de estratégias de manejo adequadas. Sendo assim, o objetivo desse estudo foi o de analisar a técnica de FLOTAC na detecção de parasitos zoonóticos em solo e hortaliças e comparar com as técnicas de Sedimentação espontânea e Centrífugo-sedimentação. Para isso, foram analisadas e comparadas 300 amostras de alface (*Lactuca sativa*) e 300 amostras de solo pelas técnicas acima propostas. Todas as técnicas utilizadas neste estudo foramcapazes de detectar larvas de parasitos tanto nas hortaliças como no solo de áreas de recreação. Sendo a técnica de FLOTAC mais eficaz nesta detecção. Os principais parasitos recuperados pertenciam as famílias Ascarididae, Ancylostomatidae e Trichuridae. Para efeito de comparação entre as técnicas foi determinado o índice kappa, e valores de sensibilidade, especificidade, valores preditivos positivos e negativos e precisão também foram calculados. Este estudo, trata-se do primeiro relato da utilização da técnica de FLOTAC para detecção de parasitos em solo e hortaliças. Tendo em vista os resultados obtidos, a técnica de FLOTAC apresentou-se mais apropriada do que as demais técnicas.

Palavras-chave: FLOTAC, saúde pública, diagnóstico, parasitos zoonóticos

ABSTRACT

Public areas such as parks, beaches and fairs are of great importance to cities, as they add economic, environmental and social benefits. In these places, cats and other animals are often represented in the spaces, which can often be a public health problem. In this, the knowledge of the degree of importance of the soil and food context is as epidemiological purposes. In thisway, the risk of exposure to frequent locations can be determined and data provided to the management establishments at these locations. Therefore, the aim of this study was to analyze the FLOTAC technique in the detection of zoonotic parasites in soil and vegetables and compare it with the Spontaneous Sedimentation and Centrifuge-sedimentation techniques.For this, 300 samples of lettuce (Lactuca sativa) and 300 soil samples were analyzed and compared by the techniques proposed above. All the techniques used in this study were ableto detect parasite larvae both in vegetables and in the soil of recreational areas. Being the mosteffective FLOTAC technique in this detection. The main parasites recovered belonged to the Ascarididae, Ancylostomatidae and Trichuridae families. For the purpose of comparison between the techniques, the kappa index was determined, and values of sensitivity, specificity, positive andnegative predictive values and precision were also calculated. This study is the first report of the use of the FLOTAC technique for the detection of parasites in soil and vegetables. In viewof the results obtained, the FLOTAC technique was more appropriate than the other techniques.

Keywords: FLOTAC, public health, diagnosis, zoonotic parasites

1.INTRODUÇÃO

As doenças parasitárias intestinais classificadas como geo-helmintíases fazem parte de um grupo de doenças negligenciadas que afetam mais de 1 bilhão de pessoas em todo o mundo prevalecendo em 149 países. Assim, segundo a Organização Panamericana de Saúde estima que 820 milhões de pessoas estão infectadas por *Ascaris lumbricoides*, 460 milhõespor *Trichuris trichiura* e 440 milhões por ancilostomídeos (OPAS, 2016). Nesse contexto, há ainda outras parasitoses intestinais causadas por protozoários e classificadas como protozooses. Essas, têm como principais agentes *Entamoeba* spp., *Giardia* spp., *Cryptosporidium* spp., entre outros. Tanto as geo-helmintíases quanto as protozooses intestinais são apontadas pela OMS como importantes causas de doenças entéricas em todo o mundo, principalmente de países em desenvolvimento (Rey, 2003).

No Brasil, estas infecções estão presentes em todas as regiões, principalmente nas periferias de centros urbanos e zona rural, visto que a realidade das populações que vivem sob essa situação é a de falta de saneamento básico e ambiental (SNIS, 2018). Entre os anos de 2005 e 2016, inquéritos sobre essas zoonoses e outras doenças negligenciadas foram realizados em todo o território nacional. Os mesmos apontaram uma diminuição na infecção por esses agentesnas regiões Sul e Sudeste, mas, apontaram que não houve diminuição nas regiões Norte e Nordeste (OPAS, 2016).

Durante muito tempo, cães, gatos e outros animais vêm sendo apontados como reservatórios ou hospedeiros definitivos de endoparasitas de importância zoonótica. Suas fezesem locais públicos como parques, praias e feiras livres são prováveis fontes de contaminação ambiental. O ser humano expõe-se a esses parasitos em contato direto com esses animais ou através da água, alimentos contaminados, mãos e solo. Uma vez que há, no Brasil, um grande número de animais errantes que circulam livremente por essas áreas expondo a população às infecções por zoonoses. Bem como, estudos (MELO, 2011; FRIAS, 2012; GREGÓRIO, 2012)relatam a correlação entre manipuladores de alimentos e contaminação parasitária em hortaliças decorrentes da água utilizada no manejo desses vegetais, do adubo utilizado no trato cultural etambém de hábitos inadequados de higiene. Desse modo, faz-se necessário a implementação demedidas para o controle dessas doenças.

Há décadas o controle dessas doenças negligenciadas vem sendo discutidas em âmbito mundial, através de tratamentos, métodos diagnósticos, estratégias de controle, estabelecimentos de metas para cada país e região em questão. Muitas dessas parasitoses intestinais poderiam ser eliminadas com melhores condições de saneamento, acesso a água tratada, controle de vetores, tratamentos disponíveis, campanha de educação ambiental e sanitária e quando recomendado, administração de medicamentos em massa, tanto em humanoscomo em animais (OPAS, 2016).

Assim sendo, torna-se tão importante quanto necessário o correto diagnóstico dessas parasitoses que acometem tanto humanos como animais, afim de diminuir o risco de tratamentoinadequado e agravamento da situação dos mesmos, passando pela escolha da técnica diagnóstica mais adequada e comparando com outros métodos. Não apenas de um método, masde vários, com diferentes fundamentos, seja flutuação, centrifugação, termo- hidrotropismo, sedimentação entre outros, que sejam capazes de forma eficiente avaliar as condições ambientais e prevenir possíveis contaminações. Muitos autores já descreveram sobre a falta de padronização de técnicas nessa área. E sugerem que os pesquisadores não só escolham determinadas técnicas, mas as testem e comparem (COUVILLION, 1993).

Atualmente, a técnica de FLOTAC tem sido utilizada para detecção de ovos, larvas e oocistos de parasitos gastrointestinais de diferentes espécies animais, incluindo o homem (CRINGOLI et al., 2010) apresentando alta sensibilidade e especificidade quando comparado as demais técnicas (LIMA et al., 2015). No entanto, não existem relatos da utilização desta técnica para detecção de ovos, cistos, larvas de parasitos em hortaliças e solos. Diante do exposto, o presente estudo teve como objetivo comparar a técnica de FLOTAC na detecção delarvas e ovos presentes em hortaliças e solos de área parques e praias da Região Metropolitanado Recife.

2. REVISÃO DE LITERATURA

2.1Geo-helmintoses e Protozooses

As geo-helmintíases constituem um grupo de doenças parasitárias que acometem tanto humanos como animais. Em linhas gerais, esses parasitos apresentam dois ciclos de vida que constituem duas fases: uma de vida livre que ocorre no solo, sendo dependente de inúmeras condições edafoclimáticas; e outra fase evolutiva que ocorre dentro do hospedeiro. Sendo os cães, gatos e outros animais considerados reservatórios/hospedeiros definitivos desses parasitose o homem um hospedeiro acidental (OMS, 2018).

Já as protozooses são de ciclo biológico diferenciado para cada agente etiológico, que de modo geral são de veiculação hídrica ou por alimentos. As pessoas e os animais se contaminam através da ingestão de água e alimentos contaminados por cistos, esses cistos evoluem normalmente no intestino do hospedeiro, esses se multiplicam e são liberados nas fezes. Sejam geo-helmintos ou protozoários, ambos alternam as fases do seu ciclo evolutivo emvida livre no ambiente e vida parasitária no hospedeiro (SILVA et al, 2012).

Dentre as principais espécies de parasitos encontrados em diversos substratos (fezes, solos, vegetais, superfícies entre outros) destacam-se frequentemente: *Ascaris* spp., *Trichuris* spp., ancilostomídeos (NOLLA et al., 2005; MATESCO et al., 2006; LIMA et al. 2015) e também os protozoários como *Entamoeba* sp., *Giardia* sp., entre outros. Todos podem causar situações graves de saúde com doenças que afetam humanos e animais devido ao seu potencialzoonótico (PEREIRA et al, 2016).

2.2 Alguns agentes causadores de zoonoses parasitárias

2.2.1*Ascaris* sp. (Linnaeus, 1758)

Considerado um geo-helminto, conhecido popularmente como lombriga ou “bicha”, quando adulto tem corpo cilíndrico, alongado e pode chegar até 40 cm de comprimento, as fêmeas são maiores e mais robustas que os machos (Rey et al, 2001).

Esses parasitos se caracterizam por apresentar um ciclo de vida homoxeno, envolvendoa participação de um único hospedeiro. Esse ciclo ocorre quando os ovos são eliminados no ambiente através das fezes do hospedeiro que pode ser humano ou animal. No ambiente, em condições ideais de temperatura e umidade após 15 dias os embriões estão formados dando origem a primeira larva do tipo L1. Após mais 1 semana essa larva se transforma em L2 e posteriormente em L3 tornando-se infectante. O hospedeiro ingere o ovo contendo a larva infectante no solo, essa larva percorre o trato digestivo e eclode no intestino delgado, seguindo todo o seu ciclo evolutivo até L4 e L5 agora dentro do hospedeiro (Rey et al,2001).

A contaminação ocorre acidentalmente a partir da ingestão de ovos contendo a larva infectante L3 por meio de solo, água e alimentos. Quanto a prevenção é de grande importânciao saneamento ambiental, higienizar os alimentos, principalmente os que serão consumidos in natura e adoção de medidas de higiene básicas.

2.2.2 *Ancylostoma* sp.

Agente causador da doença conhecida como Ancilostomíase Ancilostomose, Necatoríase ou “Amarelão”. É um geo-helminto, quando adultos atingem 1 cm de comprimento, sendo as fêmeas maiores que os machos. O corpo é cilíndrico, rígido e afilado nas extremidades (Rey, 2008).

O *Ancylostoma* sp. tem ciclo direto, que ocorre quando os ovos são eliminados no meioexterno através das fezes de cães e gatos. No meio ambiente, ocorrendo condições propícias aodesenvolvimento desses ovos, ocorre o embrionamento e formação da larva L1. Esta larva eclode em 1 a 2 dias permanecendo ativa no solo e ingerindo alimentos. Após formar uma novacutícula e perder a cutícula externa evolui para L2 e após 5 a 10 dias transforma-se em L3 tornando-se infectante para o hospedeiro (Rey, 2008).

Entretanto, em humanos esses parasitos não conseguem completar o seu ciclo, por isso realizam a migração pela pele. Ao penetrar na pele do hospedeiro causam lesões semelhantesa um mapa conhecido como “bicho geográfico” ou Larva Migrans Cutânea (LMC), a larva é oprincipal agente etiológico da LMC em humanos (Júnior et al., 2015). Essas lesões ocorrem geralmente em membros inferiores e nádegas. No Brasil a principal fonte de contaminação com essas larvas são solos de parques públicos, orla das praias, caixas de areias em área de recreaçãoe jardins (Silva et al.

2012).

2.2.3 *Trichuris* sp. (Roederer, 1761)

Esse é o agente causador da doença Tricuríase, popularmente conhecida como "chicote". Os cães selvagens e domésticos assim como os humanos podem ser infectados por parasitos pertencentes a família Trichuridae. Os vermes adultos medem cerca de 4 cm, possuem morfologicamente um aspecto de chicote sendo as fêmeas maiores que os machos, outra característica é o dimorfismo sexual. Os ovos são facilmente reconhecíveis ao microscópio devido ao seu formato de barril, composto por três cascas sendo a externa mais espessa e de cor marrom e duas cascas internas mais claras e de aspecto hialino (DiCesare et al, 2012).

É um geo-helminto e seu ciclo biológico ocorre quando esses ovos chegam ao meio externo contendo apenas uma massa de células. Encontrando um ambiente sombreado, úmido e com temperatura adequada, inicia-se o processo de embriogênese que se completa em torno de 15 a 30 dias. Os ovos larvados L1 permanecem infectantes no solo por um ano e em laboratório por cerca de cinco anos. Esses ovos podem ser disseminados por insetos, poeira e a infecção em humanos se dá por ingestão acidental através de alimentos contaminados e maus hábitos de higiene (Traversa, 2011).

2.2.4 *Entamoeba* sp.

De nome popular "ameba" este é o agente causador da doença conhecida por amebíase. Há duas espécies, a *E. dispar* e a *E. histolytica*, sendo esta última a espécie patogênica para humanos. Sua transmissão ocorre por via oral, através de água e alimentos contaminados (Schuster et al. 2004), principalmente se ingeridos crus como hortaliças.

Seu ciclo biológico ocorre quando a partir da ingestão do cisto, passa pelo estômago, sofre com a ação do suco digestivo e se encaminha ao intestino delgado. Lá, esse cisto sofre o desencistamento, eclode e libera o metacisto, este por sua vez, passa por sucessivas divisões nucleares originando trofozoítos. Estes trofozoítos saem do intestino delgado, migram para o intestino grosso e formam colônias aderidas a mucosa do intestino. Essas colônias formam os pré-cistos que se transformam em cistos e esses são eliminados pelas fezes. A ausência de saneamento básico, associado a inadequados hábitos de higiene, estão ligados a propagação desse agente biológico.

2.2.5 *Giardia* sp.

Agente causador da “giardíase”, embora a infecção ocorra geralmente através de águae alimentos contaminados é também considerada zoonose pois os humanos podem se infectar ingerindo acidentalmente cistos eliminados por outros animais (Coelho et al., 2001; Nolla et al., 2005).

A *Giardia* sp. apresenta duas formas evolutivas, trofozoíto e cisto. O trofozoíto é a formavegetativa de multiplicação, habita o intestino delgado do hospedeiro e causa a doença giardíase. Já o cisto é a forma de resistência e é responsável pela transmissão do parasito. Nestafase, a membrana cística formada de glicoproteínas protege o parasito contra as adversidades do meio ambiente como temperatura, umidade e produtos químicos (Thompson, 2004). Emboraa fonte de contaminação seja muitas vezes difícil de ser determinada, os riscosde infecção são variados e tem como critérios deterimantes a falta de saneamento básico, condições de higienedo ambiente e nível socioeconômico da população (Almeida et al, 2010).

2.3 Contaminação do Solo

Os espaços públicos destinados às atividades recreativas, esportivas e educativas,constituídos por matrizes ambientais de contato primário como solo e água, podem oferecer riscos à saúde humana devido ao crescimento populacional de animais errantes e domiciliadosem todo Brasil (SOTERO-MARTINS et al., 2014). A contaminação do solo pode ocorrer tanto pela falta de hábito dos proprietários desses animais ao não recolherem seus dejetos, comopela presença de cães e gatos errantes nos espaços urbanos (CASSENOTE et al., 2011). Mas também, pelo destino dado aos dejetos humanos, das relações de higiene pessoal e condições de saneamento básico e ambiental das cidades (SILVA et al., 1991).

Inúmeros fatores como natureza do solo, temperatura, umidade, entre outros, podem influenciar direta ou indiretamente na relação de sobrevivência desses parasitos no solo. Uma vez que tanto os geo-helmintos como alguns protozoários passam parte do seu ciclo evolutivo no meio ambiente. Entretanto, o sucesso da continuidade desse ciclo depende de sua capacidade de resistir as intempéries edafoclimáticas por meio de características inerentes a cada espécie (PEDROSA et al., 2014). O solo funciona como hospedeiro intermediário, recebe fezes ou água contaminada por parasitos em estádios não-infectantes. E assim oferece- lhes suporte e condições para o desenvolvimento para posteriormente transmití-los aos humanos (SILVA et al, 1991).

No Brasil, estudos tem demonstrado a relevância de algumas espécies como *Toxocara* spp., *Ancylostoma* sp. e *Ancylostoma* spp. entre os principais geo-helmintos presentes no solo (BORTOLLATO et al., 2013; RÊGO et al., 2017). Em diversos países do mundo como Cuba, Polônia, Chile e Iraque também têm demonstrado a prevalência desses parasitos no solo, mas também *Ascaris* spp. e *Trichuris* spp. têm sido bastante relatado (MELIN et al. 2016; PEÑA etal. 2017; NOORALDEEN et al, 2015).

2.4 Mecanismos ecológicos de sobrevivência desses parasitos

O solo é um dos principais compartimentos da biosfera em termo de reservatório biológico dinâmico (BERENDSEN et al. 2012). Encontra-se estruturado de maneira heterogênea, o que possibilita a ocorrência de micro-hábitats de muitos microorganismos, parasitos de vida livre e com potencial zoonóticos (DUCHIELA et al. 2013). A formação desses micro-hábitats encontra-se associados diretamente as proporções de areia, silte e argila que funcionam como suporte físico para desenvolvimento e sobrevivência desses organismos. Assim como condições de temperatura, umidade, matéria orgânica, entre outras (MOREIRA; SIQUEIRA, 2006).

Com as alterações do clima vem ocorrendo mudanças relevantes nas coleções naturais de água, nos alimentos e no ambiente, o que afeta diretamente a sobrevivência e disseminação de ovos e larvas de parasitos (LAFFERTY, 2009). Por exemplo, estudos apontam que as larvas de ancilostomídeos são mais ativas a temperaturas entre 30 e 35˚C (Udonsi & Atata 1987). Outros autores (CAMERON, 1963; REY, 1991) destacaram que para ovos de *Ascaris* spp. não ocorre embrionamento se a temperatura estiver abaixo de 18˚C, e a 68˚C ocorre morte instantânea. Entretanto, se expostos a uma temperatura de 23 a 35˚C por 40 dias as larvas se desenvolvem normalmente. Há estudos (RUDOLFS et al. 1951; STEVENSON P., 1979) que revelam que ocorre inativação de ovos em 10 minutos quando a temperatura atingir 55 e 60˚C.

O tipo de solo e umidade também tem influência sobre a sobrevivência e desenvolvimento desses parasitos. Para alguns estudiosos (MABASO et al. 2003; RIESS et al.2013) solos arenosos e pouco úmidos facilitam o deslocamento de larvas que podem se protegerda dessecação. Todavia, há outros (GUNAWARDENA et al. 2005; RIESS et al. 2013) que indicam ser os solos com maior teor de argila e umidade os que favorecem a sobrevivência e deslocamento dessas larvas. As plantas também tem um papel importante no mecanismo de sobrevivência, uma vez que sua copa, auxilia diminuindo a ação direta do sol no solo, protegendo ovos e larvas de temperaturas extremas. E também, dependendo do tipo de raiz, essas plantas podem tornar o solo mais ou menos aerado, influenciando no balanço entre oxigênio e gás carbônico do mesmo (COURA L. C, 1971).

2.5 Contaminação de vegetais

Devido ao seu alto teor nutritivo, as hortaliças constituem um importante alimento na dieta diária (SHARMA et al. 2014). Além de apresentar ação antioxidante ainda contribui parao bom funcionamento adequado do organismo. Entre as diversas hortaliças há as que são consumidas cruas, como as folhosas de modo geral. Sendo alface, agrião e rúcula as mais consumidas pela população (MONTANHER et al. 2007).

