TEC | Tecnológico de Costa Rica

Experiencias de investigación en salud humana

Presencia de la bacteria Helicobacter pylori en el agua y cáncer gástrico en Costa Rica.

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Instituto Tecnológico de Costa Rica

Temas

- Que me llevo a escoger este tema
- Que trabas pude encontrar a nivel político
- Resultados
- Logros con esta investigación

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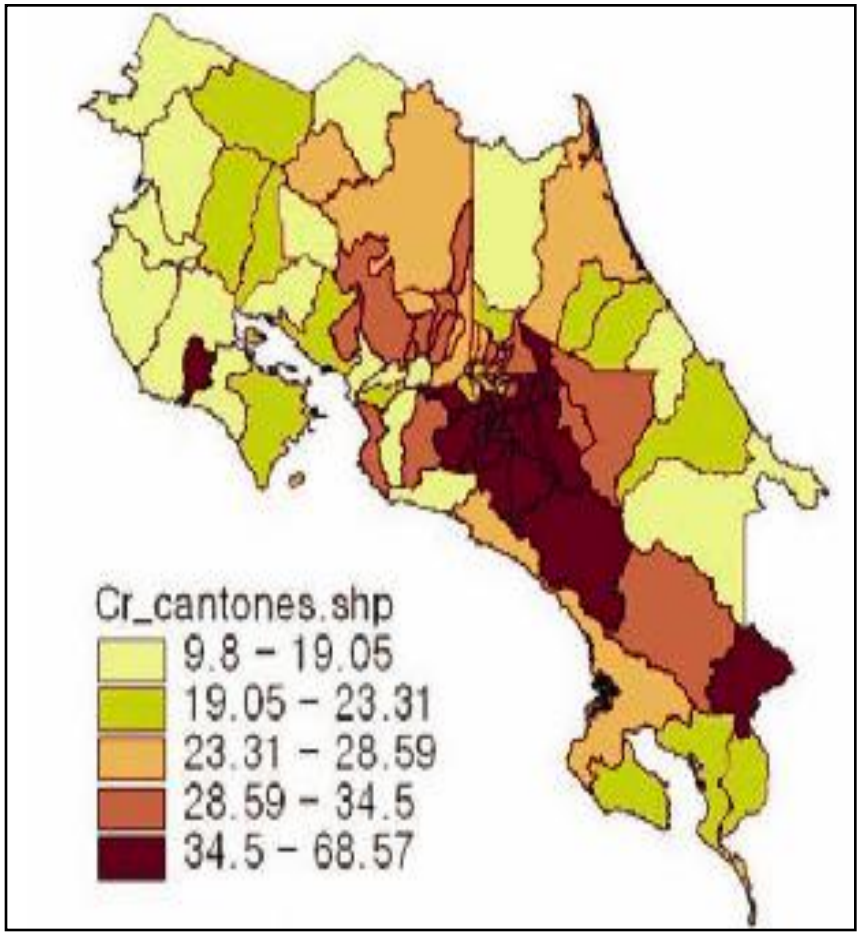
Elección de los sitios de muestreo

QUINTIL 1		QUINTIL 2		QUINTIL 3		QUINTIL 4		QUINTIL 5	
Cantón	Tasa	Cantón	Tasa	Cantón	Tasa	Cantón	Tasa	Cantón	Tasa
León Cortés	68,57	Jiménez	34,50	Tilarán	28,59	Guácimo	23,28	Nicoya	18,97
Turrazú	55,12	Grecia	34,25	Sto Domingo	28,55	Orotina	23,02	Talamanca	18,41
El Guarco	48,48	La Unión	32,16	Barva	28,35	Upala	22,94	Los Chiles	18,16
Paraíso	43,12	Naranjo	31,50	Goicoechea	27,59	Escazú	22,76	Abangares	17,95
Coto Brus	42,57	Coronado	30,89	San Carlos	27,48	Santa Ana	22,26	La Cruz	17,88
Alvarado	39,59	Puriscal	30,65	Sta Bárbara	27,48	Montes de Oca	21,86	Turrubares	17,74
Hojancha	38,10	Buenos Aires	30,57	Alfaro Ruiz	27,35	Heredia	21,57	Liberia	17,01
Alajuelita	37,85	Turrialba	30,55	Poás	26,75	Barqueras	20,74	Atenas	16,06
Aserrí	36,97	San Ramón	30,17	Aguirre	26,36	Corredores	20,65	Santa Cruz	15,90
Cartago	36,91	Flores	30,12	Palís	25,82	Cañas	20,57	Tilarán	15,49
Acosta	36,68	Valverde Vega	29,92	San José	24,78	Golfito	20,41	Matina	15,04
Pérez Zeledón	36,44	Garabito	29,65	Moravia	24,72	Curridabat	20,09	Nandayure	14,25
Mora	35,50	San Isidro	29,59	Guatuso	24,55	Siquirres	19,95	Sarapiquí	14,06
Oreamuno	35,22	Montes Oro	29,08	Belén	23,86	Puntarenas	19,92	Parrita	12,94
Desamparados	34,67	Palmares	29,05	Osa	23,65	Limón	19,13	San Mateo	11,94
Dota	34,58	San Rafael	29,03	Alajuela	23,71	Esparza	19,05	Carrillo	9,80
				San Pablo	23,31				



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July 17-18, 2017 | Lisbon, Portugal

Quantitative Detection of Helicobacter pylori by Real Time PCR in Drinking Water— Environmental and Public Health Risk Significance.

Virginia Montero-Campos, Shirley Arias-Cordero, Benedicto Valdés-Rodríguez, Monserrat Jarquín-Cordero.

Technological Institute of Costa Rica

Health, environment and sustainable
development are closely linked;
an environment not polluted or degraded,
ecologically friendly to physical and mental
health is not only a necessity
is a right of every individual

V.Montero

Whale Bay, Costa Rica

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Introduction

Drs. Barry Marshall and Robin Warren from Perth, Australia.

