

Refractory Giardia – mysteries of the organism

Michael Libman MD
J. D. MacLean Centre for Tropical Diseases
McGill University
Montreal, Canada



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Disclosures

- No disclosures related to any topics discussed in this presentation.
- Several off-label indications or non-licensed medications are discussed here



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Outline and objectives

- Very brief overview of Giardia
- Diagnostic issues
- Treatment, and resistance
- Post Giardia IBS
- Amazing Giardia Trivia



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Giardia in Travellers

- About 5% of TD in specialized clinics.
 - Less in TD surveys
 - Endless debate on Rx of test negatives
 - 100% of LMIC kids infect <2yr
- Giardia not deadly, but:
 - Drug refractory is emerging,
 - protracted infection increased morbidity



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Pathology

- Cysts
- Trophozoites
- Attach to microvilli, not invasive
- Mechanisms of pathophysiology not well known, ++complicated
 - Innoculum 10-100 cysts, incubation/prepatent = 7/14 days
 - Occ. microvillus atrophy, loss of enzymes, malabsorption.
 - Little inflammation
 - Poor pathology/symptom correlation (Nash T 1987)
- Animal reservoirs not clear



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Risk for illness

- All studies show many are asymptomatic, and may be carriers for variable period
- About 2% of acutely ill become asymptomatic carriers
- Mice with different microbiota are more or less resistant to infection
 - Antibiotics makes all susceptible
- CD4, IgA are essential for control of most strains
- Most common reported comorbidities associated with chronicity and treatment failure
 - Hypogamma, IgA deficiency, HIV, Coeliac, cystic fibrosis, cancer chemo

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Natural history

THE EXPERIMENTAL TRANSMISSION OF HUMAN INTESTINAL PROTOZOAN PARASITES

II. *GIARDIA LAMBLIA* CYSTS GIVEN IN CAPSULES

By
ROBERT C. RENDTORFF¹

(Received for publication October 13, 1953)

- Symptoms
 - Diarrhea, abdominal pain, nausea, vomiting, flatulence, foul smelling stool (!), anorexia, weight loss (Nygard BMC 2006)
- Experimental infection 1980's: strain differences (Nash 1987)
- Volunteer prisoners
 - Asymptomatic 40-50%, most mild (difficult to interpret!)
 - Spontaneous clearance of parasites (5-41 days): 85%
 - Chronic infection 15% mostly mildly symptomatic
 - Rendtorff Am J Hyg 1954



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Natural History – things we don't understand

- Reports of food allergies
 - ?related to translocation of antigens across GI mucosa
- Global Enteric Multicenter Study:
 - Giardia PROTECTED against severe diarrhea
 - MAL-ED: MTZ exposure was a confounder
 - Cross protective immunity, reduced inflammation, microbiome changes
- Neither WASH intervention nor 6 monthly ALB seem to affect Giardia prevalence (mostly asymptomatic)
 - Most risk is direct household contacts
 - Hookworm seems to protect against Giardia



Aw J, Parasites Vectors 2019, Hanevik CID (editorial) 2016 s

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Natural History – things we don't understand

- Interactions of Malnutrition and Enteric Infection, Child health and Development: Giardia associated with stunting, wasting
- Summary:
 - From non-endemic countries: strong association with acute diarrhea
 - From endemic countries: acute if <1yr, "protective" if >5yrs, assoc with chronic diarrhea, impaired growth. Reduced cognitive function
 - Enhanced pathogenicity in rotavirus coinfection



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Natural History Hypothesis – endemic regions

- Immune response established before age 2
- Immunomodulatory capabilities – diarrhea is rare
- Prevalence continues to rise, but antibodies decrease
 - Suggests immune tolerance to non-invasive parasite
 - Becomes a commensal
 - May have an impact on gut function, micronutrient uptake
- First infection later in life sets up more aggressive immune response, intestinal inflammation, villous blunting
- Reminiscent of HAV epidemiology



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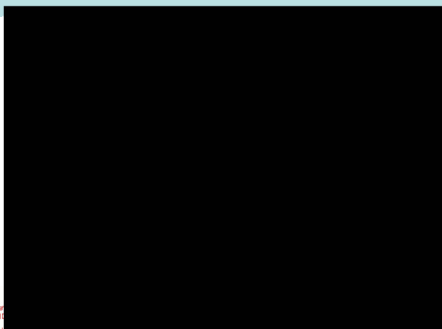
Summary of Pathology

- In residents of non-endemic countries, association with illness is clear
- Malnutrition and reduced cognitive function in children
- Stunting
- Enhanced pathogenicity in coinfection with rotavirus
- Role of strains (“assemblages”) –confusing data
 - Zajackowski P Curr Res Parasitol Vector Borne Dis 2021