A principal forma de contaminação de hortaliças por enteroparasitos ocorre principalmente pelo uso de água contaminada. Muitas vezes essa água está poluída pordejetos fecais de origem humana ou animal. Esses dejetos, por sua vez, acabam sendo disseminados através de água de irrigação, adubo orgânico dirigido ao plantio contaminado com material fecal(SILVA et al. 2010). Além disso há a forma inadequada de como essas hortaliças são manipuladas e transportadas (NOLLA E CANTO, 2005). Pouco mencionado, mas não menos importante, há também a contaminação com animais vetores e transmissores de doenças comobarata, moscas e ratos.

Enfim, como as fontes de contaminação de hortaliças são amplas, logo, torna-seindispensável como medida fundamental, a boa higiene. Seja por parte de quem produz o alimento, de quem o manipula no campo, nos mercados, na indústria da alimentação etambémdo consumidor final que deve desenvolver uma postura crítica acerca da segurança alimentar daquilo que consome.

2.6 Métodos diagnósticos para detecção de parasitos

Dentre os métodos diagnósticos para detecção de parasitos, é possível destacar Willis (1921), Faust (1938), Hoffman (1934), Baermann (1917), entre outros. Todos esses métodos são amplamente difundidos e recomendados por laboratórios de referência em todo mundo. Sãotécnicas baseadas nos princípios de flotação e/ou centrifugação que com o passar do tempo foram passando por modificações e adaptações. E com essas modificações, as técnicas foram consequentemente evoluindo em diversos critérios científicos.

Começando por Willis-Mollay, no ano de 1921, seu método é baseado no princípio da flutuação espontânea. É indicado para pesquisa de ovos leves, principalmenteancilostomídeos. Depois vem Hoffman, método proposto por Pons e Janer em 1934, com sua técnica baseada na sedimentação espontânea, que permite a pesquisa de ovos mais densos, larvas de helmintos e cistos de protozoários. Já em 1939, foi realizada a primeira citação da técnica de McMaster. Essa citação foi feita pelos pesquisadores Gordon e Whitlock e consiste na utilização de uma lâmina especial denominda "câmara de McMaster". Essa câmara permite a contagem de OPG (ovos por grama de fezes), e conhecendo-se esse número é possível calcular o número de vermes e a partir daí adotar medidas de manejo que reduzam esse quantitativo.

Há ainda outra técnica que consiste na contagem de ovos por grama de fezes, que é a técnica de Kato-Katz. Esse método foi desenvolvido por Kato & Miura (1954), publicado no ano de 1960, estudado e aperfeiçoado por Kato ainda no mesmo ano. Estudado por Komiya & Kobayashi (1966); Martin & Beaver (1968); Chaia et al. (1968); Katz, Coelho & Pellegrino (1970) e enfim modificado por Katz, Chaves & Pellegrino (1972). Conforme esses autores trata- se de um procedimento muito simples e eficiente, considerado padrão ouro para o diagnóstico de esquistossomose devido ao seu alto grau de sensibilidade.

Em 1955 nasce a técnica baseada no princío da sedimentação por centrifugação, denominda Blagg ou MIFC, indicada tanto para estruturas parasitárias leves como pesadas. É um método rápido, sensível e de fácil execução, entretanto sua desvantagem ficacorrelacionada ao uso de centrífuga. Enfim, são inúmeros os métodos diagnósticos, todos apresentam vantagens e desvantagens. E as estruturas parasitárias apresentam peso, sobrevida,sensibilidade diferentes, não há um único método capaz detectá-las todas ao mesmo tempo. Principalmente quando a origem dessa estrutura é distinta. Faz-se necessário detectar parasitos em fezes, no solo, na água, na vegetação, alimentos e outras diversas fontes. Outra ferramenta desenvolvidaem 2010 denominda FLOTAC vem se consolidando nos últimos anos como uma técnica quantitativa e qualitativa na pesquisa de parasitos. Desenvolvida inicialmente para detecção deparasitos em fezes de animais, teve seu uso estendido a ciência humana e vegetais.

2.7 FLOTAC

O FLOTAC trata-se de uma nova ferramenta para pesquisa e diagnóstico utilizada para detecção de ovos, larvas e oocistos de parasitos gastrointestinais de diferentes espécies animais. Segundo Cringoli et al. (2010), os resultados parasitológicos obtidos por meio desta técnica sãoaltamente precisos, aumentando em até 10 vezes a possibilidade de detecção de ovos, oocistos e larvas, quando comparado com técnicas clássicas. Outro fator que contribui para alta eficácia do FLOTAC na detecção de parasitos gastrointestinais está relacionado ao desenho do aparelho, o qual foi projetado com duas câmaras amostrais de flutuação com capacidade para 5 mililitros cada, ambas recobertas por uma grade contendo 12 colunas distribuídas em um quadrante de 18x18 milímetros, a fim de aumentar a área de fixação dos ovos e larvas após o processo de flotação. A alta sensibilidade, especificidade e acurácia da técnica FLOTAC está relacionada também a variação das soluções de flotação utilizadas que são o cloreto de sódio e o sulfato de zinco. Apresentando especificidade gravitacional de 1.20 e 1.35 respectivamente sobre a mesma amostra, aumentando a detecção e quantificação dos diferentes gêneros de parasitos gastrointestinais.

3. REFERÊNCIAS BIBLIOGRÁFICAS

ALMEIDA C. G.; MARQUES S. M. T.; MIQUELLUTI D. J.; QUADROS R. M. Giardíase em crianças e cães do mesmo domicílio e de bairros periféricos de Lages, Santa Catarina. Revista Ciência & Saúde, Porto Alegre, v. 3, n. 1, p. 9 - 13, 2010.

BERENDSEN R. L.; PIETERSE C. M. J.; BAKKER P. A. H. M. The rhizosphere microbiome and plant health. Trends in Plant Science, Oxford, v. 8, p. 478 - 486, 2012.

BAERMANN, G. Eine einfache methode zur auffindung von ankylostomum (Nematoden) larven in erdproben. Geneeskunding Tijdschrift voor Nederlandsch-Indië, v. 57, p. 131-137, 1917.

BORTOLATTO J. M; SNIEGOVSKI M. M; BERNARDI S. T; CRIPPA L. B; RODRIGUES A. D. Prevalence of parasites with zoonotic potential in soil from the main public parks andsquares in Caxias do Sul, RS, Brazil. Revista Patologia Tropical, v. 46, n. 1, p. 85 – 93, 2017.

CAMERON T. W. M. — Environmental factors related to the free-living stages. WHO - WorldHealth Organization, Helminth, 15, 1963.

CASSENOTE A. J. F.; NETO J. M. P.; LIMA-CATELANI A. R. A.; FERREIRA A. W. Contaminação do solo por ovos de geo-helmintos com potencial zoonótico na municipalidade de Fernandópolis, Estado de São Paulo, entre 2007 e 2008. Revista da Sociedade Brasileira deMedicina Tropical, v. 44, n. 3, p. 371 - 374, 2011.

CHAIA G.; KATZ N. & GASPER D. — Coprological diagnosis of Schistosomiasis II — Comparative study of quantitative methods. Revista Instituto Medicina Tropical, São Paulo, v. 10, p. 349 - 353, 1968.

COELHO L. M. P. S.; DINI C. Y.; MILMAN M. H. S. A. & OLIVEIRA S. M. Toxocara spp eggs in public squares of Sorocaba, São Paulo State, Brazil. Revista do Instituto de Medicina Tropical de São Paulo, v. 43, p. 189 - 191, 2001.

COUVILLION C. E. Estimation of the numbers of trichostrongylid larvae on pastures. Veterinary Parasitology, v. 46, p. 197 – 203, 1993.

COURA L. C. Fatores relacionados a transmissão de geohelmintíases. Revista da Sociedade Brasileira de Medicina Tropical, v. 8, p. 223 – 233, 1971.

CRINGOLI G., RINALDI L., MAURELLI M. P., UTZINGER J. FLOTAC: new multivalente techniques for qualitative and quantitative copromicroscopic diagnosis of parasitesin animals and humans. Nature, v. 5, p. 503 - 515, 2010.

DI CESARE A.; CASTAGNA G.; MELONI S.; OTRANTO D.; TRAVERSA D. Mixed trichuroid infestation in a dog from Italy. Parasites & Vectors, 5:128, 2012.

DUCHICELA, J. Soil aggregate stability increase is strongly related to fungal community succession along an abandoned agricultural field chronosequence in the Bolivian Altiplano. Journal of Applied Microbiology, Oxford, v. 50, p. 1266 - 1273, 2013.

DINIZ D. S.; TEIXEIRA E. S.; ALMEIDA W. G. R.; SOUZA M. S. M. COVID-19 and Neglected Diseases in the face of inequalities in Brazil: a matter of sustainable development. Revista Saúde Debate FIOCRUZ – Rio de Janeiro, v. 45, p. 43 – 55, 2021.

FAUST, E. C., D’ANTONI, J. S., ODOM, V., MILLER, M. J., PERES, C., SAWITZ, W., THOMEN, L. F., TOBIE, J., WALKER, H. A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. I. Preliminary communication. American Journal of Tropical Medicine and Hygiene, v. 18, p. 169-183, 1938.

FRIAS A. A. T.; SILVA J. B.; TOZATO H. C. Ocorrência de ovos de helmintos em hortaliçascomercializadas na cidade de Apucarana (PR). Semina: Ciências Biológicas e da Saúde, Londrina, v. 33, n. 1, p. 35 - 42, 2012.

GREGÓRIO D. S. Estudo da contaminação por parasitas em hortaliças da região leste de São Paulo. Science in Health, São Paulo, v. 3, n. 2, p. 96 - 103, 2012.

GUNAWARDENA G. S. A; KARUNAWEERA N. D; ISMAIL M. M. Effects of climatic, socio-economic and behavioural factors on the transmission of hookworm (Necator americanus) on two low-country plantations in Sri Lanka. Annals of Tropical Medicine and Parasitology, v. 99, p. 601 - 9, 2005.

HOFFMAN W. A.; PONS J. A.; JANER J. L. The sedimentation concentracion methods in Schistosomiases masoni. Puerto Rico Journal of Public Health and Tropical Medicine, v. 9, p. 283-289, 1934.

JÚNIOR A. L. F.; ARAÚJO K. B. S.; MEDEIROS V. S. Ocorrência de parasitas com potencialzoonótico em fezes de cães coletadas em vias públicas da cidade de Natal. Revista Humano Ser
– UNIFACEX, Natal-RN, v. 1, n. 1, p. 52 - 59, 2015.

LAFFERTY K. D. The ecology of climate change and infectious diseases. Ecology, v. 90, p.888 - 900, 2009.

LIMA V. F. S.; CRINGOLI G.; RINALDI L.; MONTEIRO M. F. M.; CALADO A. M. C.; RAMOS R. A. N.; SANTOS P. O. M.; ALVES L. C. A comparison of mini-FLOTAC and FLOTAC with classic methods to diagnosing intestinal parasites of dogs from Brazil. Parasitology Research, v. 114, p. 3529 – 3533, 2015.

KATO K.; MIURA M. Comparative examinations. Japão. Journal Parasitology, v. 3, p. 35,1954.

KATZ N.; CHAVES A.; PELLEGRINO J. A simple device for quantitative stool thick-smeartechnique in schistosomiasis mansoni. Revista Instituto Medicina Tropical, São Paulo, v. 14, p. 397 - 400, 1972.

KATZ N.; COELHO P. M. Z. & PELLEGRINO J. — Evaluation of Kato's quantitative method through the recovery of S. mansoni. The Journal Parasitology, v. 56, p.1032 - 1033,1970.

KOMIYA Y. & KOBAYASHI A. — Evaluation of Kato's thick smear technique with cellophane cover for helminth eggs in feces. Japan Journal Medicine Science Biology, v. 19, p. 59 - 64, 1966.

MABASO M. L. H; APPLETON C. C.; HUGHES J. C.; GOUWS E. The effect of soil type and climate on hookworm (Necator americanus) distribution in KwaZulu-Natal, South Africa.Tropical Medicine and International Health, v. 8, p. 722 - 7, 2003.

MARTIN L. K. & BEAVER P. C. — Evaluation of Kato thick-smear technique for diagnosisof Helminth infections. American Journal Tropical Medicine & Hygiene, v. 17, p. 382 - 391, 1968.

MATESCO V. C.; MENTZ M. B.; ROTT M. B.; SILVEIRA C. O. Contaminação sazonal porovos de helmintos nas praias de Ipanema, em Porto Alegre, Rio Grande do Sul, Brasil. Revista de Patologia Tropical, v. 35, p. 135 - 141, 2006.

MELIN-COLOMA M.; VILLAGUALA-PACHECO C.; LISBOA-NAVARRO R.; LANDAETA-AQUEVEQUE C. Estudio de la presencia de huevos de Toxocara sp. en suelos de áreas públicas de la ciudad de Chillán, Chile. Revista Chilena de Infectologia, v. 33, p. 428 – 432, 2016.

MELO A. C. F. L.; FURTADO L. F. V.; FERRO T. C.; BEZERRA K. C.; COSTA D. C. A.; COSTA L. A.; SILVA L. R. Contaminação parasitária de alfaces e sua relação com enteroparasitoses em manipuladores de alimentos. Revista Trópica – Ciências Agrárias e Biológicas, v. 5, p. 47 - 52, 2011.

MONTANHER C. C.; CORADIN D. C.; FONTOURA S. E. Avaliação parasitológica em alfaces (*Lactuca sativa*) comercializadas em restaurantes self-service por quilo, da cidade de Curitiba, Paraná, Brasil. Estudos de Biologia, v. 29, p. 63 - 71, 2007.

MOREIRA, F. M. S.; SIQUEIRA, J. O. Microbiologia e bioquímica do solo. Lavras: Editora UFLA, p. 744, 2006.

NOLLA, A. C.; CANTO, G. A. Prevalência de enteroparasitoses em manipuladores de alimentos, Florianópolis, SC. Revista Sociedade Brasileira de Medicina Tropical, v. 38, p.524 - 525, 2005.

NOORALDEEN K. Contamination of public squares and parks with parasites in Erbil city, Iraq. Annals of Agriculture and Environmental Medicine, v. 22, p. 418 - 420, 2015.

ORGANIZAÇÃO MUNDIAL DE SAÚDE (WHO). Infectious diseases of potential risk for travelers. 2018.

ORGANIZAÇÃO DAS NAÇÕES UNIDAS (ONU). O direito humano, a água e saneamento. 2010.

ORGANIZAÇÃO PAN-AMERICANA DA SAÚDE (OPAS). Plano de ação para a eliminação de doenças infecciosas negligenciadas e pós eliminação 2016 - 2022. 68ª Sessãodo Comitê Regional da OMS para as Américas, 2016.

PEDROSA E. F. N. C.; CABRAL B. L.; ALMEIDA P. R. S. F.; MADEIRA M. P.; DE CARVALHO B. D.; BASTOS K. M. S. & VALE J. M. Contaminação ambiental de areia depraias de Fortaleza - Ceará. Journal of Health & Biological Sciences, v. 2, p. 28 - 35, 2014.

PEREIRA A.; MARTINS A.; BRANCAL H.; VILHENA H.; SILVA P.; PIMENTA P. Parasitic zoonoses associated with dogs and cats: a survey of Portuguese pet ownersawareness and deworming practices. Parasites & Vectors, 9:245, 2016.

PEÑA I. G; VIDAL F. F, TORO A. R; HERNÁNDEZ A.; ZAPATA M. M. R. Zoonosis parasitarias causadas por perros y gatos, aspecto a considerar en salud pública de Cuba.Revista Electronica de Veterinaria, v. 18, p. 1 - 11, 2017.

RÊGO F. F; AZEVEDO H. C. N; RÊGO T. Y. M.; DANTAS D. S.; COSTA E. R. G. Avaliação da influência sazonal na incidência de ovos e larvas de parasitos intestinais empraças no município de Macapá-AP. Vigilância Sanitária Debate, v. 5, p. 72 - 76, 2017.

REY, L. Parasitologia. Parasitos e doenças parasitárias do homem nas Américas e na África.2°ed. Rio de Janeiro: Guanabara Koogan, 731p, 1991.

REY, L. Parasitologia: parasitos e doenças parasitárias do homem nos trópicos ocidentais.4°ed. Rio de Janeiro: Guanabara Koogan, 883p, 2008.

REY L. Parasitologia. Rio de Janeiro. Editora Koogan, 2001.

RIESS H., CLOWES P., KROIDL I., KOWOUR D. O., NSOJO A., MANGU C. Hookworm infection and environmental factors in Mbeya region, Tanzania: a cross-sectional, population-based study. PLOS Neglected Tropical Diseases, v. 7, p. 1 – 11, 2013.

RUDOLFS W.; FALK L. L.; RAGOTZKIE R. A. Contamination of vegetables-grown in polluted soil. V. Helminthic Decontamination. Sewage and Industrial Wastes, v.23, p. 853 - 860, 1951.

SCHUSTER F. L.; VIVESVARA G. S. Amoebae and ciliated protozoa as a causal agent of waterborne zoonotic disease. Veternary Parasitolology, v. 126, p. 91 - 120, 2004.

SHARMA S.; SHEEHY T.; KOLONEL L. Sources of vegetables, fruits and vitamins A, C andE among five ethnic groups: results from a multiethnic cohort study. Europe Journal Clinical Nutrition, v. 68, p. 384 – 391, 2014.

SILVA J. P., MARZOCHI M. C. C. & SANTOS E. C. L. Avaliação da contaminação experimental de areias de praias por enteroparasitas: pesquisa de ovos de Helmintos. Cadernosde Saúde Pública, v. 7, p. 90 - 99, 1991.

SILVA J. R. M.; SILVA R. R.; BARROS L. A.; ALMEIDA F. B. Introdução à helmintologia.Conceitos e Métodos para a Formação de Profissionais em Laboratório de Saúde. Rio de Janeiro, v.5, p.191-249, 2012.

SILVA, L. P.; SILVA, R. M. G. Ocorrência de enteroparasitos em centros de educação infantilno município de patos de minas, MG, Brasil. Bioscience Journal, Uberlândia, v. 26, p.147 - 151, 2010.

SISTEMA NACIONAL DE INFORMAÇÕES SOBRE SANEAMENTO (IBGE/SNIS) -

snis.gov.br. Ano 2018.

SOTERO-MARTINS A.; DUARTE A. N.; CARVAJAL E.; SARQUIS M. I. M.; FERNANDES O. C. C. Controle da qualidade microbiológica e parasitária em áreas de recreação. Revista Eletrônica Gestão e Saúde, v. 5, p. 2059 – 2078, 2014.

STEVENSON, P. The influence of environmental temperature of the rate of development of *Ascaris suum* eggs in Great Britain. Research in Veterinary Science, v. 27, p. 193-196, 1979.

TRAVERSA D. Are we paying too much attention to cardio-pulmonary nematodes and neglecting old-fashioned worms like Trichuris vulpis. Parasites & vectors, v. 4:32, 2011.

THOMPSON R. C. A. The zoonotic significance and molecular epidemiology of Giardia and giardiasis. Veterinary Parasitology, v.126, p.15 - 35, 2004.

UDONSI J. K, ATATA G. Necator americanos: temperature, pH, light, and larval development,longevity, and desiccation tolerance. Experimental Parasitology, v. 63, p. 136- 142, 1987.

WILLIS H. H. A simples levitation method for detection of hookworm ova. Medical Journal ofAustralia, v.2, p. 375-376, 1921.

4. OBJETIVOS

OBJETIVO GERAL

- Comparar a técnica de FLOTAC com outros métodos clássicos na detecção de parasitos zoonóticos em hortaliças e solos.