Nobel Prize in Medicine and Physiology at 2005

For the discovery of "the bacterium Helicobacter pylori and its role in gastritis and peptic ulcer disease"

Introduction

International Agency for Research on Cancer

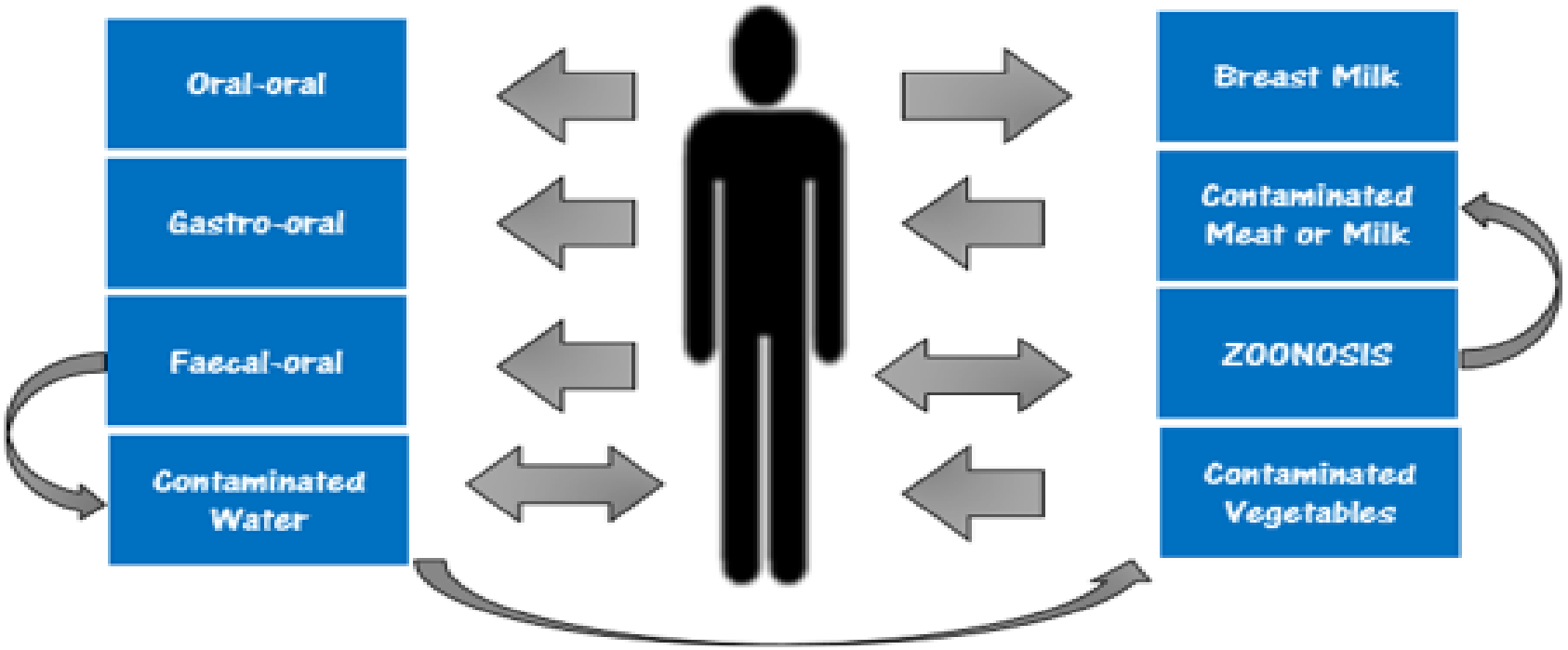
World Health Organization

IARC

Helicobacter pylori
belongs to group 1 IARC

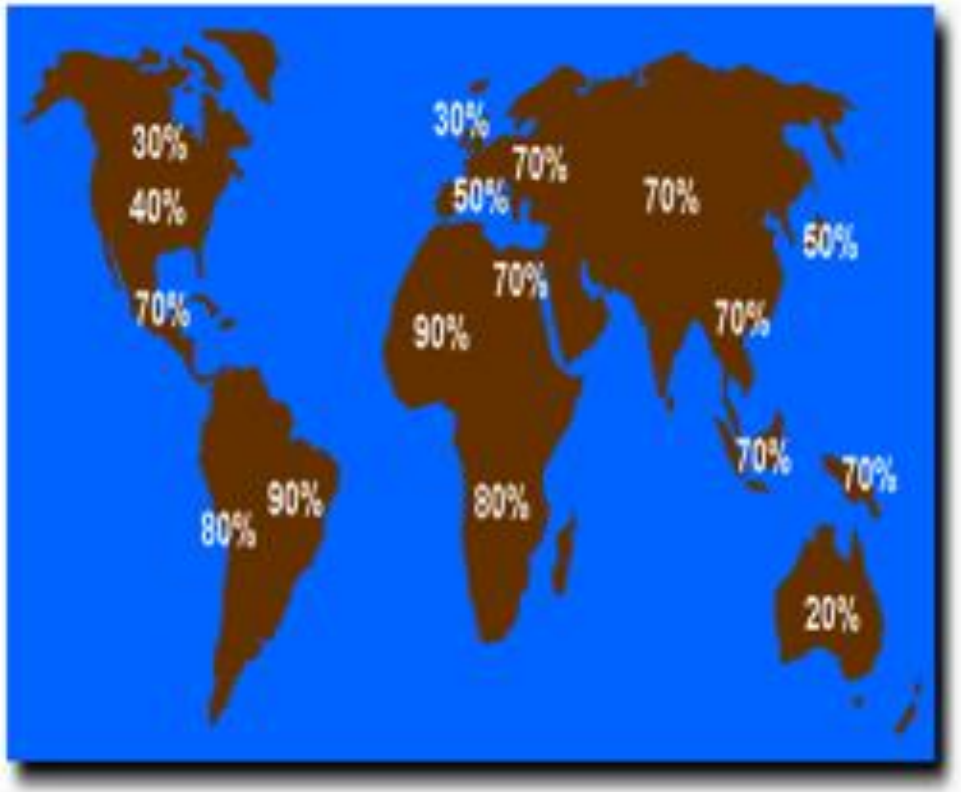
CAS No	Agent	Group	Volume	Year
002871-01-4	HC Red No. 3	3	57	1993
059820-43-8	HC Yellow No. 4	3	57	1993
	Helicobacter pylori (infection with)	1	61, 100B	2012
	Hepatitis B virus (chronic infection with)	1	59, 100B	2012
	Hepatitis C virus (chronic infection with)	1	59, 100B	2012
	Hepatitis D virus	3	59	1994
000076-44-8	Heptachlor	2B	79	2001
000118-74-1	Hexachlorobenzene	2B	79	2001
000087-68-3	Hexachlorobutadiene	3	73	1999
	Hexachlorocyclohexanes	2B	20, Sup 7	1987
000067-72-1	Hexachloroethane	2B	73	1999
000070-30-4	Hexachlorophene	3	20, Sup 7	1987
000142-83-6	2,4-Hexadienal	2B	101	in prep.
000680-31-9	Hexamethylphosphoramide	2B	15, Sup 7, 71	1999
	High-temperature frying (see Frying)			
	Hot mate (see Mate, hot)			
	Household combustion of biomass fuel (see Biomass fuel, indoor emissions from household combustion of)			
	Household combustion of coal (see Coal, indoor emissions from household combustion)			
	Human herpesvirus type 4 (see Epstein-Barr virus)			
	Human herpesvirus type 8 (see Kaposi sarcoma herpesvirus)			
	Human immunodeficiency virus type 1 (infection with)	1	67, 100B	2012
	Human immunodeficiency virus type 2 (infection with)	2B	67	1996
	Human papillomavirus genus beta (except types 5 and 8) and genus gamma	3	90, 100B	2012
	Human papillomavirus types 5 and 8 (in patients with epidermodysplasia verruciformis)	2B	100B	2012
	Human papillomavirus types 6 and 11	3	90, 100B	2012
	Human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 (NB: The HPV types that have been classified as *carcinogenic to humans* can differ by an order of magnitude in risk for cervical cancer)	1	64, 90, 100B	2012
	Human papillomavirus types 26, 53, 66, 67, 70, 73, 82	2B	100B	2012
	Human papillomavirus types 30, 34, 69, 85, 97 (NB: Classified by phylogenetic analogy to the HPV genus alpha types classified in Group 1)	2B	100B	2012
	Human papillomavirus type 68	2A	100B	2012
	Human T-cell lymphotropic virus type I	1	67, 100B	2012
	Human T-cell lymphotropic virus type II	3	67	1996
023255-93-8	Hycanthone mesylate	3	13, Sup 7	1987
000086-54-4	Hydralazine	3	24, Sup 7	1987
000302-01-2	Hydrazine	2B	4, Sup 7, 71	1999
007647-01-0	Hydrochloric acid	3	54	1992
000068-83-5	Hydrochlorothiazide	3	50	1990
007722-84-1	Hydrogen peroxide	3	36, Sup 7, 71	1999
000123-31-9	Hydroquinone	3	15, Sup 7, 71	1999
000129-43-1	1-Hydroxyanthraquinone	2B	82	2002
001689-82-3	4-Hydroxyazobenzene	3	8, Sup 7	1987
000148-24-3	8-Hydroxyquinoline	3	13, Sup 7	1987