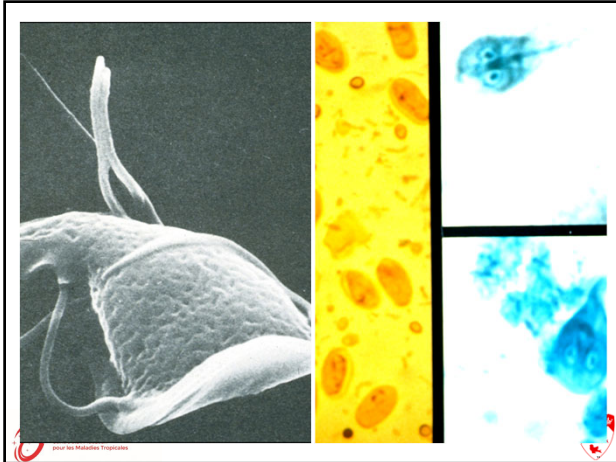


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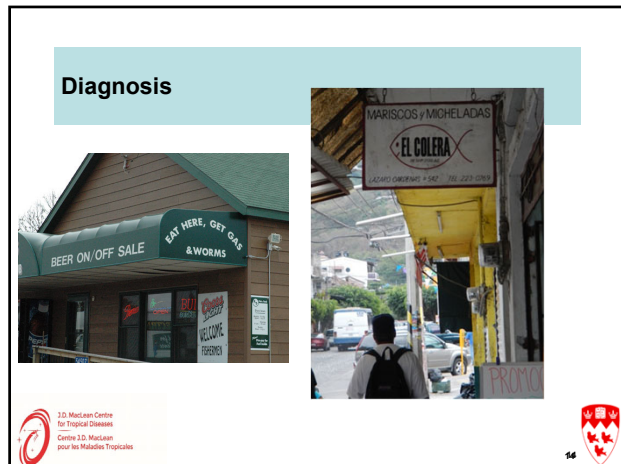
Diagnosis



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Diagnosis

- Classic microscopy: 2-3 specimens
- Training - almost insurmountable problem
- Technique (increasing yield)
 - Wet mount
 - Simple (iodine) stain
 - Rapid fixation (preserve trophozoites)
 - Concentration techniques (Labour intensive, slow, multiple toxic reagents)
 - "Permanent" stains (iron hematoxylin, Trichrome) = gold standard

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Diagnosis

- Test-retest concordance = 40%! (Libman 2008)
- Astonishing day-to-day variation
- Stool antigen tests: better than non-expert microscopy
 - Never as good as expert microscopy
- Duodenal aspirates – unclear benefit
 - String test
- Molecular tests: best answer so far
 - 1 specimen, fresh/frozen/fixation,
 - Travellers 5% to 6%, General practice 6% to 9% (real life studies)

– Ryan Trends parasitol 2017, Yansouni CIDR 2014



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Diagnosis: molecular

- Becoming cost-effective
- Highly multiplex – across “disciplines”
- High throughput, fast,
- Reproducibility issues, non-specificity of rare pathogens
- Soon:
 - Quantitation, Strain typing
- One problem seems to be DNA extraction (removal of inhibitors):
 - Structure of cysts, especially Cryptosporidium vs Giardia
 - What gets DNA out of one shears DNA of other
 - Low end of sensitivity no better than microscopy, high end better by 10 fold (quant)

Authier B, Parasite 2018, Paulos S PLOSOne 2019, Hitchcock M Diag Micro Inf Dis 2019



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Molecular: not perfect

- Problems:
 - DNA extraction/inhibitors
 - Predefined pathogens
 - Inadequate sensitivity due to primer mismatch??
 - Inability to modify system when new primers/probes needed – lack of genomic data
 - Failure to detect could lead to more carriers



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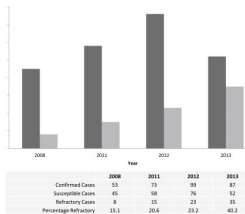


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Treatment... and failures (persistent lab+)

- Nitroimidazole is first line
- Spain 2007-09
 - 22% total, Asia 33% Gutierrez TMID 2013
- Bergen outbreak – homogeneous strain
 - MTZ failure 3%
- London:
 - Increase from 15% to 40% (India 50%)
 - 39% of cases were from India
 - (asymptomatic children may have 50% carrier rate in India)



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50 studies, different designs, followup, etc