OBJETIVOS ESPECÍFICOS

- Avaliar a técnica de FLOTAC para recuperação parasitos zoonóticos em alface (*Lactuca sativa*)

- Comparar as técnicas de FLOTAC, Sedimentação espontânea e Centrífugo-Sedimentação para recuperação parasitos zoonóticos em solos de praias e parques da Região Metropolitana do Recife **-** PE

Capítulo I

An additional asset for the FLOTAC® technique: detection of gastrointestinal parasites in vegetables

Capítulo estruturado em formato de artigo e publicado na revista *Acta Parasitologica* (v. 64, p. 423-425, 2019)

An additional asset for the FLOTAC® technique: detection of gastrointestinal parasites in vegetables

ABSTRACT

Introduction: Gastrointestinal parasites are considered amongst the most important threats to veterinary and human health worldwide. The transmission of these parasitic diseases usually involves the accidental ingestion of oocysts, cysts, eggs, and larvae, whose detection is generally achieved by classical coproparasitological methods, based on sedimentation and/or flotation techniques. These procedures might be inefcient or display a low sensitivity when performed on food matrices. This study aimed to assess the value of the FLOTAC technique for the detection of diferent parasitic stages of gastrointestinal parasites of domestic animals in fresh lettuce. **Material and methods**: Samples (n=100) were purchased from public markets located in the metropolitan region of Recife (Brazil). **Results**: A total of 79% scored positive for eggs and/or cysts of gastrointestinal parasites. Protozoa and nematodes were detected in 32% (32/100) and 64% (64/100) of samples, respectively, with cysts of the genus Entamoeba (32%) and eggs of nematodes of the order Strongylida (30%) being themost frequently diagnosed. **Conclusions**: The findings herein reported demonstrate that the FLOTAC technique can be successfully applied for recovering food-borne parasites of medical and veterinary concern in food matrices.

Keywords: FLOTAC®, Helminths, Vegetables, Contamination, Entamoeba.

1. Introduction

Infections by gastrointestinal parasites in animals and humans are considered important threats to public health worldwide. The transmission of these pathogens can be associated to the ingestion of contaminated food, including fresh vegetables [**14**]. This basic pattern jeopardises people living in areas lacking standard hygienic conditions [**9**].

Nowadays, salads based on lettuce (Lactuca sativa) are widely consumed and recommended as an importance source of micronutrients, vitamins and minerals [**13**]. The same vegetables, however, if improperly washed, disinfected or prepared, represent an important contamination source of gastrointestinal parasites, such as Angiostrongylus cantonensis and Giardia duodenalis, which may cause intestinal alterations (e.g., malabsorption, nutritional imbalance, diarrhoea), coagulative disorders, anaemia, andsometimes death [**7**]. Vegetables can indeed harbour infective stages of several parasites, withthe contamination occurring during diferent steps of the food chain, including at the source, due to the contact with spoil waters, polluted soil, or organic fertilizers, and during the manufacturing, associated to unsanitary food handling [**4**].

The World Health Organization (WHO) guidelines for the microbiological quality of treated wastewater used in agriculture indicate that the limit for nematode eggs should be less than 1 egg/l. However, this cannot be considered adequate in conditions that favour the survival of nematode eggs (lower mean temperatures and the use of surface irrigation). Insuch a case, the threshold should be reduced to less 0.1 egg/l [**1**]. It becomes evident that the detection of these parasitic stages in vegetables is of great importance for public health, as its sensitivity represents a guarantee on the hygienic conditions involved in aliment production, storage, transport, and handling [**12**]. The laboratory diagnosis of these parasites is often based on sedimentation methods, which may show a low sensitivity. Over the last years, a new tool known as the FLOTAC technique has been developed to detect oocysts, cysts, eggs and larvae of gastrointestinal parasites in diferent animal species, including humans [**3**]. More recently, this technique has also been employed to recovering equine strongyle larvae in the pasture [**11**], but has never been used to assess the presence of parasites in vegetables used for human consumption. Therefore, the aim of this study was to assess the use of the FLOTAC to detect parasite stages (oocysts, cysts, eggs, and larvae) of gastrointestinal parasites of animals in smooth lettuces.

2. Material and methods

2.1 Samples

Smooth lettuces (n=100) obtained in public markets located in the metropolitan region of Recife (08°03′14″ South and 34°52′52″ West), Northeast of Brazil, were used in this study. The number of samples was based on the number of public markets identifed in the study area (n=50), with two samples collected per market.

Samples were randomly selected, placed in plastic bags and stored in isothermal boxes (8 °C) until laboratory processing. Leaves that were inappropriate for human consumption (i.e., inedible leaves or stems) were discarded. Each sample was defoliated in distilled water (300 ml), rubbed with a brush and left to rest for 5 min. Subsequently, leaves were removed, the liquid was fltrated, and the material divided into two portions (150 ml each) for further analysis using the FLOTAC technique.

2.2 FLOTAC technique and data analysis

The FLOTAC technique was performed using 150 ml of the water from the washing process. The material was transferred into two plastic tubes, and centrifuged at 1000 rpm for 5 min. Afterwards, the supernatant was discarded, and 6 ml of S2 (sodium chloride) and S7 (zinc sulfate) solutions were added to each tube. Then, the material was homogenized and distributed into both FLOTAC chambers which, were centrifuged for 5 min at 1000 rpm, and finally microscopically examined at diferent magnifcations (100× and 400×) [**3**].

Relative and absolute frequency of positivity was calculated for each parasite detected.

3. Results

Out of all the samples analyzed, 79% (79/100) scored positive for the presence of oocysts, cysts, eggs and/or larvae of gastrointestinal parasites. In particular, protozoan and nematodes were detected in 32% (32/100) and 64% (64/100) of the samples, respectively, with the most frequently observed being the cysts of the genus Entamoeba (32%) and nematode eggs of order Strongylida (30%) (**Table 1**). The mean number of eggs and cysts retrieved per sample were 24.7 (±9.1) and 11.2 (±2.1), respectively.

4. Discussion

This study investigated the use of the FLOTAC technique to detect immature forms of gastrointestinal parasites of animals in smooth lettuces purchased in public markets in the Northeast of Brazil. The findings demonstrated that almost all samples were contaminated by some parasite stages, refecting the poor sanitary conditions of cultivation and inadequate post-harvest handling at the public markets [**8**]. In general, in these environments vegetables are more exposed to contamination, since in several cases their storage is inappropriate.

Several immature forms of gastrointestinal parasites were detected in this study (**Table 1**). However, it is important to highlight that some of them (i.e., nematodes of the genus Ascaris and strongylid nematodes) include species of particular public health concern. For example, larvae of Ancylostoma spp. from dog feces have been recognized as the causative agent of Cutaneous Larva Migrans in human patients [**2**]. Similarly, Ascaris lumbricoides is considered by the WHO as some of the most important gastrointestinalparasites in humans. This parasite is widespread throughout the world, especially in tropical areas lacking basic sanitation [**5**].

Table 1. Relative and absolute frequency of parasitic structures detected in lettuce using the FLOTAC technique

Flotac technique	Parasites (genus)	Positivity% (n/N)
	Order Strongylida	30% (30/100)
	Ascaris	16% (16/100)
	Strongyloides	22% (22/100)
	Entamoeba	32% (32/100)

Interestingly, no tapeworms and oocysts were detected in this study. The absence of these parasites is worth of note, since they have been considered important contaminants in vegetables [**4**].

Data on protozoa and nematodes is of great importance for public health, as it provides indirect information on the hygienic conditions involved in the production, storage, transport and handling of food products. Although the exact source of contamination of lettuces is difcult to achieve, it is known that animal feces are traditionally used as manure, accounting for a primary source of contamination. In addition, in several cases the cultivation areas donot present an appropriate basic sanitation, being human feces found in the environment and contaminating water and soil used in the production of vegetables. In this study, the FLOTAC technique was able to detect immature forms of gastrointestinal parasites of mammals in contaminated lettuces. For a long time, the microscopic examination of sediment was the only quantitative technique used to detect parasite stages in vegetables [**4**]. However, this present study demonstrates that the FLOTAC technique may be used to diagnose the contamination of lettuces by immature forms of gastrointestinal parasites of animals, and perhaps permit overcoming the limits presented by the classical sedimentation method extensively used. The

importance of the FLOTAC technique for the detection of gastrointestinal parasites of animals is indisputable [**6**, **10**]; however, the present study opens new possibilities for the use of this technique, beyond its use only in animals.

In conclusion, this is the frst study performed to detect vegetal contamination by gastrointestinal parasites of animals with the use of the FLOTAC technique. The fndings herein reported, open new perspectives for the use of this method on the detection of food- borne parasites of medical and veterinary concern.

Acknowledgements Authors would like thank Irma Y. T. López and Raphael Lepold for their support on the laboratory analyses. This article is based on the PhD thesis (Postgraduate Program in Tropical Animal Science) of the frst author, developed at the Federal Rural University of Pernambuco.

5. References

1. Blumenthal UJ, Mara DD, Peasey A, Ruiz-Palacios G, Stott R (2000) Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bull World Health Organ 78:1104–1116
2. Caliskan E, Uslu E, Turan H, Başkan E, Kılıç N (2016) Cutaneous larva migrans: report of three cases from the Western Black Sea Region. Turk Microbiol Bull 50:165–169
3. Cringoli G, Rinaldi L, Maurelli MP, Utzinger J (2010) FLOTAC: newmultivalente techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc 5:503–515. https://doi.org/10.1038/nprot.2009.235

4. Eraky MA, Rashed SM, Mel-S Nasr, El-Hamshary AM, Salah El-Ghannam A (2014) Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. J Parasitol Res 613960:1–7. https://doi.org/10.1155/2014/613960 5.

5. Fowler AC, Déirdre HT (2016) The dynamics of Ascaris lumbricoides infections. Bull Math Biol 78:815–833

6. Lima VF, Ramos RA, Lepold R, Cringoli G, Rinaldi L, Faustino MA et al (2016) Use of the FLOTAC technique to diagnosing parasites of the urinary tract of dogs. Parasitol Res 115:1737–1739. https://doi.org/10.1007/s00436-016-4950-4

7. Liu LX, Weller PF (1993) Strongyloidiasis and other intestinal nematode infection. Infect Dis Clin N Am 7:655–682

8. Mohamed MA, Siddig EE, Elaagip AH, Edris AM, Nasr AA (2016) Parasitic contamination of fresh vegetables sold at central markets in Khartoum state, Sudan. Annu Clin Microbiol Antimicrobiol 15:17. https://doi.org/10.1186/s12941-016-0133-5

9. Ojha SC, Jaide C, Jinawath N, Rotjanapan P, Baral P (2014) Geohelminths: public health signifcance. J Infect Dev Ctries 8:5–16. https://doi.org/10.3855/jidc.3183

10. Ramos RA, Lima VF, Monteiro MF, Santana MA, Lepold R, Faustino MA et al (2016) New insights into diagnosis of Platynosomum fastosum (Trematoda: Dicrocoeliidae) in cats. Parasitol Res 115:479–482. https://doi.org/10.1007/s00436-015-4763-x

11. Ramos ICN, Ramos RA, Lima VFS, Giannelli A, Lopez YIT, Alves LC (2018) Applicability of FLOTAC® technique in recovering equine strongyle larvae in the pasture: a comparison study. Vet Parasitol 250:68–70. https://doi.org/10.1016/j.vetpar.2017.12.014

12. Shahnazi M, Jafari-Sabet M (2010) Prevalence of parasitic contamination of raw vegetables in villages of Qazvin Province, Iran. Foodborne Pathog Dis 7:1025–1030. https://doi.org/10.1089/ fpd.2009.0477

13. Sharma S, Sheehy T, Kolonel L (2014) Sources of vegetables, fruits and vitamins A, C and E among fve ethnic groups: results from a multiethnic cohort study. Eur J Clin Nutr 68:384–391. https ://doi.org/10.1038/ejcn.2013.271

14. Sharma R, Singh BB, Gill JP (2015) Larva migrans in India: veterinary and public health perspectives. J Parasit Dis 39:604–612. https://doi.org/10.1007/s12639-013-0402-6

Capítulo I I

The application of the FLOTAC® technique for detection of immatures forms of parasites of medical and veterinary concern on the soil

Capítulo estruturado em formato de artigo e publicado na revista *Experimental Parasitology* v. 242. 108379, 2022

ABSTRACT

The FLOTAC technique was initially developed to detected immature forms of parasites in animals and human feces. Recently, its applicability has been extended by the use in pastures and vegetables for human consumption. However, its use for detection of immature forms of parasites on the soil has never been investigated. In this study we assessed the performance of the FLOTAC and compare with spontaneous sedimentation (SST) and centrifugal flotation (CFT) techniques. A total of 50 soil samples were collected from the Metropolitan region of Recife, Northeastern Brazil. Initially, samples were standardized, and then assessed in duplicate by the FLOTAC, SST and CFT. Cohen's kappa coefficient, sensitivity, specificity, positive and negative predictive values and accuracy of the FLOTAC and SST were assessed using the CFT as "gold standard". Out of all analyses performed, in 32.6% (98/300) immature forms of parasites were detected. In particular, 58% (58/100) of samples analyzed through the FLOTAC technique, 26% (26/100) for CFT and 14% (14/100) for SST were positive. Nematode eggs of the Ancylostomatidae and Ascarididae families were those more detectedin this study. Overall, the FLOTAC technique presented the best performance, which candidates this method for detection of immature forms of parasites in soil samples. This study opens new possibilities for the use of the FLOTAC technique everywhere, highlighting its role as tool for detecting environmental contamination by parasites of medical and veterinary concern.

Keywords: Diagnosis, environmental contamination, nematode, public health

1. Introduction

Currently, the soil-transmitted helminthes (STHs) affect more than one billion of people worldwide and approximately 300 millions present a severe disease originated from these parasitic infections **(OPAS, 2018)**. They are present worldwide, especially in areas where the population is socially vulnerable due to lack of basic sanitation **(WHO, 2017)**. For instance, in some African, American and Asian countries the prevalence of STHs may achieve more than 50%, which markedly contrast with some areas of Europe and North America where this kind of infection is almost inexistent due to measures of basic sanitation **(WHO, 2010)**.

Undoubtedly, STHs has been more frequently detected in rural areas or in peripheral regions of urban centers. Nonetheless, over the last years the increase of contamination of soil from the shore of beaches, parks and squares had been responsible for the increase of cases also in large urban centers (**Brener et al., 2008; Azian et al., 2008; Silva et al., 2009; Sprenger et al., 2014, Macedo et al., 2019)**. In urban areas, this contamination occurs mainlydue to the deposition of feces from dogs and cats, that depending on the species of helminthes may excrete up to 15.000 eggs per gram of feces (**Ribeiro, 2004**). In the soil, these immature forms of parasites change into infective forms and may infect humans, especially children thatare more exposed (**Pedrassani et al., 2008, Silva et al., 2019**).

Various studies have been conducted to detect the contamination of soil by immature forms of medical and veterinary concern **(Steinbaum et al., 2016)**. It is consensus of the scientific community that the proper identification of these parasites at species level has beena challenge, due the absence of information about putative definitive host. Even though, nematodes belonging to the families Ascarididae, Ancylostomatidae and Thricuridae have been those more frequently reported (**Moro et al., 2008; Silva et al., 2009**). Studies aiming the health status of humans and animals are pivotal, especially in critical areas where the contamination of the environment is a trouble. In this context, the monitoring of contamination of soil on beaches, parks and squares is an important tool of vigilance based on the One Health approach that has been employed worldwide (**Guimarães et al., 2005;Devera et al., 2008; Dado et al., 2012)**.

Although developed to detect parasites in feces, the techniques of Hoffman, Pons and Janer (1934), as well as Faust (1938), and Willis (1921) have been used for some time to detect parasites in soil (**Hoffman et al., 1934; Faust et al., 1938; Willis, 1921**). In general, these methodologies are based on the flotation and/or sedimentation of eggs and/or oocysts, and have passed for slightly modifications over the time. Although, they present low cost and are easily performed, some limitations may reduce the efficacy of these techniques. Most of them presented a low sensitivity and the level of detection of eggs in animal's feces is dependent of capacity of the helminthes in excretion eggs. For instance, *Toxocara* spp. may produce up to 200,000 eggs per day (**Glickman and Schantz, 1981**), which will contributefor high levels of environmental contamination. However, it is important to note that in soil samples the contamination is reduced compared with fresh feces, for this reason the detection and identification of immature forms of parasites has been a challenge.

Over the last decade, the FLOTAC technique has been employed for detection of immature forms of parasites in several animals and human feces (**Cringoli et al., 2010**). Morerecently, the applicability of this tool was enlarged for detection of parasites of medical and veterinary concern on the pasture (**Ramos et al., 2018**) and in vegetables (**Ramos et al.,2019**), butits use on soil samples has never been assessed. Therefore, the aim of this study was to apply the FLOTAC technique for detection of immature forms of parasites of medical and veterinary concern on the soil.

2. Material and Methods

2.1 Study area

The study was conducted using soil samples collected on shore of beaches and parks of the Metropolitan Region of Recife – MRR (8º 3'15'" South and 34º 52' 53" West), state of Pernambuco, Northeastern Brazil. The collection points on the beaches encompassed an area of approximately 38.14 km of seafront.

This area is characterized by a hot and humid tropical climate (*As*) with average annual temperature around 27 ºC, relative humidity mean of 78%, and rainfall of 2000 mm/year, unevenly distributed in dry and rainy periods. Climatic conditions were obtained from the Pernambuco Water and Climate Agency (PWCA). Approximately 3.975,411 people live in the MRR, and only 46.6% of this population has access to basic sanitation and treated water **(IBGE/SNIS, 2018).**

2.2 Soil samplings

A total of 50 sites of collections were chosen by convenience, and divided into 42 points on the beaches and 8 points on parks. For each site for sample collection an area of 100 m^2 (10 m X 10 m) was delimited and five samplings (sub-points) performed, one in each corner and one in the central part of the area (**Milano and Oscherov, 2002**; **Sousa et al., 2014**). The soil was extracted from the superficial layer (5 to 10 cm of depth) with the aid of a plastic spatula; 100 g was collected for each sub-point totaling 500 g in total **(Santarém et al., 1998)**. The material was kept in plastic bags, stored in isothermal boxes at 4 °C and processed within 24 hours. All samplings were carried out from 5 to 7 am in areas with intense movement of human beings and animals. The sampling was defined probabilistically by convenience **(Reis, 2003).**

2.3 Laboratorial procedures

2.4 Standardization of samples

All five samples (sub-points) of each point were homogenized, sieved (149µm) and 300 g weighted for standardization. Afterwards, the sample was placed in a plastic box and 900 mL of distilled water added. The material was homogenized in a shaker for 30s, decanted for one minute, and then the supernatant was versed in two sieves of 149µm and 38µm, respectively. Finally, the sieve of 38µm, where immature forms of parasites were retained, was washed with 150 mL of distilled water and the material divided in three aliquots of 50 mL each **(Jenkins, 1964).**

2.5 Spontaneous sedimentation technique (SST), Centrifugal flotation technique (CFT) and FLOTAC technique

Samples were evaluated by SST using saturated sucrose solution (s.g. 1.20) (Hoffman et al., 1934) and CFT method using zinc sulphate solution (s.g. 1.18) (Jenkins, 1964). Four slides were prepared using 50 µl each of material and analysed at different magnifications (100X and 400X). Finally, the FLOTAC was performed using NaCl (s.g. 1.20) as flotation solution (Cringoli et al., 2010). The FLOTAC chamber was microscopically analysed at different magnifications (100X and 400X). All three techniques were performed in duplicate, totaling 300 analyses, 100 for each technique. A sample was considered positive when eggs were detected in at least one analysis.