Based on epidemiological and microbiological evidence several routes of transmission have been proposed

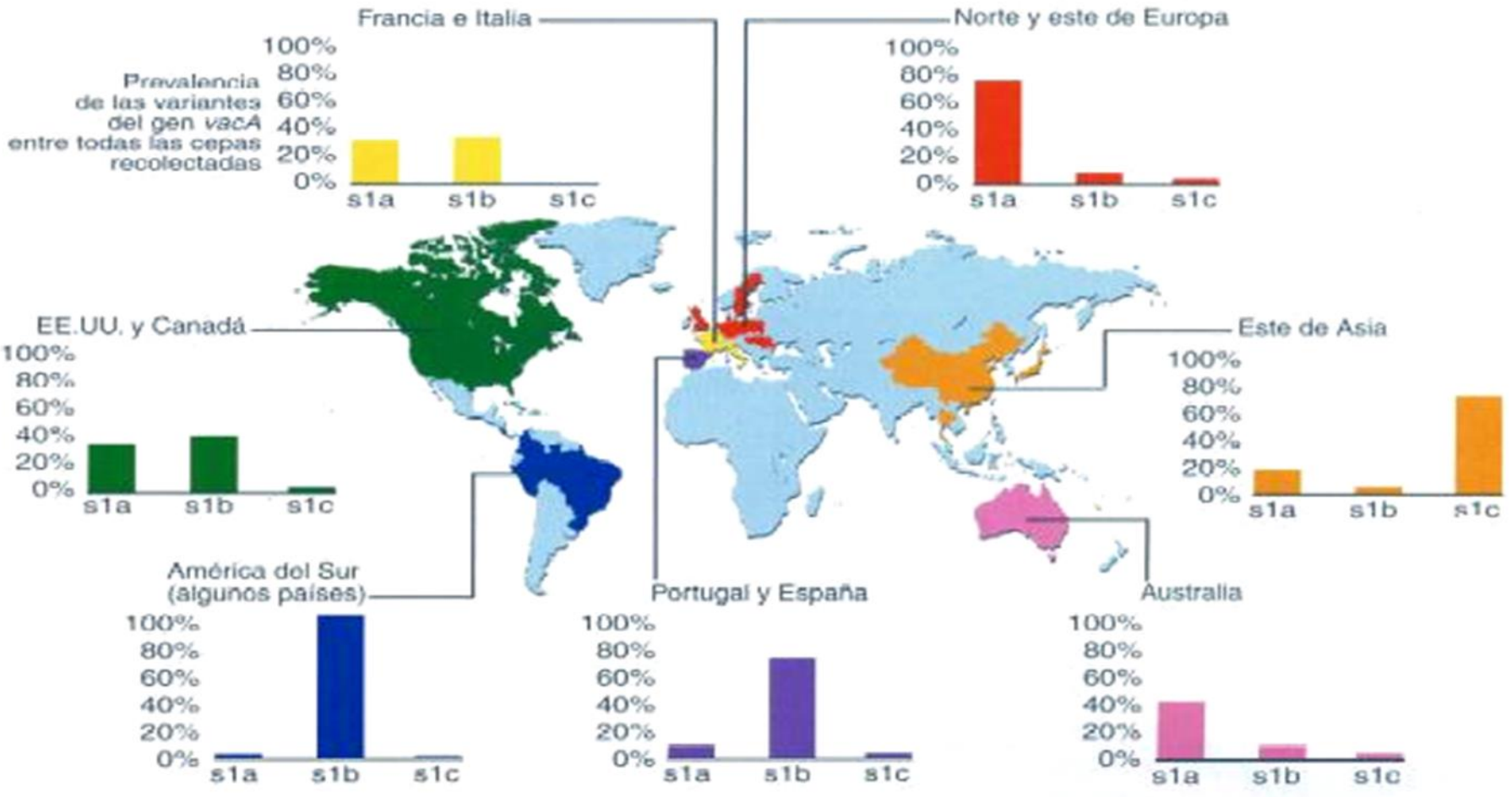


Background of the Problem

The infection with Helicobacter pylori is considered a public health problem in the world