Antigiardial agents	Efficacy clinical studies (%)	Effective dosage adults	Comments
Metronidazole	36 - 100	200 – 500mg tid x 5-7d	Efficacy low when shorter than 5 d
Tinidazole	74 - 100	1.5 – 2g sd x 1d	Single dose treatment as effective as longer course due to longer half-life
Ornidazole	90 - 100	1-2g sd x 1d	
Secnidazole	79 - 100	2g sd x 1d	
Nitazoxanide	56 - 94	500mg bid x 3d	
Furazolidone	20 - 92	100mg qid x 10d	
Albendazole	62 - 96	400mg qid x 5d	3 RCTs: Less effective than tinidazole ^a 10 RCTs: Similar effectiveness to metronidazole ^b
Mebendazole	0 - 95	200mg tid x 3-5d	
Quinacrine	77 - 100	100mg tid x 5d	Risk neuropsychiatric side effects
Paromomycin	40 - 92	500mg tid x 7d	Only drug recommended in first trimester of pregnancy
Chloroquine	86	10mg/kg bid x 5d	
Bacitracin zinc	95	120 000 U bid 10d	One RCT 1995

March 2016; PLoS Negl Trop Dis 2016; 10(4): e0004810. Grandjean: Cochrane review 2012. ^aEscherichia Acta Tropica 2016.
Poupart: PLoS Negl Trop Dis 2016.

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50 studies, different designs, followup, etc

Agent	Efficacy (%)	Dose	Comments
Metronidazole	36-100	2-500mg tid 5-7d	Low efficacy if <5d
Tinidazole	74-100	1.5-2g qD x1	Single dose due to long half life
Ornidazole	90-100	1-2g qD x1	
Secnidazole	79-100	2g qD x1	
Nitazoxinide	56-94	500mg bid x5d	
Furazolidone	20-92	100mg qid x 10d	
Albendazole	62-96	400mg qD x5d	5 RCT inf to tinidazole 10 RCT equiv MTZ
Mebendazole	0-95	200mg tid x3-5d	2 RCT Europe- no effect
Quinacrine	77-100	100mg tid x5d	Neuropsych AE's. 1 st drug
Paromomycin	40-92	500mg tid x7d	Safe 1 st trim pregnancy
Chloroquine	86	10mg/kg bid x 5d	Cuba
Bacitracin	95	120,000U bid 10d	21 kids

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The drugs: random comments

- Lower doses often associated with the lower effectiveness
- Furazolidone needed 10 days
- Albendazole – conflicting
- 2 small studies in Spain and Italy no effect of mebendazole, studies in developing countries showed good effect – treating something else?
- Quinacrine was first drug. Potential side effects
- Paromomycin ok in pregnancy
- Chloroquine 2 studies from Cuba in children
- Bacitracin 20/21 children



EDITOR'S CHOICE
Treatment strategies for nitroimidazole-refractory giardiasis: a systematic review

Carole L. Bourque, MD, PhD, J. Andrew Neumayr, PhD, MD, DMT & H, MCTM,
Michael Libman, MD, Lili H. Chen, MD

Journal of Travel Medicine, Volume 29, Issue 1, January 2022, taab120.

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Where are we with the science?

- Structural modifications of mtz, nitazoxanide, benzimidazoles shows promise, Emodepside (vet)
- Auranofin (approved for RA) effective in vitro and rodents
- Fumagillin
 - Orphan drug (bees) used for microsporidium
 - Effective in vitro and mice
- Mechanisms of resistance not known, and testing using in vitro models not well worked out
- Whole genome sequencing suggesting of markers of resistance



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Why can't we figure this out?

- Need to convert MTZ to toxic metabolite
 - 4 enzymes identified
- Binucleate, but tetraploid organisms (4 alleles each)
- Strains mostly non-culturable, grow at various rates
- Broad adaptive response identified
 - Post-transcriptional and post-translational changes
- One study with lab induced resistance – each strain had own adaptive strategy
- Recent increasing failures suggest something transmissible

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Postulated mechanisms of resistance

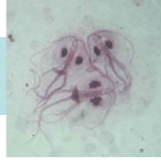
- Need to convert MTZ into toxic metabolites:
 - Maybe inability to "activate" MTZ (multiple enzymes)
 - Must reduce a nitrile group
 - Low PFOR pyruvate-ferredoxin oxidoreductase associated with some but not all resistant strains.
- Nitroreductase 1 also downregulated
- Nitroreductase 2 upregulated – seems to reduce MTZ to a non-toxic metabolite
- Transmissible (not in lab strain)

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McGill Case Review



- 147 consecutive cases of Giardia proven in our lab
 - MTZ first line Rx
- Clinical failure: symptoms and confirmed Giardia+ stool >2 weeks post treatment completion
- Treatment refractory =47 (32%)
- Cured =76
- Incomplete f/u =24 (clinically improved?)
 - Referral bias, proportion stable over 6 years
- Worst regions: Indian sc 28 fail vs 22, Asia 4 vs 6