2.6 Data analyses

Descriptive statistical analysis was performed. The Lilliefors test was used to verify the normality of the data. Overall data on the positivity of three techniques were analyzed using the Chi-square test. Conversely, Chi-square of Partition test was used to compare the occurrence of families of parasites detected. A 5% significance level was considered. Both analyses were performed using the BioEstat software version 5.3 (**Ayres et al., 2007**).

The Cohen's Kappa coefficient (k) was calculated to evaluate the agreement between different techniques **(Landis and Kock, 1977)**. In addition, The CFT method was considered as Gold Standard for the calculation of sensitivity, specificity, positive and negative predictive values, and accuracy of each technique (**Herbert, 2013**). For the Kappa calculation all samples were considered in a single analysis, whereas for the other parameters (sensitivity, specificity, predictive values, and accuracy) data of parks and beaches were assessed separately.

3. Results

Out of all analyses performed, in 96% (48/50) helminth eggs were detected. In particular, FLOTAC technique detected more positive samples (96%; 48/50) followed by CFT (76%; 38/50) and SST method (28%; 14/50) ($\chi 2 = 71.141$; $p < 0.0001$).

Parasites of the families Ancylostomatidae, Trichuridae, Ascarididae and Dipylidiidae were detected in at least one technique employed. Ancylostomatidae and Ascarididae were the parasites most frequently retrieved, regardless of the technique utilized ($\chi 2 = 0.8553$; p = 0.6520). Simultaneous contamination by two or more parasites were observed in 68% (34/50) of soil samples (Table 1). It is important to note that helminth eggs were detected in 100% (8/8) and 95.23% (40/42) of samples from parks and beaches, respectively. Additionally, 26%(13/ 50) of the samples were positive for all methods, whereas 4% (2/50) were negative.

Overall, the kappa analysis revealed the following results: FLOTAC versus SST (k = 0.09; $p < 0.001$): poor concordance, FLOTAC versus CFT (k = 0.53; $p < 0.001$): moderate concordance, and CFT versus SST (k = 0.28; $p < 0.001$): considerable concordance. Values of sensitivity, specificity, predictive positive and negative values, as well as accuracy for samples from parks and beaches are reported on Table 2.

Table 1 – Helminth eggs of parasites detected in soil samples through SST, CFT and FLOTAC techniques.

Method of diagnosis	Parasites (Family)	% (n/N)
SST	Ancylostomatidae	14 (7/50)
	Ascarididae	14 (7/50)
CFT	Ancylostomatidae	10 (5/50)
	Ascarididae	14 (7/50)
	Trichuridae	2 (1/50)

	Ancylostomatidae + Ascarididae	26 (13/50)
FLOTAC	Ancylostomatidae	22 (11/50)
	Ascarididae	40 (20/50)
	Trichuridae	10 (5/50)
	Dipylidiidae	2 (1/50)
	Ancylostomatidae + Ascarididae	24 (12/50)
	Ancylostomatidae + Trichuridae	10 (5/50)
	Ascarididae + Trichuridae	4 (2/50)
	Ancylostomatidae + Trichuridae + Ascarididae	4 (2/50)

Table 2.

Table 2 - Sensitivity, specificity, negative and positive predictive values obtained for FLOTAC and SST techniques.

Method of diagnosis	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
Parks			%		
FLOTAC	100 (71.51 – 100)	100 (2.50 – 100)	100 (2.50 – 100)	100 (2.50 – 100)	100 (73.54 – 100)
CTF	81.82 (48.22–97.72)	100 (2.50 – 100)	100 (2.50 – 100)	33.33 (12.49-63.66)	83.33(51.59-97.91)
SST	36.36 (10.93-69.21)	100 (2.50 – 100)	100 (2.50 – 100)	12.50 (8.37–18.25)	41.67 (15.17– 72.33)
Beaches					
FLOTAC	100 (90.51–100)	100 (2.50 – 100)	100 (2.50 – 100)	100 (2.50–100)	100 (90.75–100)
CTF	78.38 (61.79–90.17)	100 (2.50 – 100)	100 (2.50 – 100)	11.11 (6.34–18.76)	78.95 (62.68–90.45)
SST	27.03 (13.79–44.12)	100 (2.50 – 100)	100 (2.50 – 100)	3.57 (2.95–4.31)	28.95 (15.42–45.90)

CI confidence intervals, PPV positive predictive value, NPV negative predictive value

4. Discussion

This study demonstrated that the FLOTAC presented a higher sensitivity when compared with two classic methods (CFT and SST) for detection of helminth eggs in soil samples. Overall, samples analysed by the FLOTAC presented a high positivity (FLOTAC - 96%) when compared with all other methods (CFT - 76% and SST - 28%).

Although different families of parasites had been detected, the families Ancylostomatidae and Ascarididae predominated. Eggs of these nematodes are frequently detected in sandy soils with the presence of organic matter. The soil characteristic is very important for the egg's evolution to the larval stage, as well as its migration in different types of soils (**Eisen et al., 2019**). In this study, soil samples from beaches (n = 42) and parks (n = 8) were evaluated. It is believed that these kinds of soils may present different characteristics, which may be important for the development and survival of parasites.

It is important to note that the Ascarididae family corresponds to the first family of parasites more retrieved. Eggs of these nematodes have a thick and resistant membrane, whichallow them to survive in high temperatures. For instance, about 30% of Ascarididae eggs survive at temperatures up to 65◦C (**Cameron, 1963**). Considering that in the region of study the temperature mean is about 27◦C and large variations has not been observed, eggs may be present in the environment throughout the year, representing a risk for animal and human health.

The FLOTAC method (96%) presented a higher positivity when compared with CFT (76%) and the SST (28%) techniques. It has been already proved that the FLOTAC exhibits a good performance when compared with other classical methods, but in the detection of parasites in feces (**Lima et al., 2015; Duthaler et al., 2010**). Several reasons for the higher efficiency of the FLOTAC have been speculated, including the density of solutions that influence flotation of parasitic structures. It has been proved that NaCl flotation solution may present a limited performance when compared with sucrose-based solution used for the detection of some helminth eggs (**Cringoli et al., 2004**). However, the use of NaCl in the present study did not difficult the retrieval of helminth eggs, since a sensitivity of 100% has been observed for the FLOTAC technique.

Despite of the difference in the number of samples of parks (n = 8) and beaches (n = 42), the assessment of sensitivity, specificity, predictive values and accuracy presented the same results for the FLOTAC. In fact, for both types of samples, the FLOTAC exhibited 100% values for all parameters assessed. Although, CFT and SST had presented specificity and PPVof 100%, all other parameters were lower than those presented by the FLOTAC technique(see Table 2).

It is important to note that this study retrieved helminth eggs in soil samples, which are more difficult than in fresh fecal samples. Even though, the FLOTAC presented a high sensitivity (100%), indicating its role as a potential tool to assess the environment contamination by animal or human feces. It is known that an ideal diagnostic method with 100%of sensitivity and 100% of specificity is difficult to achieve as the increase of sensitivity implies in a reduction of specificity and vice and versa. Although, values of 100% for specificityand PPV had been achieved for all techniques, low sensitivities were observed for CFT and SST.

The FLOTAC (n = 4) was also able to detect a greater variety of families of parasites when compared with CFT (n = 3) and SST (n = 2), demonstrating that it is a more complete diagnostic tool than classical methods currently utilized for this purpose. Unfortunately, the quantification of helminth eggs was not performed, which is considered an important limitation of this study. Actually, this data would provide important information for the assessment of all techniques herein employed. It is important to note that the moderate concordance kappa value was observed between FLOTAC versus CFT (k = 0.53), therefore the use of both techniques in combination will increase the efficiency of detection of helmintheggs in the soil.

For a long time, the FLOTAC had been used only for the detection of parasites in fresh fecal samples (**Allam et al., 2021**). Recently, its use has been enlarged for detection of parasites on the pasture (**Ramos et al., 2018**) and also in vegetables used for human consumption (**Ramos et al., 2019**). It is known that the methods of diagnosis have improved faster, tests areconstantly developed, and the technology of existing tests is continuously being improved (**Bossuyt et al., 2003**). Nonetheless, most of parasitological methods used for detection of intestinal helminths and protozoan infections have passed for few modifications (**Carvalho et al., 2012**). This is the first study that investigated the use of the FLOTAC to the detection of parasites in the soil. The knowledge of the presence of these parasites in the environment is pivotal for the establishment of preventive measures against STHs. Finally, data herein presented enlarge the applicability of the FLOTAC technique demonstrating that may be also employed for monitoring the environmental contamination by helminth eggs of parasites of medical and veterinary concern.

Acknowledgements

This article is based on the PhD thesis (Graduate Program in Animal Bioscience) of the first author, developed at the Federal Rural University of Pernambuco, and supported by a grant fellowship from the Coordination for the Improvement of Personnel of Higher Education (CAPES).

5. References

Allam, A.F., Farag, H.F., Lotfy, W., Fawzy, H.H., Elhadad, H., Shehab, A.Y., 2021. Comparison among FLOTAC, Kato-Katz and formalin ether concentration techniques for diagnosis of intestinal parasitic infections in school children in an Egyptian rural setting. Parasitol. 148, 289–294.

Ayres, M., Ayres, M. Jr., Ayres, D.L., Santos, A.A., 2007. BIOESTAT – statistical applications on the biomedical sciences. Ong. Mamirauá.

Azian, M.Y., Sakhone, L., Hakim, S.L., Yursi, M.Y., Nurulsyamzawaty, Y., Zuhaizam, A.H., Rodi, I.M., Maslawaty, M.N., 2008. Detection of helminth infections in dogs and soil contamination in rural and urban areas, Southeast Asian. J. Trop. Med. Pub. Health. 39, 205–212.

Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P., Irwig, L.M. Lijmer, J.G., Moher, D., Rennie, D., Henrica, C.W., 2003. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Clin. Chem. 326, 41– 44

Brener, B., Mattos, D.P.B.G., Mattos, G., Millar, P.R., Arshiro, E.K.N., Duque-Ferreira, V., Sudré, A.P., 2008. Evaluation of the contamination of public squares of three regions of the State of Rio de Janeiro, Brazil, by eggs and larvae of helminths. Braz. J. Pathol. 37, 247–254.

Cameron T.W.M., 1963. Environmental factors related to the free and living stages helminth. World Health Organization - WHO.

Carvalho, G.L., Moreira, L.E., Pena, J.L., Marinho, C.C., Bahia, M.T., Machado-Coelho, G.L., 2012. A comparative study of the TF Test, Kato–Katz, Hoffman Pons–Janer, Willis and Baermann-Moraes coprologic methods for the detection of human parasitosis. Mem. Inst. Oswaldo Cruz, 107, 80–84

Cringoli, G., Rinaldi, L., Maurelli, M.P., Utzinger, J., 2010. FLOTAC: new multivalente techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat. Protoc. 5, 503–515.

Dado, D., Izquierdo, F., Vera, O., Montoya, A., Mateo, M., Fenoy, S., Galván, A.L., 2012. Detection of zoonotic intestinal parasites in public parks of Spain. Zoon. Pub. Heal. 59, 8–23.

Devera, R., Blanco, Y., Hernández, H., Simoes, D., 2008. *Toxocara* spp. and other helminths in squares and parks of Ciudad Bolivar (Venezuela). Enf. Infec. Microbiol. Clin. 26, 23–26.

Duthaler, U., Rinaldi, L., Maurelli, M.P., Vargas, M., Utzinger, J., Cringoli, G., Keiser, J., 2010. Fasciola hepatica: comparison of the sedimentation and FLOTAC techniques for the detection and quantification of faecal egg counts in rats. Exp. Parasitol. 126, 61–166.

Eisen, A.K.A., Demoliner, M., Oliveira, K.G., Troian, E.A., Mallmann, L., Filippi, M., Almeida, P.R., Spilki, F.R., 2019. Soil contamination of a public park by human and canine mastadenovirus, as well as hookworms and *Toxocara* spp eggs. J. São Paulo Inst. Trop. Med. 25, e60.

Faust, E.C., D'Antoni, J.S., Odom, V., Miller, M.J., Peres, C., Sawitz, W., Thomen, L.F., Tobie, J., Walker, H., 1938. A critical study of clinical laboratory technics for thediagnosis of protozoan cysts and helminth eggs in feces. Am. J. Trop. Med. Hyg. 18, 169–183.

Glickman, L.T., Schantz, P.M., 1981. Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol. J. 3, 230–250.

Guimarães, A.M., Alves, E.G.L., Rezende, G.F., Rodrigues, M.C., 2005. *Toxocara* sp. eggs and *Ancylostoma* sp. larva in public parks, Brazil. Pub. Health. J. 39, 293–295.

Herbert, R., 2013. Confidence Interval Calculator. https://pedro.org.au/ Accessed on 03 August 2021.

Hoffman, W.A., Pons, J.A., Janer, J.L., 1934. The sedimentation concentration methods in *Schistosomiases masoni*. Puerto Rico J. Pub. Health Trop. Med. 9, 283–289.

IBGE/SNIS (Sistema nacional de informação sobre saneamento) ano 2018. Available at: <www.snis.gov.br>. Accessed on 15 March 2021.

Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Dis. Rep. 48, 692.

Landis, J.R., KocK, G.G., 1977. An application of hierarchical Kappa- type statistics in the assessment of majority agreement among multiple observers. Int. Biom. Soc. 33, 363– 374.

Lima, V.F., Cringoli, G., Rinaldi, L., Monteiro, M.F., Calado, A.M., Ramos, R.A., Meira-Santos, P.O., Alves, L.C., 2015. A comparison of mini-FLOTAC and FLOTAC with classic methods to diagnosing intestinal parasites of dogs from Brazil. Parasitol. Res. 114, 3529–3533.

Macedo, L.O., Bezerra-Santos, M.A., Santana, B.B., Pereira, T.A., Silva, N.M.M., Alves, L.C., Ramos, R.A.N., Carvalho, G.A., 2019. Parasites with zoonotic potential in canine fecal samples from Garanhuns, Pernambuco, Brazil. Vet. Med. 13, 227–231.

Milano, A.M.F., Oscherov, E.B., 2002. Contamination by canine parasites of zoonotic importance on beaches of the city of Corrientes, Argentina. Latinoam. J. Parasitol. 57, 119–123.

Moro, F.C.B., Pradebon, J.B., Santos, H.T., Querol, E., 2008. *Ancylostoma* spp. and *Toxocara* spp. Biodiv. Pampeana. 6, 25–29.

ORGANIZAÇÃO PAN-AMERICANA DE SAÚDE (OPAS). Plano de ação para eliminação de doenças infecciosas negligenciadas e pós eliminação 2016-2022. Sessão do Comitê Regional da OMS para as Américas. Washington, D.C., EUA, 26 a 30 de setembro de 2018.

Pedrassani, D., Viera, A.M., Thiem, E.M.B., 2008. Contamination by *Toxocara* spp. and *Ancylostoma* spp. in areas of leisure from Canoinhas County, Santa Catarina state. Arch. Vet. Sci. 13, 110–117.

Ramos, I.C.N., Ramos, R.A.N., Giannelli, A., Lima, V.F.S., Cringoli, G., Rinaldi, L., Carvalho, G.A., Alves, L.C., 2019. An additional asset for the FLOTAC technique: detection of gastrointestinal parasites in vegetables. Acta Parasitol. 64, 423–425.

Ramos, I.C.N., Ramos, R.A.N., Giannelli, A., Lima, V.F.S., López, I.Y.T., Alves, L.C., 2018. Applicability of FLOTAC technique in recovering equine strongyle larvae in the pasture: A comparison study. Vet. Parasitol. 250, 68–70.

Reis, J.C. Estatística aplicada à pesquisa em ciência veterinária. 1a ed. Olinda 2003. 651p.

Ribeiro, V.M., 2004. Control of helminthes of dogs and cats. Braz. J. Vet. Parasitol. 13, 88–95.

Santarém, V.A.; Sartor, I.F.; Bergamo, F.M.M., 1998. Contamination by *Toxocara* spp. eggs in public parks and squares in Botucatu, São Paulo State, Brazil. Rev. Soc. Bras. Med. Trop. 31, 529-533.

Silva, P.F., Cavalcanti, I.M.D., Irmão, J.I., Rocha, F.J., 2009. Common beach sand contamination due to enteroparasites on the southern coast of Pernambuco state, Brazil. J. São Paulo Inst. Trop. Med., 51, 217–218.

Silva, N.M.M., Bezerra-Santos, M.A., Santana, B.B., Macedo, L.O., Alves, L.C., Ramos, R.A.N., Carvalho, G.A., 2019. Soil contamination by zoonotic gastrointestinal parasites of mammals in Garanhuns, Pernambuco, Brazil. Vet. Med. 13, 65–69.

Sousa, J.O., Santos, E.O., Lira, E.M., Sá, I.C., Hirsch, C.M., 2014. Parasitological analysis of sand from urban beaches of João Pessoa - PB Braz. J. Health Scie. 18, 195.

Sprenger, L.K., Green, K.T., Molento, M.B., 2014. Geohelminth contamination of public areas and epidemiological risks factors in Curitiba, Brazil. Braz. J. Vet. Parasitol. 23, 69– 73.

Steinbaum, L., Njenga, S.M., Kihara, J., Boehm, A.B., Davis, J., Null, C., Pickering, A.J., 2016. Soil-transmitted helminth eggs are present in soil at multiple locations within households in rural Kenya. PLoS One. 11, e0157780.

Willis, H.H., 1921. A simples levitation methods for detection of hookworm ova. Austral. Med. J. 2, 375–376.

World Health Organization. The human right for water and sanitation. WHO, Genebra, 2010.

World Health Organization. Infectious diseases of potential risk for travelers. WHO, 2017.

Capítulo III

Contamination by eggs of nematodes (Nematoda) of public health concern in tropical beaches

Capítulo estruturado em formato de artigo e publicado em 1 de Maio de 2022 na revista *Parasitologia* (v. 2, p. 95-100, 2022).

Contamination by eggs of nematodes (Nematoda) of public health concern in tropical beaches

ABSTRACT

Public spaces such as beaches develop an important role in the welfare of the human population in Brazil, where leisure and safety spaces are increasingly scant. Knowledge about the possible role of contaminated sand of beaches on the health of animals and humans is pivotal to determine risk areas and preventing future cases. Therefore, the aim of this study was to assess the presence of eggs of nematodes of public health interest on tropical beaches. Soil samplings (n = 42) from 10 different beaches located in the Metropolitan region of Recife, Northeastern Brazil, were analyzed through the FLOTAC technique. In 80% (8/10) of beaches, eggs of nematodes were detected. In particular, if we consider each individual sample (n = 42) a positivity rate of 90.4% (38/42) was observed. Eggs belonging to the families Ascarididae (47.6%; 20/42), Ancylostomatidae (26.1%; 11/42), and Trichuridae (11.9%; 5/42) were detected. Based on these data, it is necessary to implement preventive measures and educational actions for the population in order to mitigate the potential risk of exposition to parasites of zoonotic importance. Additionally, the FLOTAC technique has beenshown to be a reliable technique for assessing soil contamination by nematode eggs.

Keywords: Ascarididae; Ancylostomatidae; feces; animals; humans; beaches.