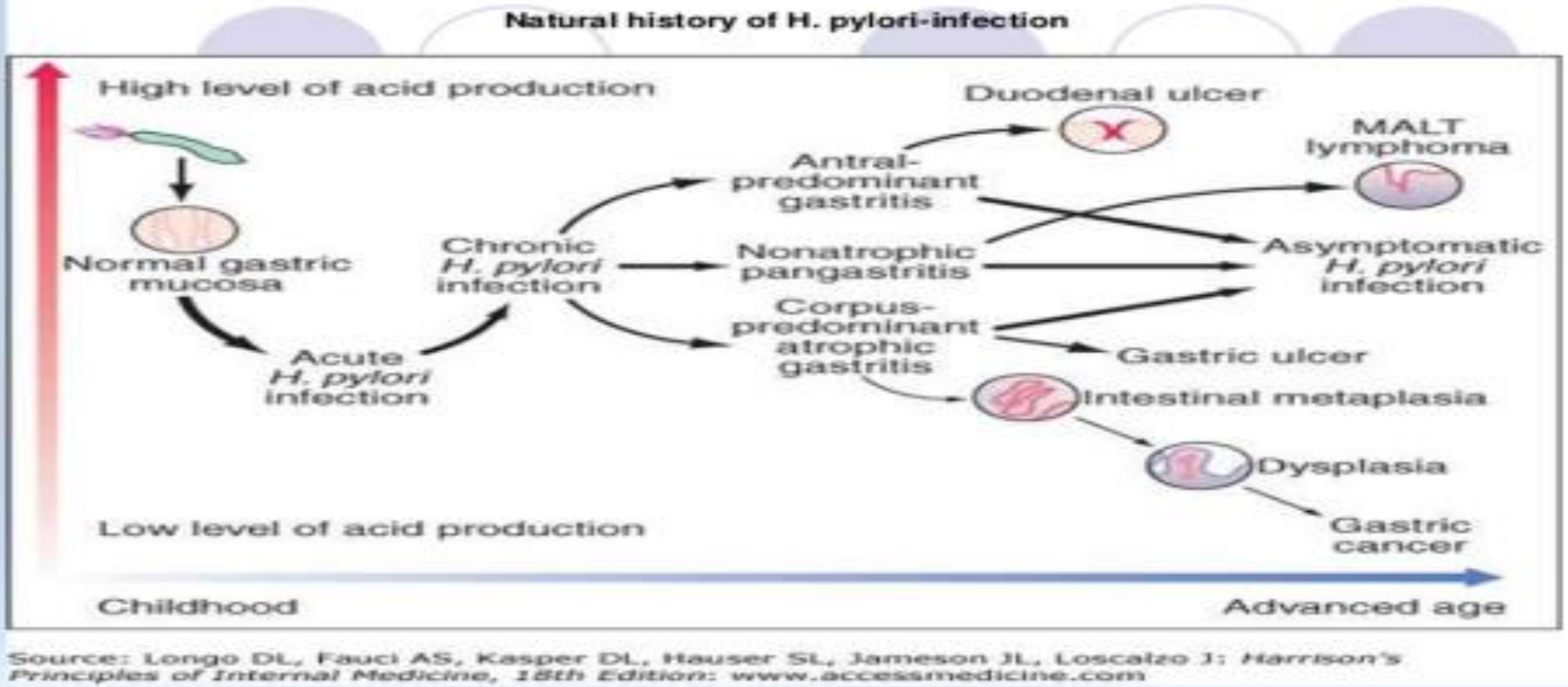


Regarding the prevalence strains of H. pylori infection and its worldwide geographic distribution.



