A Molecular Approach to the Epidemiology of *Giardia duodenalis* in a Peruvian Shantytown

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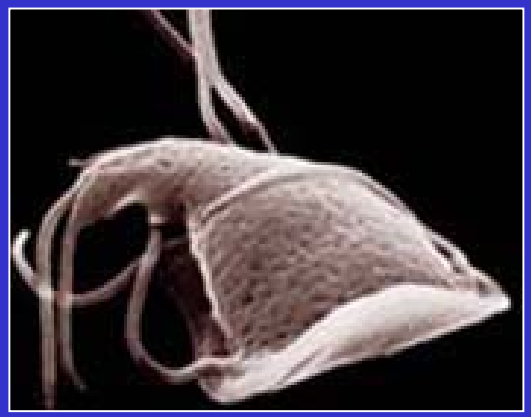

Picture from Purestream Water Filters

History of Las Pampas

- Outskirts of Lima, Peru
- 1980s Sendero Luminoso
- Government

- Squalor living conditions
- Lack of sanitation
- Population growth

Goal of Study

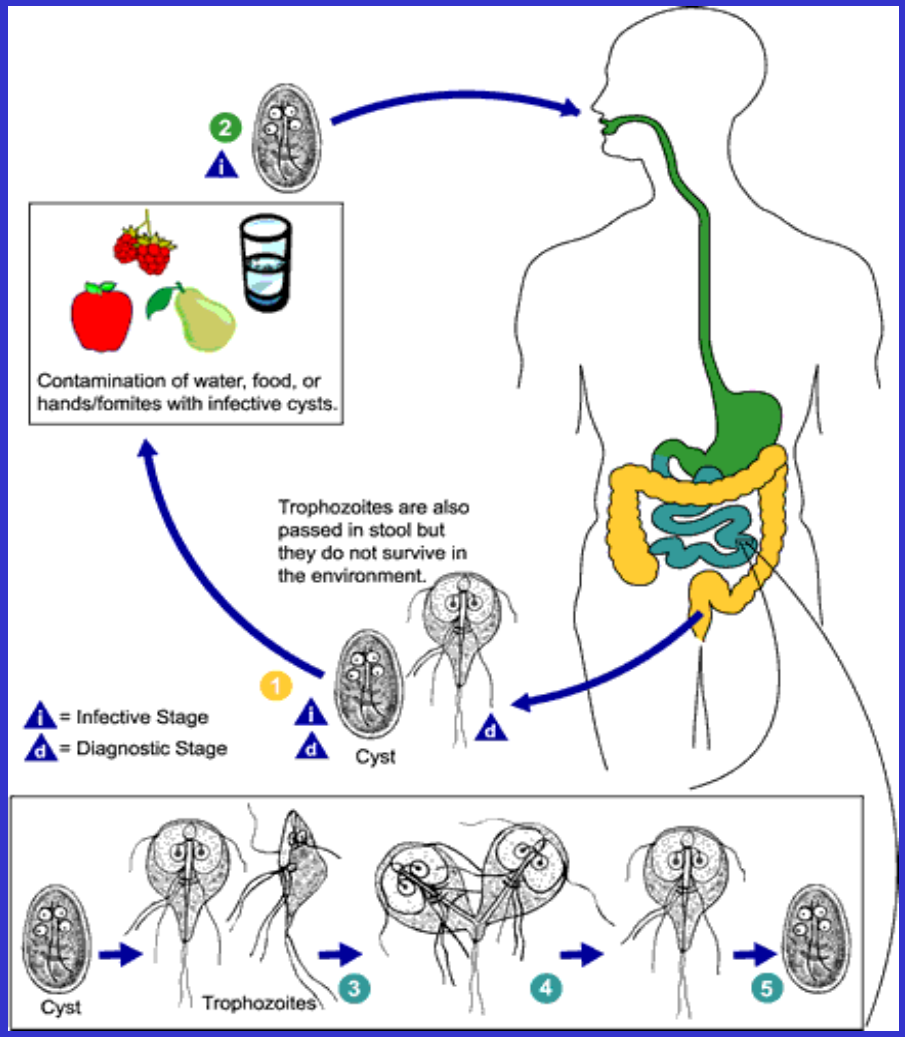

Figure from Parasites and Health

Provide insight into the transmission cycles, patterns of infection, and infecting strains of the parasite, *G. duodenalis*, within a community in Lima, Peru.

Project Design

- Identify and enroll appropriate Las Pampas households
- Collect fecal samples from household members over time (May 20 to July 19, 2001)
- Microscopically analyze samples for presence of cyst
- Create database tracking infection among household members

Picture from The Regional Council Water Supply

Database

Household	Member	DOB	Age	24-May	25-May	26-May	27-May	28-May	29-May	30-May	31-May	01-Jun	02-Jun	03-Jun	04-Jun	05-Jun	06-Jun	07-Jun	08-Jun	09-Jun	10-Jun
1	A	08/10/1981	20																		
1	B	08/08/1975	26		N/A																
1	C	09/15/1993	7																		
1	D	09/08/1995	5																		
1	E	07/10/2000	1																		
1	F	04/08/1979	22																		
1	G	03/28/1984	17	II																	
1	H	09/24/1998	2					N/A		N/A					N/A			N/A		II	
1	I	06/09/2000	1	II																	

Household	Member	DOB	Age	11-Jun	12-Jun	13-Jun	14-Jun	15-Jun	16-Jun	17-Jun	18-Jun	19-Jun	20-Jun	21-Jun	22-Jun	23-Jun	24-Jun	25-Jun	26-Jun	27-Jun	28-Jun
1	A	08/10/1981	20																		
1	B	08/08/1975	26																		
1	C	09/15/1993	7																		
1	D	09/08/1995	5																		
1	E	07/10/2000	1																		
1	F	04/08/1979	22																		
1	G	03/28/1984	17																		
1	H	09/24/1998	2	II							II		II		II					II	
1	I	06/09/2000	1								II										

Key

II — Positive *G. duodenalis* screen. Genotype II.