1. Introduction

Public spaces everywhere, such as parks and beaches, have played an important role in the welfare of the human population. These areas have been used for several purposes, such as sports practice, meetings, and rest, promoting a pivotal social aspect in the life of humans. In general, an intense movement of people and their pets (e.g., dogs and cats) has been observed in these areas that frequently present a ground contaminated by eggs of helminths of medical and veterinary concern **[1].**

The contamination of the soil on beaches and in parks has been extensively studied **[2]**, but over the last few years, little attention has been given to this sanitary threat that has increased **[3]**. It is important to note that the increase in soil contamination by eggs of helminths is related to the rise in the human population and their pets, especially in urbanareas **[4]**. Undoubtedly, in developing countries, this kind of environmental contamination hasbecome a public health matter due to several reasons, including health education, the absence of basic sanitation, and animal welfare **[2]**.

For instance, in Brazil, at the beginning of the current century, several studies were conducted on squares and beaches. These researches have demonstrated contamination of the soil varying from 4.69% in Pernambuco **[5]** to 40% in São Paulo **[6].** More recently, a similar study carried out in the state of Paraíba revealed that 96.4% of soil samples collected from squares were contaminated with eggs of nematodes, including Ancylostoma spp. (Dubini, 1834) (28.6%), Trichuris spp. (Morgani, 1740) (21.4%), and Strongyloides spp. (Normand, 1876) (46.4%) **[7]**.

Overall, parasites belonging to the genera Ancylostoma, Toxocara (Wilder, 1950), Trichuris, Ascaris (Linnaeus, 1758), and Strongyloides are those more frequently detected **[8,9]**. It is important to note that some of them are parasites of dogs, cats, and also humans, and in some cases, the sharing of these pathogens among different mammal species has been considered a common event. Most of these parasite species spend part of their life cycle on the soil, which is considered an important source of accidental infection of animals, including human beings **[10].** Indeed, the role of contamination of the soil on the health of animals and humans is indisputable. However, even with all knowledge acquired over the last decades, the lack of education and the absence of basic sanitation in many Brazilian regions contribute to the persistence of this issue, exposing the animal and human population to the risk of parasitic infections.

It is also important to note that public spaces such as beaches have developed an important role in the welfare of the human population in Brazil, where leisure and safety spaces are increasingly scant. In this context, knowledge about the possible role of contaminated sand of beaches on the health of animals and humans is pivotal to determining risk areas and preventing future cases of parasitic infections. Therefore, the aim of this study was to assess the presence of eggs of nematodes of public health interest in tropical beaches in Northeastern Brazil.

2. Material and Methods

2.1 Study area

The study was conducted in the Metropolitan Region of Recife (MRR) (8˚3′15″ S and 34˚52′53″ W), state of Pernambuco, Northeastern Brazil. This area is characterized by a hot and humid tropical climate (As) with an average annual temperature of around 27˚C and rainfall of 2000 mm/year, unevenly distributed in dry and rainy periods. Climatic information was obtained from the Pernambuco Water and Climate Agency (Agência Pernambucana de Águas e Clima, APAC, Recife, Brazil). Approximately 3,975,411 people live in the MRR, and only 46.6% of this population has access to basic sanitation and treated water **[10]**.

2.2 Soil samplings and laboratorial procedures

Soil samplings (n = 42) were performed on the beaches and encompassed an area of approximately 38.14 km of the seafront. Briefly, samplings were carried out at 42 points from 5 a.m. to 7 a.m. For each collection point, an area of 100 m2 was delimited and the soil was collected of five sub-points (four on the corners and one in the center). The collection was carried out in a superficial layer up to 5 cm deep with 100 g at each point, totaling 500 g of soil. In some areas of sampling was observed the presence of sewage discharge on the beach, as well as the presence of domestic animals (**Figure 1**).

Figure 1: Areas of sampling with sewage discharge on the beaches. (A=D): sewage discharge

Each sample was individually standardized according to Ramos **[11]**. Briefly, they were homogenized, sieved (149 µm), and 300 g weighed. Afterwards, the sample was placed in a plastic box and 900 mL of distilled water was added. The material was homogenized for 30 s, then left to rest for one minute, and the supernatant was separated in two sieves of 149 µm and 38 µm, respectively. Finally, the sieve of 38 µm, where eggs of nematodes were retained, was washed with 50 mL of distilled water and the material analyzed through the FLOTAC technique **[12]**.

2.2 *Data analyses and map construction*

All data were firstly described by descriptive statistics and the relative and absolute frequencies were obtained. Afterwards, the distribution of parasites was performed using choropleth maps with color intensity levels, using the Quantum Geographic Information System (QGIS 3.16, Hannover, Germany).

3. Results

Soil samples from 10 different beaches were analyzed, and nematode eggs were detected in 80% (8/10) of them. In particular, if we consider each individual sample (n = 42), a positivity rate of 90.4% (38/42) was observed. Eggs belonging to the families Ascarididae (47.6%; 20/42), Ancylostomatidae (26.1%; 11/42), and Trichuridae (11.9%; 5/42) were detected **(Table 1)**.

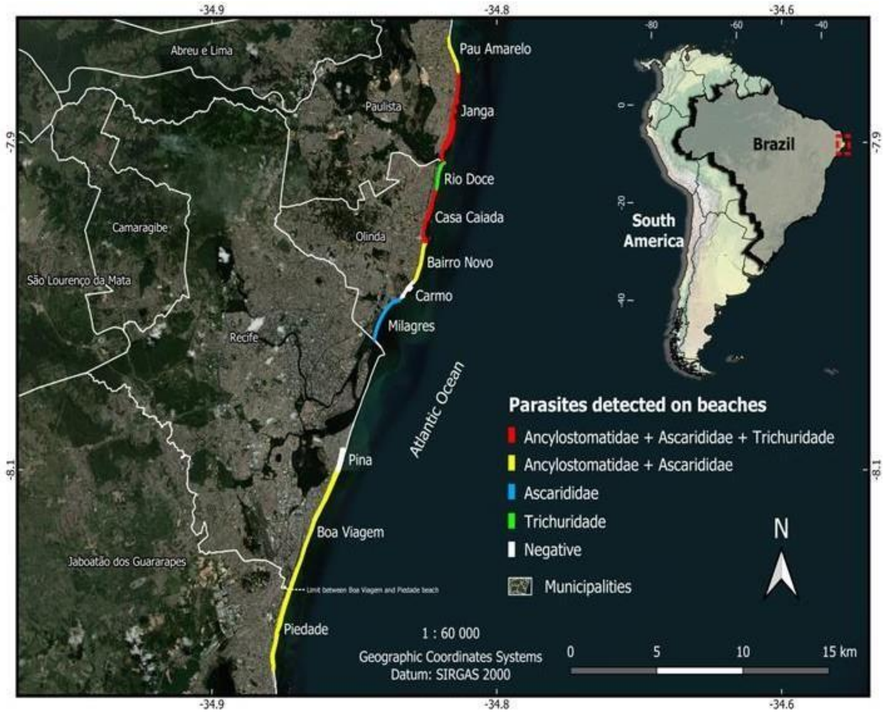
Table 1. Positivity for eggs of nematodes in the sand of beaches located in the Metropolitan Region of Recife

Beach	**Collection Points (N)**	**Overall Positivity (%; n/N)**	**Family of nematodes (%; n/N)**	**Municipality**
Pau Amarelo	3	100% (3/3)	Ancylostomatidae (100%; 3/3) Ascarididae (100%; 3/3)	Paulista
Janga	9	100% (9/9)	Ancylostomatidae (88,8%; 8/9) Ascarididae (100%; 9/9) Thichuridae (77,8%; 7/9)	Paulista
Rio Doce	3	100% (3/3)	Thichuridae (100%; 3/3)	Olinda

Casa Caiada	6	100% (6/6)	Ancylostomatidae (100%; 6/6) Ascarididae (100%; 6/6) Thichuridae (83,3%; 5/6)	Olinda
Bairro Novo	3	100% (3/3)	Ancylostomatidae (100%; 3/3) Ascarididae (100%; 3/3)	Olinda
Carmo	2	0 (0/2)	Negative	Olinda
Milagres	3	100% (3/3)	Ascarididade (100%; 3/3)	Olinda
Pina	2	0 (0/2)	Negative	Recife
Boa Viagem	5	100% (5/5)	Ancylostomatidae (100%; 5/5) Ascarididade (100%; 5/5)	Recife
Piedade	6	100% (6/6)	Ancylostomatidae (100%; 6/6) Ascarididade (100%; 6/6)	Jaboatão dos Guararapes

It is important to note that the higher diversity of eggs of nematodes was observed in Janga and Casa Caiada beaches (three distinct families), followed by Pau Amarelo, Bairro Novo, and Boa Viagem beaches (two distinct families), and finally Rio Doce and Milagres beaches with only one family of nematodes. No eggs of nematodes were retrieved in Carmo and Pina beaches. The graphical distribution of eggs of nematodes on the shore of Metropolitan Region of Recife is represented on Figure **2**.

Figure 2. Graphical distribution of nematode families on the shore of Metropolitan Region of Recife



4. Discussion

This study revealed that eggs of nematodes of medical and veterinary concern are present in sand of the shore of the Metropolitan Region of Recife, with some areas presenting a high risk of exposition for the animal and human population.

Overall, the eggs of nematodes were detected in 80% (8/10) of beaches and in 90.4% (38/42) of soil samples. This high positivity has been a trend observed in other public spaces throughout Brazil. For instance, in the municipality of Sousa, in the state of Paraíba, Helmith eggs were detected in 90% of the analyzed soil samples of public squares **[4]**, whereas 96.4% of positivity was recorded in public squares from João Pessoa, Paraíba **[2]**.

It is important to note that all families of parasites herein detected have been frequently observed in previous studies, where in most cases, the predominance of Ancylostomatidae eggs has been reported **[1,4,13]**. In comparison, in the present study,Ascarididae eggs predominated. These eggs may have as their source of contamination sewage and waste discharges, as well as feces of animals (e.g., dogs, cats, and human beings) excreted directly on the sand. It is known that Ascarididae eggs are very resistant in the environment. In general, they have a layer formed by chitin, proteins, and lipids, which may resist adverse environmental conditions **[14]**.

Regardless of the family retrieved, both of them (e.g., Ascarididae or Ancylostomatidae) may comprise species of zoonotic concern causative agents of Larva Migrans syndrome, as well as other infections of medical and veterinary importance **[1]**. The high frequency of eggs of nematodes reported in this study represents an important finding from an epidemiological perspective. The study area presents suitable environmental conditions for the establishment and development of these nematodes on the soil. In addition, the regulations regarding the access of animals to beaches are nonexistent or poorly supervised, allowing the unrestricted access of stray animals in these places. Unfortunately, the lack of basic sanitation with the deposition of sanitary sewage on beaches (see **Figure 1**) and also poor hygiene behavior of the population are still common practice in these areas and contributes to the retrieval of these parasites.

Although less frequent eggs of the Trichuridae family were also detected, parasites belonging to this group have been already observed in previous studies **[4,15]**, and it seems that eggs are very resistant and remain viable in hot and humid soil for **months [16]**. Despite the differences in the data observed in different surveys performed worldwide, it is the consensus that parasites with zoonotic potential are frequently present.

However, in this study, two beaches (Carmo and Pina) scored negatively. This was an unexpected finding, especially because the area presents the same features and hygienic conditions of the other beaches assessed. However, it is important to note that at Pina beach, the soil is turned as a process of cleaning twice a day. This event will facilitate the contact of deep layers of soil with the sun, reducing the survival of eggs of helminths. In addition, it is signposted as a Protected Area of Natural Preservation that probably inhibits the movement of people and the presence of domestic animals, and consequently the deposition of organic matter.

It is believed that the hygienic conditions of each beach and the population density observed in these areas may influence the results herein obtained. In addition, it is important to highlight that due to the advance of the sea, some beaches present a process called“fattening”of the sand strip, in which clayey soil is added, modifying the natural composition of the shore, which may influence the presence and survival of eggs of nematodes.

Data herein presented confirms that the FLOTAC technique should be considered a reliable technique to assess the soil contamination by eggs of nematodes. Recently, the applicability of this technique in the assessment of this kind of sample has been validated in a study where FLOTAC presented a better performance than the spontaneous sedimentation technique (SST) and centrifugal flotation technique (CFT) **[11].**

Based on these data, it is necessary to implement preventive measures and educational actions for the population in order to mitigate the potential risk of exposition to parasites of zoonotic importance. In addition, the main issue of this study (soil contamination) needs to be worked through a “One Health” approach, to establish the equilibrium among human, animals, and environmental conditions.

5. References

1. Santarém, P.A.; Guiffrida, R.; Zanin, D.A. Larva migrans cutânea: Ocorrência de casos humanos e identificação de larvas de *Ancylostoma* spp. em parque público do município de Taciba, São Paulo. Rev. Soc. Bras. Med. Trop **2019**, 37, 179–181. [CrossRef] [PubMed]

2. Silva, M.L.F.; Melo, V.S.P.; Milken, V.M.F.; Seixas, F.N. Parasitic contamination of public squares in the City of João Pessoa, Paraíba. Res. Soc. Dev. **2021**, 10, e74101018643. [CrossRef]

3. Melo, M.V.C.; Oliveira, T.R.; Málaga, S.M.R.; Cruz, D.R.S.; Jonas, J.M. Environmental contamination: The influence of seasonality in the occurrence of geohelminths in a public area of Fortaleza, Ceará. Vig. Sanit. Deb. **2020**, 8, 80–85.
4. Batista, S.P.; Silva, F.F.; Valêncio, B.A.; Carvalho, G.M.M.; Santos, A.; Costa, F.T.R. Zoonotic parasites in soils of public squares in the municipality of Sousa, Paraíba. Rev. Bras. Cie. Vet. **2019**, 26, 82–86. [CrossRef]
5. Lima, A.M.A.; Alves, L.C.; Faustino, M.A.G.; Lira, N.M.S.; Magalhães, A.; Lima, M.M. Búsqueda de huevos de anquilostomideos y toxocarídeos em el suelo de residências y escuelas em el barrio de Dois Irmãos, Recife—PE, Brasil. Parasitol. Latinoam. **2007**, 62, 89–93.
6. Cassenote, A.J.F.; Neto, J.M.P.; Lima-Catelani, A.R.A.; Ferreira, A.W. Contaminação do solo por ovos de geo-helmintos com potencial zoonótico na municipalidade de Fernandópolis, estado de São Paulo, entre 2007 e 2008. Rev. Soc. Bras. Med. Trop. **2011**, 44, 371–374. [CrossRef] [PubMed]
7. Gorgônio, A.S.; Sousa, D.L.C.; Bezerra, C.S.; Monteiro, G.D.F.; Paulo, F.S.;Costa, P.W.L. Agentes parasitários de importância em Saúde Única em solos de praças públicas em condições semiáridas. Res. Soc. Dev. **2021**, 10, 1. [CrossRef]
8. Moro, F.C.B.; Pradebon, J.B.; Santos, H.T.; Querol, E. Ancylostoma spp. e Toxocara spp. Biodivers. Pampeana **2008**, 6, 25–29
9. Cirne, F.S.L.; Silva, T.; Carvalho, A.C.F.; Dias, P.M.; Ramos, C.D.; Batista, L.C.S.O. Contaminação ambiental por ovos de Ancylostoma spp. e Toxocara spp. em áreas de seis praças públicas do município de Valença, estado do Rio de Janeiro. Acta Biol. Bras. **2017**, 8, 35–42. [CrossRef]

10. IBGE/SNIS Sistema Nacional de Informação Sobre Saneamento. Ministério do Desenvolvimento Regional. Disponível em. **2018**. Available online: www.snis.gov.br (accessed on 15 March 2021).
11. Ramos, I.C.N.; Macedo, L.O.; Ramos, R.A.N.; Carvalho, G.A.; Alves, L.C. The application of the FLOTAC technique for detection on the soil of immature forms of parasites of medical and veterinary concern. Exp. Parasitol. **2022**, in press.
12. Cringoli, G.; Rinaldi, L.; Maurelli, M.P.; Utzinger, J. FLOTAC: New multivalente techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat. Protoc. **2010**, 5, 503–515. [CrossRef] [PubMed]
13. Ferreira, I.S.; Nery, J.M.J.; Moraes, M.H.S.; Maggi, L.E.; Brilhante, A.F.; Sabino, K.C. Frequência de geo-helmintos em áreas recreativas da cidade de Rio Branco, estado do Acre, Brasil. South Am. J. Bas. Educ. Tec. Technol. **2018**, 5, 68–77.
14. Coura, L.C. Fatores relacionados à transmissão das geohelmintíases. Rev. Soc. Bras. Med. Trop. **1974**, 8, 4.
15. Prestes, L.F.; Jeske, S.; Santos, C.V.; Gallo, M.C.; Villela, M.M. Contaminação do solo por geohelmintos em áreas públicas de recreação em municípios do sul do Rio Grande do Sul (RS), Brasil. Ann. Trop. Pathol. **2015**, 44, 155–162. [CrossRef]
16. Bowman, D. Georgis Parasitologia Veterinária. In Elsevier Health Sciences Brazil, 2nd ed.; Elsevier: São Paulo, Brazil, **2010**; p. 447

CONSIDERAÇÕES FINAIS

O FLOTAC trata-se de uma ferramenta de uso ainda muito recente no Brasil. Sendo o laboratório onde as análises deste estudo foram procedidas, o único no país a desenvolver tais pesquisas nesse âmbito.

Este estudo poderá servir como base para melhoramento da aplicabilidade da técnica deFLOTAC em avaliações parasitológicas em hortaliças e solos.

É importante também estender o uso da ferramenta e melhorar novos estudos que venham ser realizados.

Este estudo trata-se do primeiro relato da utilização da técnica de FLOTAC em amostrasde solos.

ANEXO

Acta Parasitologica
https://doi.org/10.2478/s11686-019-00059-3

SHORT COMMUNICATION

An Additional Asset for the FLOTAC Technique: Detection of Gastrointestinal Parasites in Vegetables

Ingrid Carla do Nascimento Ramos[1] · Rafael Antonio Nascimento Ramos[2] · Alessio Giannelli[3] · Victor Fernando Santana Lima[4] · Giuseppe Cringoli[5] · Laura Rinaldi[5] · Gílcia Aparecida de Carvalho[2] · Leucio Câmara Alves[1]

Received: 16 August 2018 / Accepted: 29 March 2019

Abstract

Introduction Gastrointestinal parasites are considered amongst the most important threats to veterinary and human health worldwide. The transmission of these parasitic diseases usually involves the accidental ingestion of oocysts, cysts, eggs, and larvae, whose detection is generally achieved by classical coproparasitological methods, based on sedimentation and/or flotation techniques. These procedures might be inefficient or display a low sensitivity when performed on food matrices. This study aimed to assess the value of the FLOTAC technique for the detection of different parasitic stages of gastrointestinal parasites of domestic animals in fresh lettuce.

Material and methods Samples (n = 100) were purchased from public markets located in the metropolitan region of Recife (Brazil).

Results A total of 79% scored positive for eggs and/or cysts of gastrointestinal parasites. Protozoa and nematodes were detected in 32% (32/100) and 64% (64/100) of samples, respectively, with cysts of the genus *Entamoeba* (32%) and eggs of nematodes of the order Strongylida (30%) being the most frequently diagnosed.

Conclusions The findings herein reported demonstrate that the FLOTAC technique can be successfully applied for recovering food-borne parasites of medical and veterinary concern in food matrices.