Source: Blazer, 2005. H pylori

H. pylori chronically colonizes the stomachs of over half the world's population.

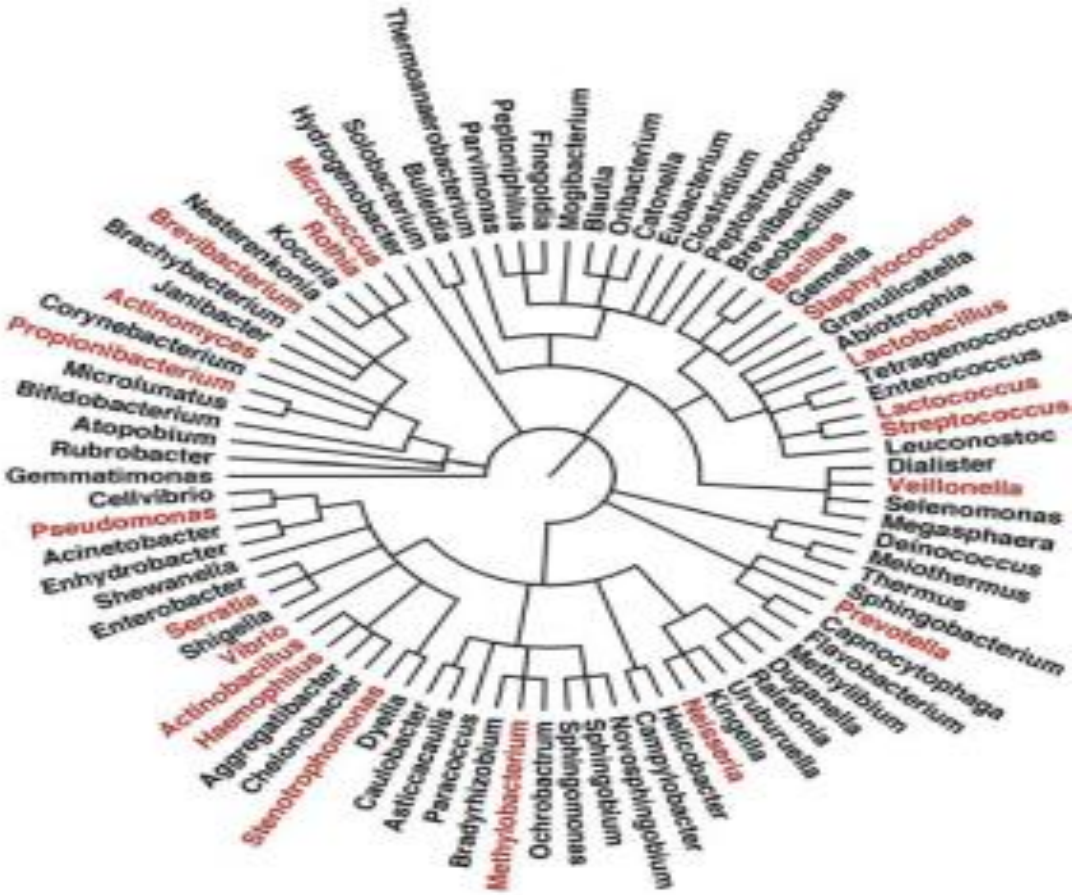


Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

- Metagenomics studies focused on the composition and function of the gastric microbiota have associated with gastric metabolic diseases and even tumorigenesis.

The microbiota composition in normal stomach *
The dominant genera are highlighted in red.

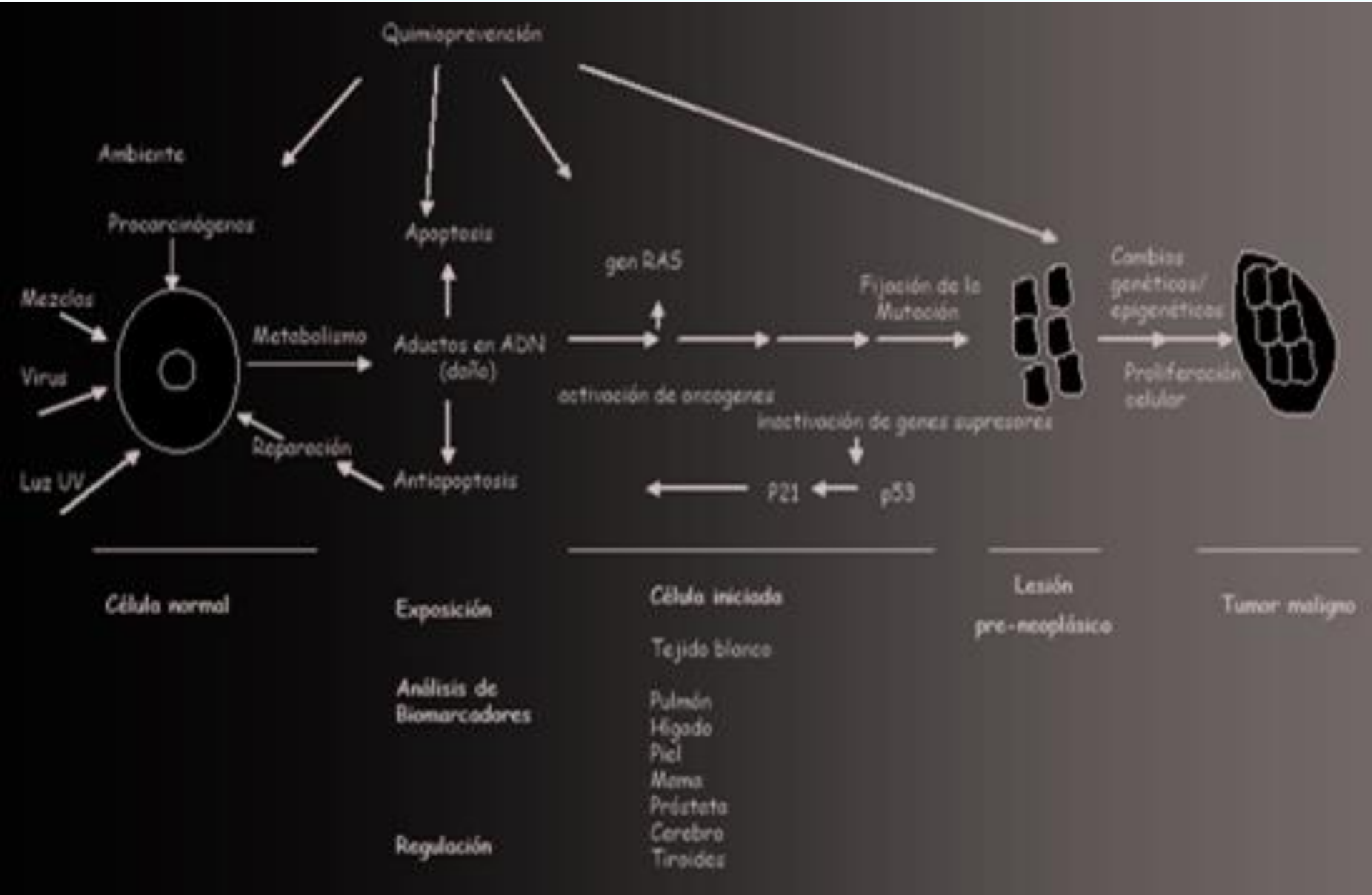
* Lei Cao Jun Yu. Gastrointest Tumors. 2015. Effect of Helicobacter pylori Infection on the Composition of Gastric Microbiota in the Development of Gastric Cancer. 2:14–25



Strains of *H. pylori* inducing factors that increase the risk in gastric cancer

- Strains CagA positive
- Strains VacA (alelos s1, m1)
- Production of fosfolipasa A
- LPS and production of ureasa
- Flagella and adhesins of surface (babA2)
- Gen ice A1/ice A2
- Protein Inductor Factor Necrosis Tumoral- Alfa (Tipalpha)