II — Mixed infection--positive screen for *G. duodenalis* and *C. parvum*.

N/A — Positive *G. duodenalis* screen. Unknown genotype.

Negative screen.

No sample received. No screen.

Project Design

- Identify and enroll appropriate Las Pampas households
- Collect fecal samples from household members over time (May 20 to July 19, 2001)
- Microscopically analyze samples for presence of cyst
- Create database tracking infection among household members
- Purify positive fecal samples
- Extract DNA from positive fecal samples using QIAmp DNA Stool Mini kit
- Nested PCR for to amplify portion of DNA known to be variable among strains
- Sequence analysis to determine differences among isolates

Molecular Analysis: Nested PCR

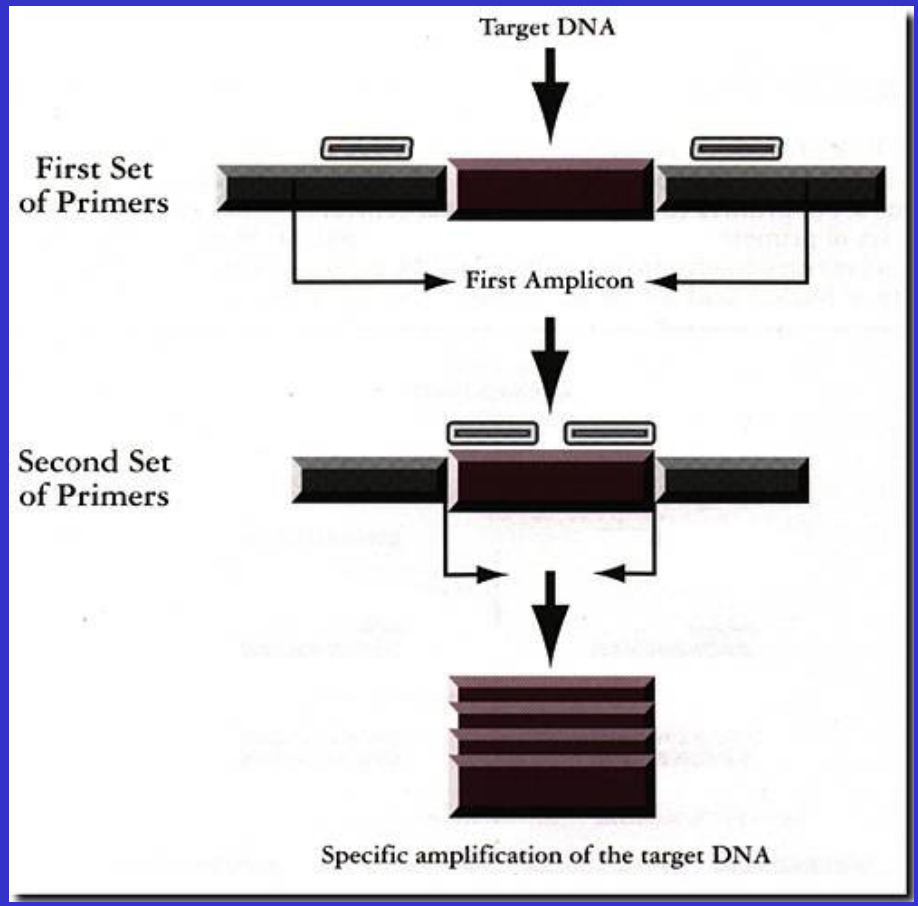

Figure from Medical Diagnostics Laboratory

First Round

Primers: FEG1, REG1

Target DNA: 600 bp SSU-rRNA

Second Round

Primers: RH4, RH11

Target DNA: 292 bp SSU-rRNA

Sequence Analysis

Genotype I (Human)

5’-TCCTGCCGGAGCGCGACGCTCTC-3’

Genotype II (Human)

5’-TCCTGCCGGAATCCGACGCTCTC-3’

Other differences between GI and GII

Position (GI base→GII base)

43(-→ T), 44 (G→ C), 62 (T→ G), 72 (C→ G),

Results

- Five of twelve participating households consistently gave fecal samples during the study. (Overall, 37 of 81 human participants microscopically screened positive. One of four dogs screened positive)
- Database analysis reveals that all age groups were affected. Children (<5yrs.) were infected most frequently
- Database analysis reveals infections in children (<5yrs.) were chronic (often lasting the length of the study). Adult infections ranged from chronic to a single positive screen
- Sequence analysis of the SSU-rRNA reveals the predominance of two distinct *G. duodenalis* genotypes within the endemic locality (the dog isolate could not be sequenced); Type I (GCG-tag) and Type II (ATC-tag)
- Two of the five families were infected with Type I, while the other two were infected with Type II. Molecular analyses have not yet been performed on the remaining family
- Only a single *G. duodenalis* genotype infected members from the same household during the study

Discussion

- Only a single genotype (I or II) infected a household, suggesting that infection is from a single source. Sources may be household members, pets, food, or water.
- Prolonged infection in children may be due to sanitation habits and underdeveloped immune systems.

Acknowledgements

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