Keywords FLOTAC · Helminths · Vegetables · Contamination · *Entamoeba*

Introduction

Infections by gastrointestinal parasites in animals and humans are considered important threats to public health worldwide. The transmission of these pathogens can be associated to the ingestion of contaminated food, including fresh vegetables [14]. This basic pattern jeopardises people living in areas lacking standard hygienic conditions [9].

Nowadays, salads based on lettuce (*Lactuca sativa*) are widely consumed and recommended as an importance source of micronutrients, vitamins and minerals [13]. The same vegetables, however, if improperly washed, disinfected or prepared, represent an important contamination source of gastrointestinal parasites, such as *Angiostrongylus cantonensis* and *Giardia duodenalis*, which may cause intestinal alterations (e.g., malabsorption, nutritional imbalance, diarrhoea), coagulative disorders, anaemia, and sometimes death [7]. Vegetables can indeed harbour infective stages of several parasites, with the contamination occurring during different steps of the food chain, including at the source, due to the contact with spoil waters, polluted soil, or organic fertilizers, and during the manufacturing, associated to unsanitary food handling [4].

The World Health Organization (WHO) guidelines for the microbiological quality of treated wastewater used in

✉ Rafael Antonio Nascimento Ramos
rafaelanramos10@yahoo.com.br

1 Department of Veterinary Medicine, Federal Rural University of Pernambuco, Recife, Brazil

2 Academic Unit of Garanhuns, Federal Rural University of Pernambuco, Av. Bom Pastor, s/n, Boa Vista, Garanhuns, Pernambuco CEP 55292-270, Brazil

3 Poulpharm BVBA, Izegem, Belgium

4 Department of Veterinary Medicine, Federal University of Sergipe, Nossa Senhora da Glória, Sergipe, Brazil

5 Department of Veterinary Medicine and Animal Productions, University of Napoli Federico II, Naples, Italy

Published online: 08 May 2019

agriculture indicate that the limit for nematode eggs should be less than 1 egg/l. However, this cannot be considered adequate in conditions that favour the survival of nematode eggs (lower mean temperatures and the use of surface irrigation). In such a case, the threshold should be reduced to less 0.1 egg/l [1]. It becomes evident that the detection of these parasitic stages in vegetables is of great importance for public health, as its sensitivity represents a guarantee onthe hygienic conditions involved in aliment production, stor-age, transport, and handling [12]. The laboratory diagnosis of these parasites is often based on sedimentation methods, which may show a low sensitivity. Over the last years, a new tool known as the FLOTAC technique has been developed to detect oocysts, cysts, eggs and larvae of gastrointestinal parasites in different animal species, including humans [3]. More recently, this technique has also been employed to recovering equine strongyle larvae in the pasture [11], but has never been used to assess the presence of parasites in vegetables used for human consumption. Therefore, the aim of this study was to assess the use of the FLOTAC to detect parasite stages (oocysts, cysts, eggs, and larvae) of gastrointestinal parasites of animals in smooth lettuces.

Materials and Methods

Samples

Smooth lettuces ($n = 100$) obtained in public markets located in the metropolitan region of Recife (08°03′14″ South and 34°52′52″ West), Northeast of Brazil, were used in this study. The number of samples was based on the number of public markets identified in the study area ($n = 50$), with two samples collected per market.

Samples were randomly selected, placed in plastic bags and stored in isothermal boxes (8 °C) until laboratory processing. Leaves that were inappropriate for human consumption (i.e., inedible leaves or stems) were discarded. Each sample was defoliated in distilled water (300 ml), rubbed with a brush and left to rest for 5 min. Subsequently, leaves were removed, the liquid was filtrated, and the material divided into two portions (150 ml each) for further analysis using the FLOTAC technique.

FLOTAC Technique and Data Analysis

The FLOTAC technique was performed using 150 ml of the water from the washing process. The material was transferred into two plastic tubes, and centrifuged at 1000 rpm for 5 min. Afterwards, the supernatant was discarded, and 6 ml of S2 (sodium chloride) and S7 (zinc sulfate) solutions were added to each tube. Then, the material was homogenized and distributed into both FLOTAC chambers, which were centrifuged for 5 min at 1000 rpm, and finally microscopically examined at different magnifications (100 × and 400 ×) [3].

Relative and absolute frequency of positivity was calculated for each parasite detected.

Results

Out of all the samples analyzed, 79% (79/100) scored positive for the presence of oocysts, cysts, eggs and/or larvae of gastrointestinal parasites. In particular, protozoan and nematodes were detected in 32% (32/100) and 64% (64/100) of the samples, respectively, with the most frequently observed being the cysts of the genus *Entamoeba* (32%) and nematode eggs of order Strongylida (30%) (Table 1). The mean number of eggs and cysts retrieved per sample were 24.7 (± 9.1) and 11.2 (± 2.1), respectively.

Discussion

This study investigated the use of the FLOTAC technique to detect immature forms of gastrointestinal parasites of animals in smooth lettuces purchased in public marketsin the Northeast of Brazil. The findings demonstrated that almost all samples were contaminated by some parasite stages, reflecting the poor sanitary conditions of cultivation and inadequate post-harvest handling at the public markets [8]. In general, in these environments vegetables are more exposed to contamination, since in several cases their storage is inappropriate.

Several immature forms of gastrointestinal parasites were detected in this study (Table 1). However, it is impor-tant to highlight that some of them (i.e., nematodes of the genus *Ascaris* and strongylid nematodes) include species of particular public health concern. For example, larvae of *Ancylostoma* spp. from dog feces have been recognized as the causative agent of Cutaneous Larva Migrans in human patients [2]. Similarly, *Ascaris lumbricoides* is considered by the WHO as some of the most important gastrointestinal parasites in humans. This parasite is widespread throughout

Table 1 Relative and absolute frequency of parasitic structures detected in lettuce using the FLOTAC technique

FLOTAC technique	Parasites (genus)	Positivity % (*n*/*N*)
	Order Strongylida	30% (30/100)
	Ascaris	16% (16/100)
	Strongyloides	22% (22/100)
	Entamoeba	32% (32/100)

the world, especially in tropical areas lacking basic sanitation [5].

Interestingly, no tapeworms and oocysts were detected in this study. The absence of these parasites is worth of note, since they have been considered important contaminants in vegetables [4].

Data on protozoa and nematodes is of great importance for public health, as it provides indirect information on the hygienic conditions involved in the production, storage, transport and handling of food products. Although the exact source of contamination of lettuces is difficult to achieve, it is known that animal feces are traditionally used as manure, accounting for a primary source of contamination. In addition, in several cases the cultivation areas do not present an appropriate basic sanitation, being human feces found in the environment and contaminating water and soil used in the production of vegetables. In this study, the FLOTAC technique was able to detect immature forms of gastrointestinal parasites of mammals in contaminated lettuces. For a long time, the microscopic examination of sediment was the only quantitative technique used to detect parasite stages in vegetables [4]. However, this present study demonstrates that the FLOTAC technique may be used to diagnose the contamination of lettuces by immature forms of gastrointestinal parasites of animals, and perhaps permit overcoming the limits presented by the classical sedimentation method extensively used. The importance of the FLOTAC technique for the detection of gastrointestinal parasites of animals is indisputable [6, 10]; however, the present study opens new possibilities for the use of this technique, beyond its use only in animals.

In conclusion, this is the first study performed to detect vegetal contamination by gastrointestinal parasites of animals with the use of the FLOTAC technique. The findings herein reported, open new perspectives for the use of this method on the detection of food-borne parasites of medical and veterinary concern.

Acknowledgements Authors would like thank Irma Y. T. López and Raphael Lepold for their support on the laboratory analyses. This article is based on the PhD thesis (Postgraduate Program in Tropical Animal Science) of the first author, developed at the Federal Rural University of Pernambuco.

References

1. Blumenthal UJ, Mara DD, Peasey A, Ruiz-Palacios G, Stott R (2000) Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bull World Health Organ 78:1104–1116
2. Caliskan E, Uslu E, Turan H, Başkan E, Kılıç N (2016) Cutaneous larva migrans: report of three cases from the Western Black Sea Region. Turk Microbiol Bull 50:165–169
3. Cringoli G, Rinaldi L, Maurelli MP, Utzinger J (2010) FLOTAC: new multivalente techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc 5:503–515. https://doi.org/10.1038/nprot.2009.235
4. Eraky MA, Rashed SM, Mel-S Nasr, El-Hamshary AM, Salah El-Ghannam A (2014) Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. J Parasitol Res 613960:1–7. https://doi.org/10.1155/2014/613960
5. Fowler AC, Déirdre HT (2016) The dynamics of *Ascaris lumbricoides* infections. Bull Math Biol 78:815–833
6. Lima VF, Ramos RA, Lepold R, Cringoli G, Rinaldi L, Faustino MA et al (2016) Use of the FLOTAC technique to diagnosing parasites of the urinary tract of dogs. Parasitol Res 115:1737–1739. https://doi.org/10.1007/s00436-016-4950-4
7. Liu LX, Weller PF (1993) Strongyloidiasis and other intestinal nematode infection. Infect Dis Clin N Am 7:655–682
8. Mohamed MA, Siddig EE, Elaagip AH, Edris AM, Nasr AA (2016) Parasitic contamination of fresh vegetables sold at central markets in Khartoum state, Sudan. Annu Clin Microbiol Antimicrobiol 15:17. https://doi.org/10.1186/s12941-016-0133-5
9. Ojha SC, Jaide C, Jinawath N, Rotjanapan P, Baral P (2014) Geohelminths: public health significance. J Infect Dev Ctries 8:5–16. https://doi.org/10.3855/jidc.3183
10. Ramos RA, Lima VF, Monteiro MF, Santana MA, Lepold R, Faustino MA et al (2016) New insights into diagnosis of *Platynosomum fastosum* (Trematoda: Dicrocoeliidae) in cats. Parasitol Res 115:479–482. https://doi.org/10.1007/s00436-015-4763-x
11. Ramos ICN, Ramos RA, Lima VFS, Giannelli A, Lopez YIT, Alves LC (2018) Applicability of FLOTAC® technique in recovering equine strongyle larvae in the pasture: a comparison study. Vet Parasitol 250:68–70. https://doi.org/10.1016/j.vetpar.2017.12.014
12. Shahnazi M, Jafari-Sabet M (2010) Prevalence of parasitic contamination of raw vegetables in villages of Qazvin Province, Iran. Foodborne Pathog Dis 7:1025–1030. https://doi.org/10.1089/fpd.2009.0477
13. Sharma S, Sheehy T, Kolonel L (2014) Sources of vegetables, fruits and vitamins A, C and E among five ethnic groups: results from a multiethnic cohort study. Eur J Clin Nutr 68:384–391. https://doi.org/10.1038/ejcn.2013.271
14. Sharma R, Singh BB, Gill JP (2015) Larva migrans in India: veterinary and public health perspectives. J Parasit Dis 39:604–612. https://doi.org/10.1007/s12639-013-0402-6

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Experimental Parasitology 242 (2022) 108379

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

The application of the FLOTAC technique for detection of helminth eggs of medical and veterinary importance in soil samples

Ingrid Carla do Nascimento Ramos [a,b], Rafael Antonio Nascimento Ramos [c,*], Lucia Oliveira de Macedo [a,c], Gílcia Aparecida de Carvalho [c], Leucio Câmara Alves [b]

[a] *Graduate Program of Animal Bioscience, Federal Rural University of Pernambuco, Recife, Brazil*
[b] *Department of Veterinary Medicine, Federal Rural University of Pernambuco, Recife, Brazil*
[c] *Laboratory of Parasitology, Federal University of the Agreste of Pernambuco, Garanhuns, Brazil*

ARTICLE INFO

Keywords:
Diagnosis
Environmental contamination
Nematode
Public health

ABSTRACT

The FLOTAC technique was initially developed to detected infective stages of parasites in animal and human feces. Recently, its applicability has been extended by the use in pastures and vegetables for human consumption. However, its use for the detection of parasites in the soil has never been investigated. In this study, we assessed the performance of the FLOTAC and compared with centrifugal flotation (CFT) and spontaneous sedimentation (SST) techniques. A total of 50 soil samples were collected from the Metropolitan region of Recife, Northeastern Brazil. Initially, samples were standardized, and then assessed in duplicate by the FLOTAC, CFT and SST. Cohen's kappa coefficient, sensitivity, specificity, positive and negative predictive values, and accuracy of the FLOTAC, CFT and SST were assessed using the results of the combination of all three techniques as gold standard. Out of all analyses performed, in 96% (48/50), helminth eggs of parasites were detected. In particular, 96% (48/50) of samples analysed through the FLOTAC technique, 76% (38/50) for CFT and 28% (14/50) for SST were positive. Nematode eggs of the Ancylostomatidae and Ascarididae families were those mostly detected in this study. Overall, the FLOTAC presented high values of sensitivity, demonstrating that this method may also be employed for detection of parasites in soil samples. This study opens new possibilities for the use of the FLOTAC technique, highlighting its role as a potential tool for detecting environmental contamination by parasites of medical and veterinary importance.

1. Introduction

Currently, the soil-transmitted helminths (STHs) affect more than one billion people throughout the world and approximately 300 million present a severe disease originated from these parasitic infections (PAHO, 2016). They are present especially in areas without basic sanitation and where the population is socially vulnerable (World Health Organization, 2017). For instance, in some African, American and Asian countries the prevalence of STHs may achieve more than 50%, which markedly contrast with some areas of Europe and North America where this kind of infection is almost inexistent due to measures of basic sanitation (World Health Organization, 2010).

Undoubtedly, STHs has been more frequently detected in rural areas or in peripheral regions of urban centers. Nonetheless, over the last years the increase of contamination of soil from the shore of beaches, parks and squares had been responsible for the rise of cases also in large urban centers (Brener et al., 2008; Azian et al., 2008; Silva et al., 2009; Sprenger et al., 2014; Macedo et al., 2019). In urban areas, this contamination occurs mainly due to the deposition of feces from dogs and cats, that depending on the species of helminths may excrete up to 15.000 eggs per gram of feces (Ribeiro, 2004). On the soil, these helminth eggs change into infective forms and may infect humans, especially children that are more exposed (Pedrassani et al., 2008; Silva et al., 2019).

Various studies have been conducted to detect the contamination of soil by infective stages of parasites of medical and veterinary importance (Steinbaum et al., 2016). It is consensus of the scientific community that the proper identification of these parasites at species level has been a challenge, due to the absence of information about putative definitive host. Even though, nematodes belonging to the families Ascarididae, Ancylostomatidae and Trichuridae have been those more frequently reported (Moro et al., 2008; Silva et al., 2009). Studies focusing on the

* Corresponding author. Federal University of the Agreste of Pernambuco. Av. Bom Pastor, s/n, Boa Vista, Garanhuns, Pernambuco, CEP: 55292-270, Brazil.
E-mail address: rafael.ramos@ufape.edu.br (R.A.N. Ramos).

https://doi.org/10.1016/j.exppara.2022.108379
Received 7 October 2021; Received in revised form 19 August 2022; Accepted 6 September 2022
Available online 16 September 2022
0014-4894/

health status of humans and animals are pivotal, especially in critical areas where the contamination of the environment is a trouble. In this context, the monitoring of contamination of soil in beaches, parks and squares is an important tool of vigilance based on the One Health approach that has been employed worldwide (Guimarães et al., 2005; Devera et al., 2008; Dado et al., 2012).

Although developed to detect parasites in feces, the techniques of Hoffman et al. (1934), as well as Faust et al. (1938), and Willis (1921) have been used for a long time to detect parasites in soil (Hoffman et al., 1934; Faust et al., 1938; Willis, 1921). In general, these methodologies are based on the flotation and/or sedimentation of eggs and/or oocysts, and have passed for slight modifications over the time. Although, they present low cost and are easily performed, some limitations may reduce the efficacy of these techniques. Most of them presented a limited sensitivity and the level of detection of eggs in animal's feces is dependent of the number of eggs excreted. For instance, *Toxocara* spp. may produce up to 200,000 eggs per day (Glickman and Schantz, 1981), which will contribute to high levels of environmental contamination. However, it is important to note that the contamination in soil samples is reduced, compared with fresh feces. For this reason, the detection and identification of infective stages of parasites in this kind of samples has been a challenge.

Over the last decade, the FLOTAC technique has been employed for detection of several parasites in animals and human feces (Cringoli et al., 2010). More recently, the applicability of this tool was enlarged for detection of parasites on the pasture (Ramos et al., 2018) and on vegetables (Ramos et al., 2019), but its use in the soil samples has never been assessed. Therefore, the aim of this study was to apply the FLOTAC technique for detection of medical and veterinary important helminth eggs in the soil.

2. Material and methods

2.1. Study area

The study was conducted using soil samples collected on shore of beaches (n = 10) and parks (n = 8) of the Metropolitan Region of Recife - MRR (8° 3′15″ South and 34° 52′ 53″ West), state of Pernambuco, Northeastern Brazil.

This area is characterized by a hot and humid tropical climate (*As*) with average annual temperature around 27 °C, mean relative humidity of 78%, and rainfall of 2000 mm/year, unevenly distributed in dry and rainy periods. Approximately 3.975,411 people live in the MRR, and only 46.6% of this population has access to basic sanitation and treated water (IBGE/SNIS, 2018).

2.2. Soil samplings

A total of 50 sites of collections were chosen by convenience, and divided into 42 points on the beaches and 8 points in parks. For each site, an area of 100 m^2 (10 m × 10 m) was delimited and five samplings (sub-points) performed, one in each corner and one in the central part of the area (Milano and Oscherov, 2002; Sousa et al., 2014). The soil was extracted from the superficial layer (5–10 cm depth) with the aid of a plastic spatula; 100 g was collected for each sub-point totaling 500 g in total (Santarém et al., 1998). The material was kept in plastic bags, stored in isothermal boxes at 4 °C and processed within 24 h. All samplings were carried out from 5 to 7 a.m. in areas with intense movement of human beings and animals. The sampling was defined probabilistically by convenience (Reis, 2003).

2.3. Laboratorial procedures

2.3.1. Standardization of samples

All five samples (sub-points) of each point were homogenized prior and after pooled, sieved (149 μm) and 300 g weighted for standardization. Afterwards, the sample was placed in a plastic box and 900 mL of distilled water was added. The material was homogenized in a shaker for 30 s, then left to rest for 1 min, and the supernatant was versed in two sieves of 149 μm and 38 μm, respectively. Finally, the sieve of 38 μm, where helminth eggs of parasites were retained, was washed with 150 mL of distilled water and the material divided in three aliquots of 50 mL each (Jenkins, 1964). For each technique described below we use a single aliquot of 50 mL.

2.3.2. Spontaneous sedimentation technique (SST), centrifugal flotation technique (CFT) and FLOTAC technique

Samples were evaluated by SST using saturated sucrose solution (s.g. 1.20) (Hoffman et al., 1934) and CFT method using zinc sulphate solution (s.g. 1.18) (Jenkins, 1964). Four slides were prepared using 50 μl each of material and analysed at different magnifications (100X and 400X). Finally, the FLOTAC was performed using NaCl (s.g. 1.20) as flotation solution (Cringoli et al., 2010). The FLOTAC chamber was microscopically analysed at different magnifications (100X and 400X).

All three techniques were performed in duplicate, totaling 300 analyses, 100 for each technique. A sample was considered positive when eggs were detected in at least one analysis.