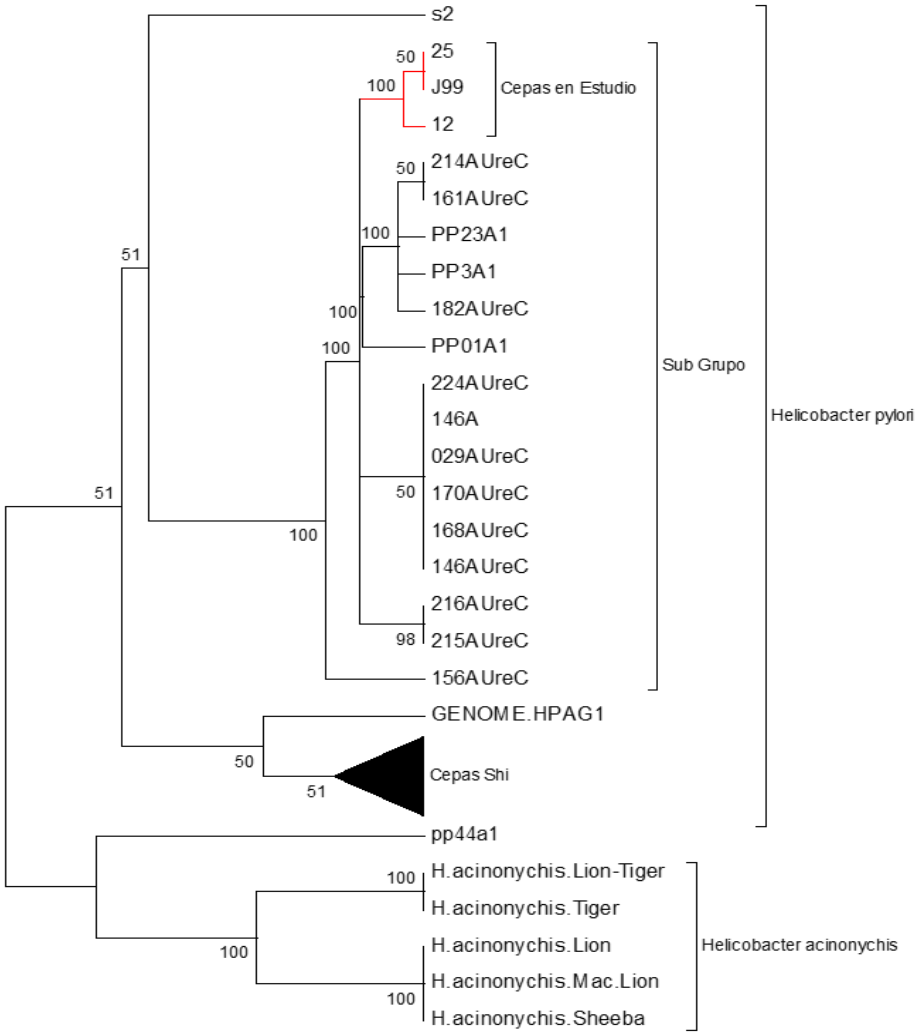
- Impairment of celullar repair system DNA- MMR (MGMT)
- Active chronic iflammation and accumulation of ROS and RNS
- Deregulation of tumor suppressor genes about citoquins and oncogenes.
- Inhibition of protein protective membrane HSP 70


Quimioprevención
Ambiente
Procarcinógenos
Mezclas
Virus
Luz UV
Metabolismo
Reparación
Apoptosis
Aductos en ADN (daño)
Antiapoptosis
gen RAS
Fijación de la Mutación
activación de oncogenes
inactivación de genes supresores
P21
p53
Cambios genéticos/ epigenéticos
Proliferación celular
Célula normal
Exposición
Análisis de Biomarcadores
Regulación
Célula iniciada
Tejido blanco
Pulmón
Hígado
Piel
Mama
Próstata
Cerebro
Tiroides
Lesión pre-neoplásico
Tumor maligno

Investigation in Costa Rica

Chronic gastritis is associated with duodenal ulcers and atrophic gastritis likely to transform into gastric cancer especially in the adenocarcinoma intestinal type.

The highest level of incidences is found in central Costa Rica. Specifically, in the province of Cartago and in the southern part of the province of San Jose.



- Phylogenetic origin of strains *Helicobacter pylori* isolated in drinking water in Costa Rica, 2010.

Source: - Montero V, Hernández A, Camacho J (2014). Culture and Molecular Identification of Helicobacter pylori in Drinking Water from Areas of High and Low Incidence of Gastric Cancer in Costa Rica. OJMM 4(4): 261-269

Methodology

- Costa Rica: The sampling strategy included the aqueducts in areas of high prevalence of gastric cancer, by collecting samples of chlorine-treated water with chlorine content lower than the established by the Water Quality Regulation in Costa Rica (n=44).

- As for Panamá, analysis of the bacteria was based on samples from aqueducts supplying untreated water for human consumption in the province of Chiriquí (n=44).

Methodology

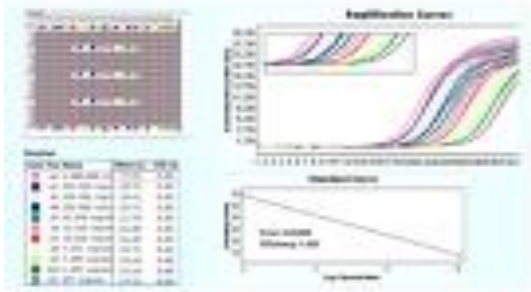
For each aqueduct, a sample of 250 mL of drinking water was collected.

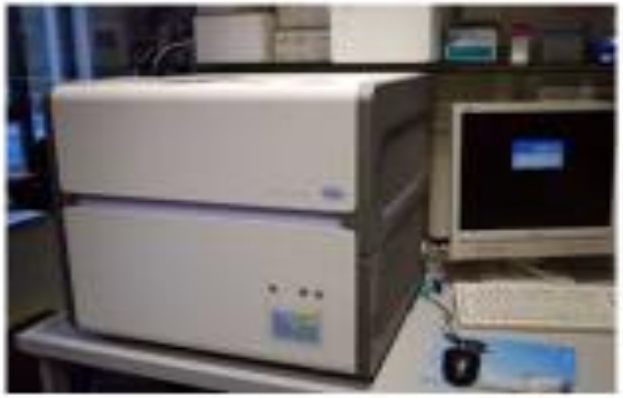
The sample was filtered in the lab using the membrane filtration system Biosart® (0.45 µm filter).

The Rapid Water DNA Isolation Kit from MO-BIO Laboratories was used for the extraction of the DNA.

Subsequently, the filter membrane was removed and placed in a tube with beads used for mechanical removal of the microorganisms; thereafter, for the DNA extraction the technique established by the manufacturer was followed.