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McGill Study Results

- Cured:
 - MTZ high dose 9/17 (6 lost)
 - Paromomycin 5/8 (3)
 - Quinacrine 13/13 (5)
 - Nitazoxanide MBZ or ALB 3/6 (1)
- Investigated for comorbidities: 37/60 refractory
 - 1 hypogammaglobulinemia found
 - 1 anti-TTG



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Treatment strategies: (Bergen, London, Israel, Cuba, McGill and few reviews)

- No RCT in 25 years
- Repeat/Higher dose same drug/class: Tinidazole 50%, MTZ 15%
- New class:
 - Albendazole, Nitazoxanide, Paromo <50%
 - Quinacrine almost 100% (if no immune problem)
- Combo
 - ALB + MTZ/TND = 60-80%, SCZ+MBZ = 87%*
 - Quinacrine + anything: success in almost all reports, even if individ failure



Munoz GI et al. Travel Med Infect Dis (2013); Nabarro LBR. Parasitology (2015); Watkins RH. Curr Infect Dis Rep (2014); Carapando RP et al. Clin Ther (1998); Marsh K. J Infect (2008); *Carter R. Clin Microbiol Infect. 2019



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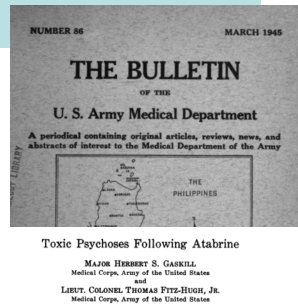
Possible algorithm

- 1st
 - MTZ 250-500 tid x 7 or TND 2gm x1
- 2nd
 - ALB 400 bid plus MTZ 250-500 bid x7
 - ALB 400 bid x 7d plus TND 2gm day 1 and 7
 - Quinacrine 100 tid x 7 if no psych "predisposition"

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Quinacrine interlude

- Widely quoted 0.4% rate of psychosis
- Psychosis was 1.4% of general medical admissions
- All but one resolved
- 16 were re-challenged with >full dose (2 with malaria)
 - 15 no reaction, 1 "mildly excited"
- Since then several case reports



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- Italy RCT n=20
- ALB + MTZ cured 9/10, ALB alone cured 2/10
- Spain retrospective (n=10)
- ALB + nitroimidazole, paromo, quinacrine all worked
- London
- Quinacrine + ALB or tinidazole 9/9 cured
- ALB + tinidazole = 12/20 cured
- (quinacrine and MTZ have synergy in vitro)

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Immune suppression

- Case series:
- Quinacrine and MTZ or tinidazole (3 weeks): 5/6 cured
- Paromo + bacitracin for 3 months: 1 cured
- Some clear spontaneously
- 1 case of immune deficiency: MTZ and quinacrine mono failed
 - Both together for 2 weeks = cure
- AIDS case
 - MTZ, ALB mono and combo failed, nitazoxanide 5-10d failed, nitazoxanide 1.5g bid x 30 d cured



Nash CID 2001, Taylor CMAJ 1987, Abboud CID 2001



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Quinacrine monotherapy also good

- Spain retrospective:
 - Paromo 0/3
 - ALB 0/2
 - Quinacrine 14/14 cured
- Family after travel to India
 - Tinidazole 1/4
 - Quinacrine 3/3
- Israel
 - ALB 5/12, nitazoxanide 1/4, paromo 1/1, quinacrine 2/2
- London
 - Quinacrine 11/11



Munoz Gutierrez TMAID 2013, Requena-Mendez Clin micro infect 2013, Meltzer EID 2014, Nabarro Clin Micro Infect 2015



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Quinacrine

- Yellow skin after > 1week
 - Common, self limited, related to cumulative dose
 - Bergen: 2/3 with 3 week Rx had this
- Neuropsychiatric effect
 - Rare, reported from d3 to 2wk post Rx
 - Commonly given anti-psychotic therapy
- Bergen: confusion, nightmares, dizziness in all 3
- Spain: 14, without major events
- McGill: 19, without major events (not published)



Genel Human psychopharmacology 2002, Lindenmayer J Clin Psychiatry 1981, Weisholtz South Med J 1982, Morch J Infect 2008, Gutierrez TMAID 2013



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New Problem – Cannot find Quinacrine

- No current manufacturers meeting USP or BPh standards
- Sold by various lab supply companies
- We have used a private Mass Spectroscopy lab to identify level of purity
 - IRB to offer to patients
 - Sigma-Aldrich sells quinacrine ">90% purity", with our batch testing as close to 100%
 - Hard to know what the impurities are