2.4. Data analyses

Descriptive statistical analysis was performed. The Lilliefors test was used to verify the normality of the data. Overall data on the positivity of three techniques were analysed using the Chi-square test. Conversely, Chi-square of Partition test was used to compare the occurrence of families of parasites detected. A 5% significance level was considered. Both analyses were performed using the BioEstat software version 5.3 (Ayres et al., 2007).

The Cohen's Kappa coefficient (k) was calculated to evaluate the agreement between different techniques (Landis and KocK, 1977). In addition, the combination of the results of all three techniques was considered as gold standard for the calculation of sensitivity, specificity, positive and negative predictive values, and accuracy of each technique (Herbert, 2013). For the Kappa calculation all samples were considered in a single analysis, whereas for the other parameters (sensitivity, specificity, predictive values, and accuracy) data of parks and beaches were assessed separately.

3. Results

Out of all analyses performed, in 96% (48/50) helminth eggs were detected. In particular, FLOTAC technique detected more positive samples (96%; 48/50) followed by CFT (76%; 38/50) and SST method (28%; 14/50) ($\chi 2 = 71.141$; $p < 0.0001$).

Parasites of the families Ancylostomatidae, Trichuridae, Ascarididae and Dipylidiidae were detected in at least one technique employed. Ancylostomatidae and Ascarididae were the parasites most frequently retrieved, regardless of the technique utilized ($\chi 2 = 0.8553$; $p = 0.6520$). Simultaneous contamination by two or more parasites were observed in 68% (34/50) of soil samples (Table 1). It is important to note that helminth eggs were detected in 100% (8/8) and 95.23% (40/42) of samples from parks and beaches, respectively. Additionally, 26% (13/50) of the samples were positive for all methods, whereas 4% (2/50) were negative.

Overall, the kappa analysis revealed the following results: FLOTAC *versus* SST ($k = 0.09$; $p < 0.001$): poor concordance, FLOTAC *versus* CFT ($k = 0.53$; $p < 0.001$): moderate concordance, and CFT *versus* SST ($k = 0.28$; $p < 0.001$): considerable concordance. Values of sensitivity, specificity, predictive positive and negative values, as well as accuracy for samples from parks and beaches are reported on Table 2.

Table 1
Helminth eggs of parasites detected in soil samples through SST, CFT and FLOTAC techniques.

Method of diagnosis	Parasites (Family)	% (n/N)
SST	Ancylostomatidae	14 (7/50)
	Ascarididae	14 (7/50)
CFT	Ancylostomatidae	10 (5/50)
	Ascarididae	14 (7/50)
	Trichuridae	2 (1/50)
	Ancylostomatidae + Ascarididae	26 (13/50)
FLOTAC	Ancylostomatidae	22 (11/50)
	Ascarididae	40 (20/50)
	Trichuridae	10 (5/50)
	Dipylidiidae	2 (1/50)
	Ancylostomatidae + Ascarididae	24 (12/50)
	Ancylostomatidae + Trichuridae	10 (5/50)
	Ascarididae + Trichuridae	4 (2/50)
	Ancylostomatidae + Trichuridae + Ascarididae	4 (2/50)

4. Discussion

This study demonstrated that the FLOTAC presented a higher sensitivity when compared with two classic methods (CFT and SST) for detection of helminth eggs in soil samples. Overall, samples analysed by the FLOTAC presented a high positivity (FLOTAC - 96%) when compared with all other methods (CFT - 76% and SST - 28%).

Although different families of parasites had been detected, the families Ancylostomatidae and Ascarididae predominated. Eggs of these nematodes are frequently detected in sandy soils with the presence of organic matter. The soil characteristic is very important for the egg's evolution to the larval stage, as well as its migration in different types of soils (Eisen et al., 2019). In this study, soil samples from beaches (n = 42) and parks (n = 8) were evaluated. It is believed that these kinds of soils may present different characteristics, which may be important for the development and survival of parasites.

It is important to note that the Ascarididae family corresponds to the first family of parasites more retrieved. Eggs of these nematodes have a thick and resistant membrane, which allow them to survive in high temperatures. For instance, about 30% of Ascarididae eggs survive at temperatures up to 65 °C (Cameron, 1963). Considering that in the region of study the temperature mean is about 27 °C and large variations has not been observed, eggs may be present in the environment throughout the year, representing a risk for animal and human health.

The FLOTAC method (96%) presented a higher positivity when compared with CFT (76%) and the SST (28%) techniques. It has been already proved that the FLOTAC exhibits a good performance when compared with other classical methods, but in the detection of parasites in feces (Lima et al., 2015; Duthaler et al., 2010). Several reasons for the higher efficiency of the FLOTAC have been suggested, including the density of solutions that influence flotation of parasitic structures. It has been proved that NaCl flotation solution may present a limited performance when compared with sucrose-based solution used for the detection of some helminth eggs (Cringoli et al., 2004). However, the use of NaCl in the present study did not difficult the retrieval of helminth eggs, since a sensitivity of 100% has been observed for the FLOTAC technique.

Despite of the difference in the number of samples of parks (n 8) and beaches (n 42), the assessment of sensitivity, specificity, predictive values and accuracy presented the same results for the FLOTAC. Infact, for both types of samples, the FLOTAC exhibited 100% values for all parameters assessed. Although, CFT and SST had presented specificity and PPV of 100%, all other parameters were lower than those presented by the FLOTAC technique (see Table 2).

It is important to note that this study retrieved helminth eggs in soil samples, which are more difficult than in fresh fecal samples. Even though, the FLOTAC presented a high sensitivity (100%), indicating its role as a potential tool to assess the environment contamination by animal or human feces. It is known that an ideal diagnostic method with 100% of sensitivity and 100% of specificity is difficult to achieve as the increase of sensitivity implies in a reduction of specificity and vice and versa. Although, values of 100% for specificity and PPV had been achieved for all techniques, low sensitivities were observed for CFT and SST.

The FLOTAC (n = 4) was also able to detect a greater variety of families of parasites when compared with CFT (n = 3) and SST (n = 2), demonstrating that it is a more complete diagnostic tool than classical methods currently utilized for this purpose. Unfortunately, the quantification of helminth eggs was not performed, which is considered an important limitation of this study. Actually, this data would provide important information for the assessment of all techniques herein employed. It is important to note that the moderate concordance kappa value was observed between FLOTAC *versus* CFT (k 0.53), therefore the use of both techniques in combination will increase the efficiency of detection of helminth eggs in the soil.

For a long time, the FLOTAC had been used only for the detection of parasites in fresh fecal samples (Allam et al., 2021). Recently, its use has been enlarged for detection of parasites on the pasture (Ramos et al., 2018) and also in vegetables used for human consumption (Ramos et al., 2019). It is known that the methods of diagnosis have improved faster, tests are constantly developed, and the technology of existing tests is continuously being improved (Bossuyt et al., 2003). Nonetheless, most of parasitological methods used for detection of intestinal helminths and protozoan infections have passed for few modifications (Carvalho et al., 2012). This is the first study that investigated the use of the FLOTAC to the detection of parasites in the soil. The knowledge of the presence of these parasites in the environment is pivotal for the establishment of preventive measures against STHs. Finally, data herein presented enlarge the applicability of the FLOTAC technique demonstrating that may be also employed for monitoring the environmental contamination by helminth eggs of parasites of medical and veterinary concern.

Financial support

This research did not receive any specific grant from funding

Table 2
Sensitivity, specificity, negative and positive predictive values obtained for FLOTAC, CTF and SST techniques.

Method of diagnosis	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
Parks	%				
FLOTAC	100 (71.51–100)	100 (2.50–100)	100 (2.50–100)	100 (2.50–100)	100 (73.54–100)
CTF	81.82 (48.22–97.72)	100 (2.50–100)	100 (2.50–100)	33.33 (12.49–63.66)	83.33 (51.59–97.91)
SST	36.36 (10.93–69.21)	100 (2.50–100)	100 (2.50–100)	12.50 (8.37–18.25)	41.67 (15.17–72.33)
Beaches					
FLOTAC	100 (90.51–100)	100 (2.50–100)	100 (2.50–100)	100 (2.50–100)	100 (90.75–100)
CTF	78.38 (61.79–90.17)	100 (2.50–100)	100 (2.50–100)	11.11 (6.34–18.76)	78.95 (62.68–90.45)
SST	27.03 (13.79–44.12)	100 (2.50–100)	100 (2.50–100)	3.57 (2.95–4.31)	28.95 (15.42–45.90)

CI confidence intervals, PPV positive predictive value, NPV negative predictive value.

agencies in the public, commercial, or not-for-profit sectors.

Credit authors statement

Ingrid C. N. Ramos: Conceptualization, Methodology, Writing - original draft. Rafael A. N. Ramos: Conceptualization, Data curation, Writing - review and editing. Lucia O. Macedo: Data curation, Formal analysis, Writing - review and editing.

Gílcia A. Carvalho: Data curation, Writing - review and editing.

Leucio C. Alves: Conceptualization, Project administration, Writing - review and editing.

Declaration of competing interest

The authors declare no competing interest.

Acknowledgements

This article is based on the PhD thesis (Graduate Program in Animal Bioscience) of the first author, developed at the Federal Rural University of Pernambuco, and supported by a grant fellowship from the Coordination for the Improvement of Personnel of Higher Education (CAPES). R.A.N.R received a research fellowship from CNPq (Bolsa de Produtividade; grant no. 305971/2021-2).

References

Allam, A.F., Farag, H.F., Lotfy, W., Fawzy, H.H., Elhadad, H., Shehab, A.Y., 2021. Comparison among FLOTAC, Kato-Katz and formalin ether concentration techniques for diagnosis of intestinal parasitic infections in school children in an Egyptian rural setting. Parasitology 148, 289–294.

Ayres, M., Ayres Jr., M., Ayres, D.L., Santos, A.A., 2007. BIOESTAT – Statistical Applications on the Biomedical Sciences. Ong. Mamirauá.

Azian, M.Y., Sakhone, L., Hakim, S.L., Yursi, M.Y., Nurulsyamzawaty, Y., Zuhaizam, A. H., Rodi, I.M., Maslawaty, M.N., 2008. Detection of helminth infections in dogs and soil contamination in rural and urban areas. Southeast Asian J. Trop. Med. Public Health 39, 205–212.

Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P., Irwig, L.M., Lijmer, J.G., Moher, D., Rennie, D., Henrica, C.W., 2003. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Clin. Radiol. 58, 575–580.

Brener, B., Mattos, D.P.B.G., Mattos, G., Millar, P.R., Arshiro, E.K.N., Duque-Ferreira, V., Sudré, A.P., 2008. Evaluation of the contamination of public squares of three regions of the State of Rio de Janeiro, Brazil, by eggs and larvae of helminths. Braz. J. Pathol. 37, 247–254.

Cameron, T.W.M., 1963. Environmental Factors Related to the Free and Living Stages Helminth. World Health Organization - WHO.

Carvalho, G.L., Moreira, L.E., Pena, J.L., Marinho, C.C., Bahia, M.T., Machado-Coelho, G. L., 2012. A comparative study of the TF test, Kato-Katz, Hoffman Pons-Janer, Willis and Baermann-Moraes coprologic methods for the detection of human parasitosis. Mem. Oswaldo Cruz Inst. 107, 80–84.

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G., Scala, A., 2004. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. Vet. Parasitol. 123, 123–131.

Cringoli, G., Rinaldi, L., Maurelli, M.P., Utzinger, J., 2010. FLOTAC: new multivalente techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat. Protoc. 5, 503–515.

Dado, D., Izquierdo, F., Vera, O., Montoya, A., Mateo, M., Fenoy, S., Galván, A.L., 2012. Detection of zoonotic intestinal parasites in public parks of Spain. Potential epidemiological role of microsporidia. Zoonoses Public Health 59, 23–28.

Devera, R., Blanco, Y., Hernández, H., Simoes, D., 2008. *Toxocara* spp. and other helminths in squares and parks of Ciudad Bolivar (Venezuela). Enferm. Infecc. Microbiol. Clín. 26, 23–26.

Duthaler, U., Rinaldi, L., Maurelli, M.P., Vargas, M., Utzinger, J., Cringoli, G., Keiser, J., 2010. Fasciola hepatica: comparison of the sedimentation and FLOTAC techniques for the detection and quantification of faecal egg counts in rats. Exp. Parasitol. 126, 161–166.

Eisen, A.K.A., Demoliner, M., Oliveira, K.G., Troian, E.A., Mallmann, L., Filippi, M., Almeida, P.R., Spilki, F.R., 2019. Soil contamination of a public park by human and canine mastadenovirus, as well as hookworms and *Toxocara* spp eggs. J. São Paulo Inst. Trop. Med. 61, e60.

Faust, E.C., D'Antoni, J.S., Odom, V., Miller, M.J., Peres, C., Sawitz, W., Thomen, L.F., Tobie, J., Walker, H., 1938. A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. Am. J. Trop. Med. Hyg. 18, 169–183.

Glickman, L.T., Schantz, P.M., 1981. Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol. Rev. 3, 230–250.

Guimarães, A.M., Alves, E.G.L., Rezende, G.F., Rodrigues, M.C., 2005. *Toxocara* sp. eggs and *Ancylostoma* sp. larva in public parks, Brazil. J. Public Health 39, 293–295.

Herbert, R., 2013. Confidence interval calculator. https://pedro.org.au/. (Accessed 3 August 2021).

Hoffman, W.A., Pons, J.A., Janer, J.L., 1934. The sedimentation concentration methods in *Schistosomiases masoni*. PR J. Public Health Trop. Med. 9, 283–289.

IBGE/SNIS - Brazilian institute of geography and statistics/National sanitation information system. Available at: www.snis.gov.br–. (Accessed 15 March 2021).

Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Dis. Rep. 48, 692.

Landis, J.R., KocK, G.G., 1977. An application of hierarchical Kappa- type statistics in the assessment of majority agreement among multiple observers. Biometrics 33, 363–374.

Lima, V.F., Cringoli, G., Rinaldi, L., Monteiro, M.F., Calado, A.M., Ramos, R.A., Meira-Santos, P.O., Alves, L.C., 2015. A comparison of mini-FLOTAC and FLOTAC with classic methods to diagnosing intestinal parasites of dogs from Brazil. Parasitol. Res. 114, 3529–3533.

Macedo, L.O., Bezerra-Santos, M.A., Santana, B.B., Pereira, T.A., Silva, N.M.M., Alves, L. C., Ramos, R.A.N., Carvalho, G.A., 2019. Parasites with zoonotic potential in canine fecal samples from Garanhuns, Pernambuco, Brazil. Vet. Med. 13, 227–231.

Milano, A.M.F., Oscherov, E.B., 2002. Contamination by canine parasites of zoonotic importance on beaches of the city of Corrientes, Argentina. Latin. Am. J. Parasitol. 57, 119–123.

Moro, F.C.B., Pradebon, J.B., Santos, H.T., Querol, E., 2008. Ocorrência de *Ancylostoma* spp. e *Toxocara* spp. em praças e parques públicos dos municípios de Itaqui e Uruguaiana, fronteira oeste do Rio Grande do Sul. Biodiversidade Pampeana 6, 25–29.

PAHO - Pan American Health Organization, September 2016. Plan of action for the elimination of neglected infectious diseases and post-elimination actions 2016-2022. In: 68th Session of the Regional Committee of WHO for the Americas. Washington, D. C., USA, 26-30.

Pedrassani, D., Viera, A.M., Thiem, E.M.B., 2008. Contamination by *Toxocara* spp. and *Ancylostoma* spp. in areas of leisure from Canoinhas county, Santa Catarina state. Arch. Vet. Sci. 13, 110–117.

Ramos, I.C.N., Ramos, R.A.N., Giannelli, A., Lima, V.F.S., Cringoli, G., Rinaldi, L., Carvalho, G.A., Alves, L.C., 2019. An additional asset for the FLOTAC technique: detection of gastrointestinal parasites in vegetables. Acta Parasitol. 64, 423–425.

Ramos, I.C.N., Ramos, R.A.N., Giannelli, A., Lima, V.F.S., López, I.Y.T., Alves, L.C., 2018. Applicability of FLOTAC technique in recovering equine strongyle larvae in the pasture: a comparison study. Vet. Parasitol. 250, 68–70.

Reis, J.C., 2003. Estatística aplicada à pesquisa em ciência veterinária, 1a ed., p. 651 Olinda.

Ribeiro, V.M., 2004. Control of helminthes of dogs and cats. Braz. J. Vet. Parasitol. 13, 88–95.

Santarém, V.A., Sartor, I.F., Bergamo, F.M.M., 1998. Contamination by *Toxocara* spp. eggs in public parks and squares in Botucatu, São Paulo state, Brazil. J. Braz. Soc. Trop. Med. 31, 529–533.

Silva, P.F., Cavalcanti, I.M.D., Irmão, J.I., Rocha, F.J., 2009. Common beach sand contamination due to enteroparasites on the southern coast of Pernambuco state, Brazil. J. São Paulo Inst. Trop. Med. 51, 217–218.

Silva, N.M.M., Bezerra-Santos, M.A., Santana, B.B., Macedo, L.O., Alves, L.C., Ramos, R. A.N., Carvalho, G.A., 2019. Soil contamination by zoonotic gastrointestinal parasites of mammals in Garanhuns, Pernambuco, Brazil. Vet. Med. 13, 65–69.

Sousa, J.O., Santos, E.O., Lira, E.M., Sá, I.C., Hirsch, C.M., 2014. Parasitological analysis of sand from urban beaches of João Pessoa – PB. Braz. J. Health Scie. 18, 195–202.

Sprenger, L.K., Green, K.T., Molento, M.B., 2014. Geohelminth contamination of public areas and epidemiological risks factors in Curitiba, Brazil. Braz. J. Vet. Parasitol. 23, 69–73.

Steinbaum, L., Njenga, S.M., Kihara, J., Boehm, A.B., Davis, J., Null, C., Pickering, A.J., 2016. Soil-transmitted helminth eggs are present in soil at multiple locations within households in rural Kenya. PLoS One 11, e0157780.

Willis, H.H., 1921. A simple levitation method for detection of hookworm ova. Med. J. Aust. 2, 375–376.

World Health Organization, 2010. The Human Right for Water and Sanitation. WHO, Geneva.

World Health Organization, 2017. Infectious Diseases of Potential Risk for Travelers. WHO, Geneva.

parasitologia

MDPI

Article

Contamination by Eggs of Nematodes (*Nematoda*) of Public Health Concern in Tropical Beaches

Ingrid Carla do Nascimento Ramos [1], Thiago Antonio Rodrigues Freire Lima [2], Rafael Antonio do Nascimento Ramos [3,*], Gílcia Aparecida de Carvalho [3] and Leucio Câmara Alves [1]

[1] Department of Veterinary Medicine, Federal Rural University of Pernambuco, Recife 52171-900, PE, Brazil; ingridcnramos@gmail.com (I.C.d.N.R.); leucioalves@gmail.com (L.C.A.)