Methodology





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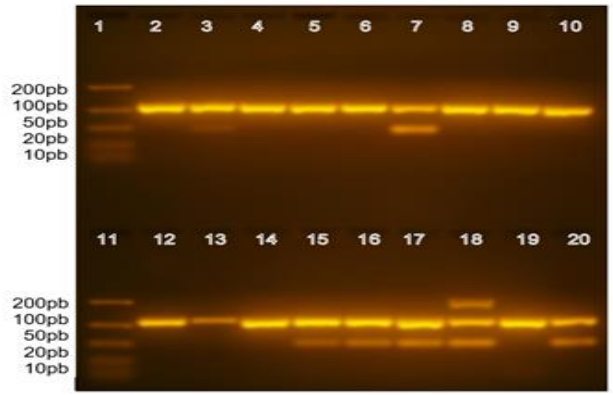
Methodology

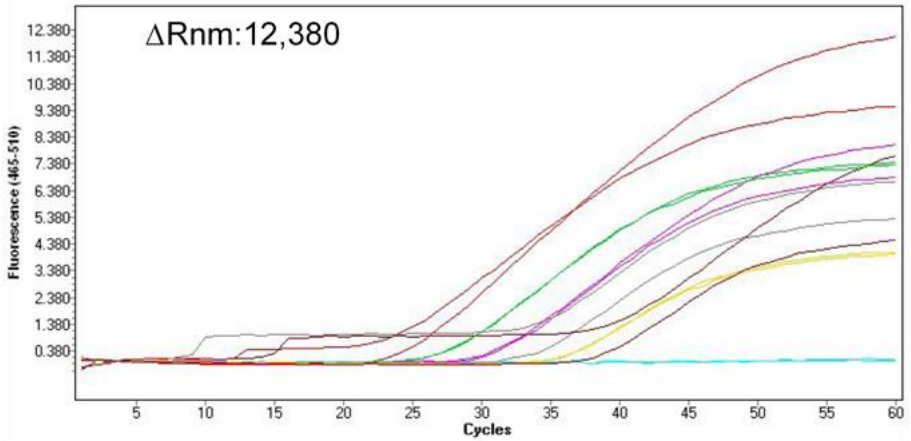
Parameter	Optimum value	Error	Efficiency	Slope	R^2
Annealing temperature for primers	52°C	0.0094	1.963	-3.415	0.9914
glmMRv primer concentration	1µM	0.0374	1.962	-3.416	0.9944
glmMFw primer concentration	1µM	0.0374	1.962	-3.416	0.9944
Probe concentration	1µM	0.0094	1.963	-3.415	0.9914

- The molecular marker of *H. pylori glmM* was used, and to optimize the qPCR technique, annealing temperature, the concentration of primers and probe were standardized.

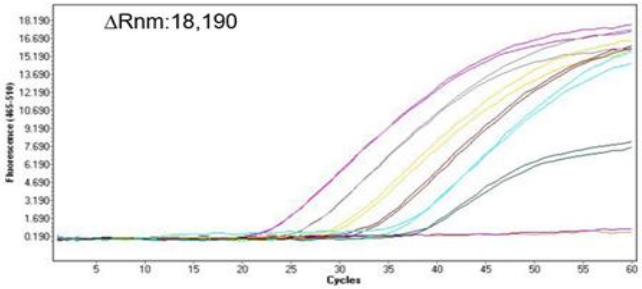
Results



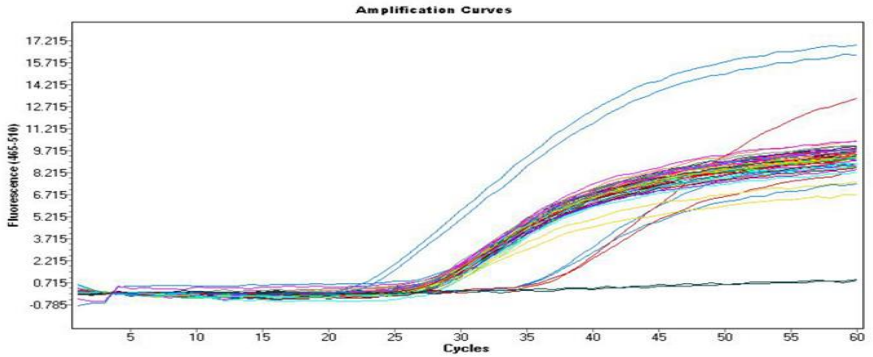
Electrophoresis of the PCR products corresponding to the standards of the three temperatures evaluated.



Amplification curves for the development of the standard curve corresponding to the concentration of 1µM of glmMFw and glmMRv primers in the reaction. Y-axis: emitted fluorescence.