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Conclusions

- If treatment failure, a second line drug from a different class, or combination therapy
- Genetic markers or in vitro tests of resistance not available (yet)
- MTZ and ALB has synergistic effect in clinical studies
- Quinacrine for >5 days should be weighed against risk (if you can get it!)
- New approaches include new drugs, peptides, microbiome manipulation, using metagenomics (quad-allelic) and metabolomic studies



Lalle M, Infect Drug Resist 2018



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Workup in Resistant Cases

- Check for IgA, HIV, Coeliac
- Check water supply, animal, household (esp children, sexual) contact
- Adherence, source of meds



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Future questions

- Maybe strain typing is important
- Adding MTZ in culture can make them resistant, but freezing causes resistance to go away
- Ivermectin: also resistance
- Bergen: duodenal biopsies were abnormal in failures
 - Household members were negative
 - Persistent diarrhea: 32% giardia; 68% neg= microscopy x 3, duodenal aspirate, and Ag test neg



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Non-acute GI complications

- Large outbreak in Bergen 2500 cases in 2004
- Leaking sewage pipes from housing on hill above water reservoir
- Heavy rainfall
- Chlorination only
- 38 refractory to Rx



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Chronic Sequelae

- Large outbreak in Bergen 2500 cases in 2004
 - Appears clonal
- Post infectious IBS, chronic fatigue common complications
- In Bergen: 2 years later, abdominal symptoms 38%, fatigue 41%
- 38 refractory cases



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Risk factors for IBS/CF

- Treatment resistant infection
- Received >1 treatment course
- Sick leave > 2weeks
- Loss of >1 semester
- IBS 39% and chronic fatigue 31% 6 years later
 - Mørch BMC inf dis 2009, Hanevik CID 2014
- QoL scores reduced vs controls 10 years after infection
 - Attributable to IBS and CF (Lilleskare S, Neurogastroenterology 2019)

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Post giardia IBS – controlled study

- age/sex matched from Bergen
- IBS 46% in giardia, 14% in control 3 years after exposure
(Wensaas Gut 2012)



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Post giardia IBS

- Primary care visits to MD within 2 years prior to outbreak associated with persistent symptoms
 - ?more inclined to seek health care?
- No correlation with milk intolerance
 - IBS not correlated with any food intolerance
- IBS and chronic fatigue/malaise highly correlated

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Giardia and Chronic Fatigue

- Several other reports of CFS, lasting for years
 - Systematic review suggests more common than other pathogens (Svendsen Scand J Gastroent 2019)
- Anecdotally, malaise can be very prominent in acute disease
 - Some cases of post travel fatigue found to have Giardia
 - Appear to improve with Rx?
 - Even if no infection found (Nissan, JTM 2018)
 - Suggest question fatigue pt re GI symptoms
 - Suggest not advertising that tired people get tested

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Amazing Giardia Trivia

Research and Reports in Tropical Medicine

Dovepress

Open Access Medical Journals

ORIGINAL RESEARCH

Intestinal parasites in paper money circulating in the city of Diamantina (Minas Gerais, Brazil)

This article was published in the following Open Access journal: Research and Reports in Tropical Medicine

Marina A Costa¹
Layane M Teodoro²

Background: Parasitosis are objects of great interest and diffusion among the population, and can be efficient mechanisms in the dissemination of several intestinal parasites. This study investigated the presence of the intestinal forms of parasitic protozoa present in circulation in the city of Diamantina (Minas Gerais, Brazil).

- 60 banknotes in each of 5 commercial establishments, including supermarket, butcher, pharmacy
- Washed, and microscopy
- 75% with *Entamoeba coli*, 4.4% *Giardia*, few tapeworm eggs, hookworm larvae

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Amazing Giardia Trivia

Parasitology International
Volume 87, Issue 6, December 2018, Pages 776–780

Methodological innovations for the study of irreplaceable samples reveal giardiasis in extinct animals (*Nothotherium maquinense* and *Palaeolama maior*)

Daniela Leite^{1,2,3,4}, Paula Cascardo⁵, Eliza Puro⁶, Beatriz Brenner⁶, Adriane Sude⁷, Elizabeth Alves⁸, Flávia Uchoa⁹, Priscilla Fajardo⁹, Patricia Miller⁹, Danuza Matos⁹, Mônica Chame⁹, Castor Carlele⁹

- Used residue from DNA extraction of coprolites
- Positive from extinct Llama and ground sloth in NE Brazil
- Dates from late Pleistocene era = first human occupation

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