[2] Department of Cartographic Engineering, Federal University of Pernambuco, Recife 50670-901, PE, Brazil; tarflima@yahoo.com.br

[3] Laboratory of Parasitology, Federal University of the Agreste of Pernambuco, Garanhuns 55292-270, PE, Brazil; gilcia.carvalho@ufape.edu.br

* Correspondence: rafael.ramos@ufape.edu.br

Abstract: Public spaces such as beaches develop an important role in the welfare of the human population in Brazil, where leisure and safety spaces are increasingly scant. Knowledge about the possible role of contaminated sand of beaches on the health of animals and humans is pivotal to determine risk areas and preventing future cases. Therefore, the aim of this study was to assess the presence of eggs of nematodes of public health interest on tropical beaches. Soil samplings (n = 42) from 10 different beaches located in the Metropolitan region of Recife, Northeastern Brazil, were analyzed through the FLOTAC technique. In 80% (8/10) of beaches, eggs of nematodes were detected. In particular, if we consider each individual sample (n = 42) a positivity rate of 90.4% (38/42) was observed. Eggs belonging to the families Ascarididae (47.6%; 20/42), Ancylostomatidae (26.1%; 11/42), and Trichuridae (11.9%; 5/42) were detected. Based on these data, it is necessary to implement preventive measures and educational actions for the population in order to mitigate the potential risk of exposition to parasites of zoonotic importance. Additionally, the FLOTAC technique has been shown to be a reliable technique for assessing soil contamination by nematode eggs.

Keywords: Ascarididae; Ancylostomatidae; feces; animals; humans; beaches

Citation: Ramos, I.C.d.N.; Lima, T.A.R.F.; Ramos, R.A.d.N.; Carvalho, G.A.d.; Alves, L.C. Contamination by Eggs of Nematodes (*Nematoda*) of Public Health Concern in Tropical Beaches. *Parasitologia* **2022**, *2*, 95–100. https://doi.org/10.3390/parasitologia2020010

Academic Editor: Geoff Hide

Received: 8 April 2022
Accepted: 27 April 2022
Published: 1 May 2022

1. Introduction

Public spaces everywhere, such as parks and beaches, have played an important role in the welfare of the human population. These areas have been used for several purposes, such as sports practice, meetings, and rest, promoting a pivotal social aspect in the life of humans. In general, an intense movement of people and their pets (e.g., dogs and cats) has been observed in these areas that frequently present a ground contaminated by eggs of helminths of medical and veterinary concern [1].

The contamination of the soil on beaches and in parks has been extensively studied [2], but over the last few years, little attention has been given to this sanitary threat that has increased [3]. It is important to note that the increase in soil contamination by eggs of helminths is related to the rise in the human population and their pets, especially in urban areas [4]. Undoubtedly, in developing countries, this kind of environmental contamination has become a public health matter due to several reasons, including health education, the absence of basic sanitation, and animal welfare [2].

For instance, in Brazil, at the beginning of the current century, several studies were conducted on squares and beaches. These researches have demonstrated contamination of the soil varying from 4.69% in Pernambuco [5] to 40% in São Paulo [6]. More recently, a similar study carried out in the state of Paraíba revealed that 96.4% of soil samples collected from squares were contaminated with eggs of nematodes, including *Ancylostoma* spp. (Dubini, 1834) (28.6%), *Trichuris* spp. (Morgani, 1740) (21.4%), and *Strongyloides* spp. (Normand, 1876) (46.4%) [7].

Overall, parasites belonging to the genera *Ancylostoma*, *Toxocara* (Wilder, 1950), *Trichuris*, *Ascaris* (Linnaeus, 1758), and *Strongyloides* are those more frequently detected [8,9]. It is important to note that some of them are parasites of dogs, cats, and also humans, and in some cases, the sharing of these pathogens among different mammal species has been considered a common event. Most of these parasite species spend part of their life cycle on the soil, which is considered an important source of accidental infection of animals, including human beings [10]. Indeed, the role of contamination of the soil on the health of animals and humans is indisputable. However, even with all knowledge acquired over the last decades, the lack of education and the absence of basic sanitation in many Brazilian regions contribute to the persistence of this issue, exposing the animal and human population to the risk of parasitic infections.

It is also important to note that public spaces such as beaches have developed an important role in the welfare of the human population in Brazil, where leisure and safety spaces are increasingly scant. In this context, knowledge about the possible role of contaminated sand of beaches on the health of animals and humans is pivotal to determining risk areas and preventing future cases of parasitic infections. Therefore, the aim of this study was to assess the presence of eggs of nematodes of public health interest in tropical beaches in Northeastern Brazil.

2. Material and Methods

2.1. Study Area

The study was conducted in the Metropolitan Region of Recife (MRR) (8°3′15″ S and 34°52′53″ W), state of Pernambuco, Northeastern Brazil. This area is characterized by a hot and humid tropical climate (As) with an average annual temperature of around 27 °C and rainfall of 2000 mm/year, unevenly distributed in dry and rainy periods. Climatic information was obtained from the Pernambuco Water and Climate Agency (Agência Pernambucana de Águas e Clima, APAC, Recife, Brazil). Approximately 3,975,411 people live in the MRR, and only 46.6% of this population has access to basic sanitation and treated water [10].

2.2. Soil Samplings and Laboratorial Procedures

Soil samplings (n = 42) were performed on the beaches and encompassed an area of approximately 38.14 km of the seafront. Briefly, samplings were carried out at 42 points from 5 a.m. to 7 a.m. For each collection point, an area of 100 m^2 was delimited and the soil was collected of five sub-points (four on the corners and one in the center). The collection was carried out in a superficial layer up to 5 cm deep with 100 g at each point, totaling 500 g of soil. In some areas of sampling was observed the presence of sewage discharge on the beach, as well as the presence of domestic animals (Figure 1).

Figure 1. Areas of sampling with sewage discharge on the beaches. (**A**–**D**): sewage discharge.

Each sample was individually standardized according to Ramos [11]. Briefly, they were homogenized, sieved (149 μm), and 300 g weighed. Afterwards, the sample was placed in a plastic box and 900 mL of distilled water was added. The material was homogenized for 30 s, then left to rest for one minute, and the supernatant was separated in two sieves of 149 μm and 38 μm, respectively. Finally, the sieve of 38 μm, where eggs of nematodes were retained, was washed with 50 mL of distilled water and the material analyzed through the FLOTAC technique [12].

2.3. Data Analyses and Map Construction

All data were firstly described by descriptive statistics and the relative and absolute frequencies were obtained. Afterwards, the distribution of parasites was performed using choropleth maps with color intensity levels, using the Quantum Geographic Information System (QGIS 3.16, Hannover, Germany).

3. Results

Soil samples from 10 different beaches were analyzed, and nematode eggs were detected in 80% (8/10) of them. In particular, if we consider each individual sample (n = 42), a positivity rate of 90.4% (38/42) was observed. Eggs belonging to the families Ascarididae (47.6%; 20/42), Ancylostomatidae (26.1%; 11/42), and Trichuridae (11.9%; 5/42) were detected (Table 1).

Table 1. Positivity for eggs of nematodes in the sand of beaches located in the Metropolitan Region of Recife.

Beach	Municipality	Collection Points (N)	Overall Positivity (%; *n*/N)	Family of Nematodes (%; *n*/N)
Pau Amarelo	Paulista	3	100% (3/3)	Ancylostomatidae (100%; 3/3) Ascarididae (100%; 3/3)
Janga	Paulista	9	100% (9/9)	Ancylostomatidae (88.8%; 8/9) Ascarididae (100%; 9/9) Thichuridae (77.8%; 7/9)
Rio Doce	Olinda	3	100% (3/3)	Thichuridae (100%; 3/3)
Casa Caiada	Olinda	6	100% (6/6)	Ancylostomatidae (100%; 6/6) Ascarididae (100%; 6/6) Thichuridae (83.3%; 5/6)
Bairro Novo	Olinda	3	100% (3/3)	Ancylostomatidae (100%; 3/3) Ascarididae (100%; 3/3)
Carmo	Olinda	2	0 (0/2)	-
Milagres	Olinda	3	100% (3/3)	Ascarididade (100%; 3/3)
Pina	Recife	2	0 (0/2)	-
Boa Viagem	Recife	5	100% (5/5)	Ancylostomatidae (100%; 5/5) Ascarididade (100%; 5/5)
Piedade	Jaboatão dos Guararapes	6	100% (6/6)	Ancylostomatidae (100%; 6/6) Ascarididade (100%; 6/6)

It is important to note that the higher diversity of eggs of nematodes was observed in Janga and Casa Caiada beaches (three distinct families), followed by Pau Amarelo, Bairro Novo, and Boa Viagem beaches (two distinct families), and finally Rio Doce and Milagres beaches with only one family of nematodes. No eggs of nematodes were retrieved in Carmo and Pina beaches. The graphical distribution of eggs of nematodes on the shore of Metropolitan Region of Recife is represented on Figure 2.

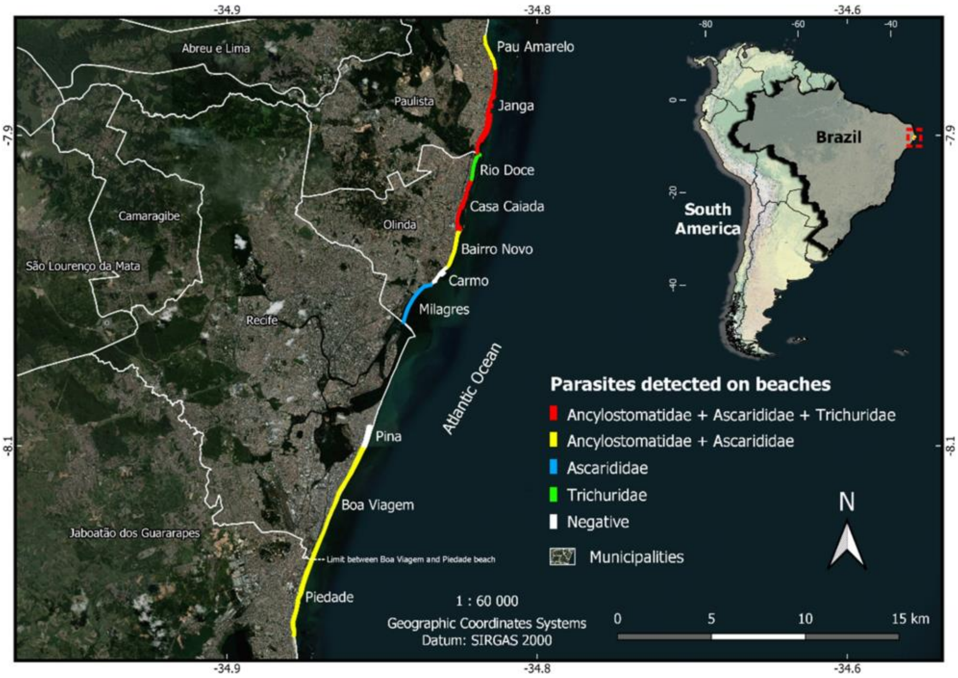


Figure 2. Graphical distribution of nematode families on the shore of Metropolitan Region of Recife.

4. Discussion

This study revealed that eggs of nematodes of medical and veterinary concern are present in sand of the shore of the Metropolitan Region of Recife, with some areas presenting a high risk of exposition for the animal and human population.

Overall, the eggs of nematodes were detected in 80% (8/10) of beaches and in 90.4% (38/42) of soil samples. This high positivity has been a trend observed in other public spaces throughout Brazil. For instance, in the municipality of Sousa, in the state of Paraíba, Helmith eggs were detected in 90% of the analyzed soil samples of public squares [4], whereas 96.4% of positivity was recorded in public squares from João Pessoa, Paraíba [2].

It is important to note that all families of parasites herein detected have been frequently observed in previous studies, where in most cases, the predominance of Ancylostomatidae eggs has been reported [1,4,13]. In comparison, in the present study, Ascarididae eggs predominated. These eggs may have as their source of contamination sewage and waste discharges, as well as feces of animals (e.g., dogs, cats, and human beings) excreted directly on the sand. It is known that Ascarididae eggs are very resistant in the environment. In general, they have a layer formed by chitin, proteins, and lipids, which may resist adverse environmental conditions [14].

Regardless of the family retrieved, both of them (e.g., Ascarididae or Ancylostomatidae) may comprise species of zoonotic concern causative agents of Larva Migrans syndrome, as well as other infections of medical and veterinary importance [1]. The high frequency of eggs of nematodes reported in this study represents an important finding from an epidemiological perspective. The study area presents suitable environmental conditions for the establishment and development of these nematodes on the soil. In addition, the regulations regarding the access of animals to beaches are nonexistent or poorly supervised, allowing the unrestricted access of stray animals in these places. Unfortunately, the lack of basic sanitation with the deposition of sanitary sewage on beaches (see Figure 1) and also poor hygiene behavior of the population are still common practice in these areas and contributes to the retrieval of these parasites.

Although less frequent eggs of the Trichuridae family were also detected, parasites belonging to this group have been already observed in previous studies [4,15], and it seems that eggs are very resistant and remain viable in hot and humid soil for months [16]. Despite the differences in the data observed in different surveys performed worldwide, it is the consensus that parasites with zoonotic potential are frequently present.

However, in this study, two beaches (Carmo and Pina) scored negatively. This was an unexpected finding, especially because the area presents the same features and hygienic conditions of the other beaches assessed. However, it is important to note that at Pina beach, the soil is turned as a process of cleaning twice a day. This event will facilitate the contact of deep layers of soil with the sun, reducing the survival of eggs of helminths.In addition, it is signposted as a Protected Area of Natural Preservation that probably inhibits the movement of people and the presence of domestic animals, and consequently the deposition of organic matter.

It is believed that the hygienic conditions of each beach and the population density observed in these areas may influence the results herein obtained. In addition, it is important to highlight that due to the advance of the sea, some beaches present a process called "fattening" of the sand strip, in which clayey soil is added, modifying the natural composition of the shore, which may influence the presence and survival of eggs of nematodes.

Data herein presented confirms that the FLOTAC technique should be considered a reliable technique to assess the soil contamination by eggs of nematodes. Recently, the applicability of this technique in the assessment of this kind of sample has been validated in a study where FLOTAC presented a better performance than the spontaneous sedimentation technique (SST) and centrifugal flotation technique (CFT) [11].

Based on these data, it is necessary to implement preventive measures and educational actions for the population in order to mitigate the potential risk of exposition to parasites of zoonotic importance. In addition, the main issue of this study (soil contamination) needs to be worked through a "One Health" approach, to establish the equilibrium among human, animals, and environmental conditions.

Author Contributions: Conceptualization, I.C.d.N.R., R.A.d.N.R. and L.C.A.; methodology, I.C.d.N.R. and T.A.R.F.L.; data curation, I.C.d.N.R., R.A.d.N.R. and G.A.d.C.; writing—original draft preparation, I.C.d.N.R. and R.A.d.N.R.; writing—review and editing, I.C.d.N.R., T.A.R.F.L., R.A.d.N.R., G.A.d.C. and L.C.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This article is based on the PhD thesis (Graduate Program in Animal Bioscience) of the first author, developed at the Federal Rural University of Pernambuco, and supported by a grant fellowship from the Coordination for the Improvement of Personnel of Higher Education (CAPES). R.A.N.R. receives a research fellowship from CNPq (Bolsa de Produtividade; grant no. 305971/2021-2).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Santarém, P.A.; Guiffrida, R.; Zanin, D.A. Larva migrans cutânea: Ocorrência de casos humanos e identificação de larvas de *Ancylostoma* spp. em parque público do município de Taciba, São Paulo. *Rev. Soc. Bras. Med. Trop* **2019**, *37*, 179–181. [CrossRef] [PubMed]
2. Silva, M.L.F.; Melo, V.S.P.; Milken, V.M.F.; Seixas, F.N. Parasitic contamination of public squares in the City of João Pessoa, Paraíba. *Res. Soc. Dev.* **2021**, *10*, e74101018643. [CrossRef]
3. Melo, M.V.C.; Oliveira, T.R.; Málaga, S.M.R.; Cruz, D.R.S.; Jonas, J.M. Environmental contamination: The influence of seasonality in the occurrence of geohelminths in a public area of Fortaleza, Ceará. *Vig. Sanit. Deb.* **2020**, *8*, 80–85.
4. Batista, S.P.; Silva, F.F.; Valêncio, B.A.; Carvalho, G.M.M.; Santos, A.; Costa, F.T.R. Zoonotic parasites in soils of public squares in the municipality of Sousa, Paraíba. *Rev. Bras. Cie. Vet.* **2019**, *26*, 82–86. [CrossRef]
5. Lima, A.M.A.; Alves, L.C.; Faustino, M.A.G.; Lira, N.M.S.; Magalhães, A.; Lima, M.M. Búsqueda de huevos de anquilostomideos y toxocarídeos em el suelo de residências y escuelas em el barrio de Dois Irmãos, Recife—PE, Brasil. *Parasitol. Latinoam.* **2007**, *62*, 89–93.

6. Cassenote, A.J.F.; Neto, J.M.P.; Lima-Catelani, A.R.A.; Ferreira, A.W. Contaminação do solo por ovos de geo-helmintos com potencial zoonótico na municipalidade de Fernandópolis, estado de São Paulo, entre 2007 e 2008. *Rev. Soc. Bras. Med. Trop.* **2011**, *44*, 371–374. [CrossRef] [PubMed]
7. Gorgônio, A.S.; Sousa, D.L.C.; Bezerra, C.S.; Monteiro, G.D.F.; Paulo, F.S.; Costa, P.W.L. Agentes parasitários de importância em Saúde Única em solos de praças públicas em condições semiáridas. *Res. Soc. Dev.* **2021**, *10*, 1. [CrossRef]
8. Moro, F.C.B.; Pradebon, J.B.; Santos, H.T.; Querol, E. *Ancylostoma* spp. e *Toxocara* spp. *Biodivers. Pampeana* **2008**, *6*, 25–29.
9. Cirne, F.S.L.; Silva, T.; Carvalho, A.C.F.; Dias, P.M.; Ramos, C.D.; Batista, L.C.S.O. Contaminação ambiental por ovos de *Ancylostoma* spp. e *Toxocara* spp. em áreas de seis praças públicas do município de Valença, estado do Rio de Janeiro. *Acta Biol. Bras.* **2017**, *8*, 35–42. [CrossRef]
10. IBGE/SNIS Sistema Nacional de Informação Sobre Saneamento. Ministério do Desenvolvimento Regional. Disponível em. 2018. Available online: www.snis.gov.br (accessed on 15 March 2021).
11. Ramos, I.C.N.; Macedo, L.O.; Ramos, R.A.N.; Carvalho, G.A.; Alves, L.C. The application of the FLOTAC technique for detection on the soil of immature forms of parasites of medical and veterinary concern. *Exp. Parasitol.* **2022**, in press.
12. Cringoli, G.; Rinaldi, L.; Maurelli, M.P.; Utzinger, J. FLOTAC: New multivalente techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nat. Protoc.* **2010**, *5*, 503–515. [CrossRef] [PubMed]
13. Ferreira, I.S.; Nery, J.M.J.; Moraes, M.H.S.; Maggi, L.E.; Brilhante, A.F.; Sabino, K.C. Frequência de geo-helmintos em áreas recreativas da cidade de Rio Branco, estado do Acre, Brasil. *South Am. J. Bas. Educ. Tec. Technol.* **2018**, *5*, 68–77.
14. Coura, L.C. Fatores relacionados à transmissão das geohelmintíases. *Rev. Soc. Bras. Med. Trop.* **1974**, *8*, 4.
15. Prestes, L.F.; Jeske, S.; Santos, C.V.; Gallo, M.C.; Villela, M.M. Contaminação do solo por geohelmintos em áreas públicas de recreação em municípios do sul do Rio Grande do Sul (RS), Brasil. *Ann. Trop. Pathol.* **2015**, *44*, 155–162. [CrossRef]
16. Bowman, D. Georgis Parasitologia Veterinária. In *Elsevier Health Sciences Brazil*, 2nd ed.; Elsevier: São Paulo, Brazil, 2010; p. 447.