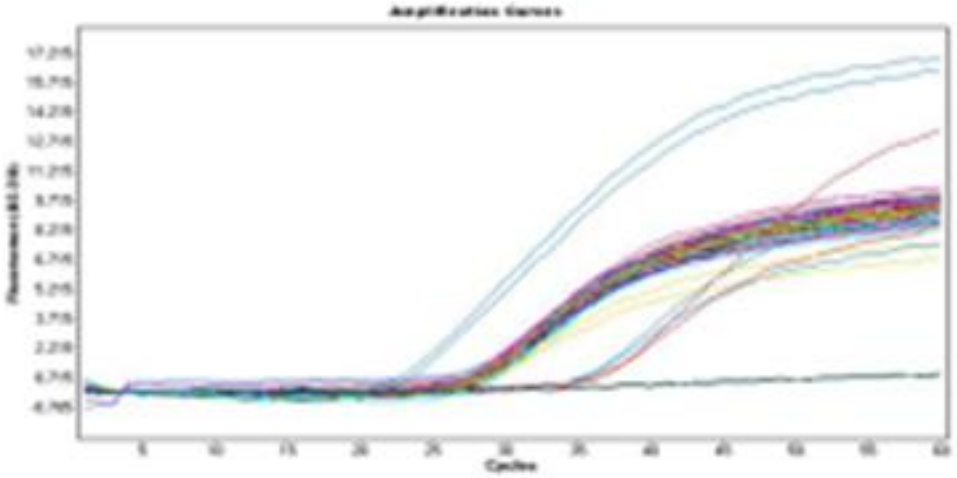


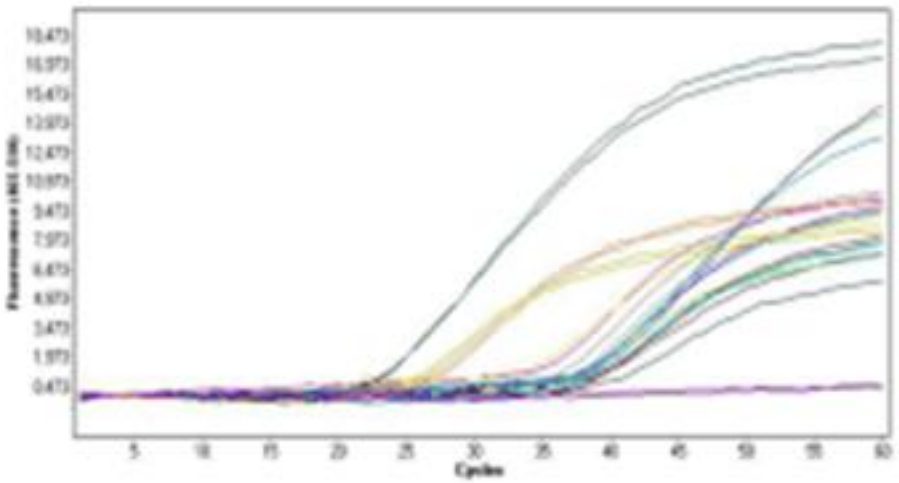
Amplification curves for the development of the standard curve corresponding to the 1 µM concentration of the probe glmMPb in the reaction.

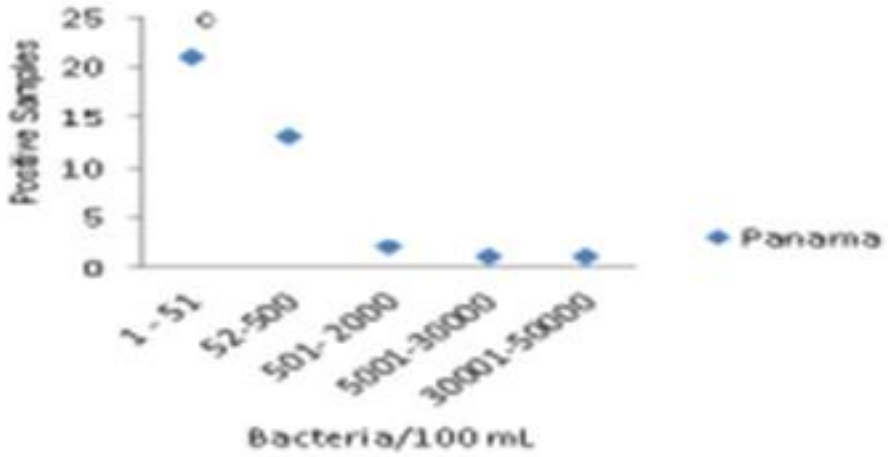


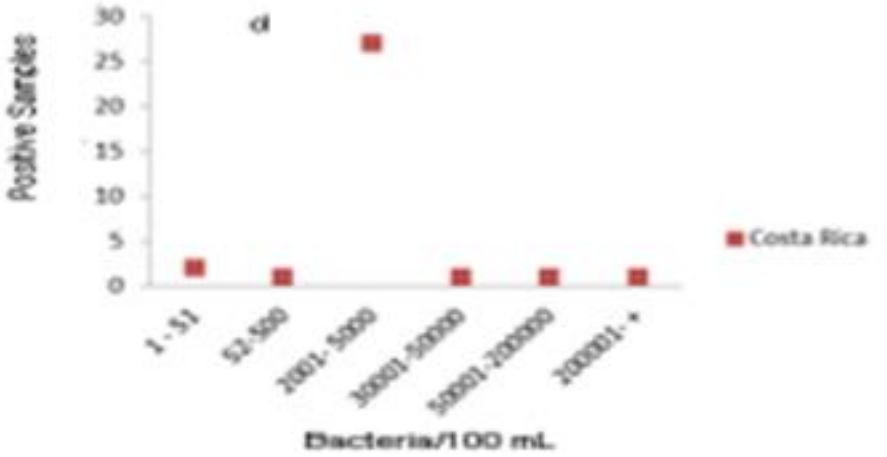
Amplification curves in the analysis of 30 samples from Costa Rica

Results





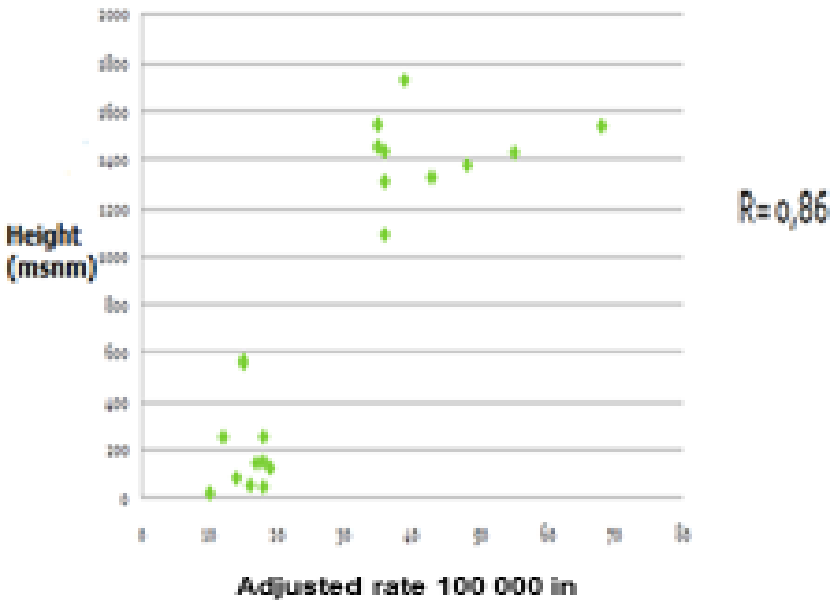

c
Positive Samples
25
20
15
10
5
0
1 - 51
52-500
501-2000
5001-30000
30001-50000
Bacteria/100 mL
Panama


d
Positive Samples
30
25
20
15
10
5
0
1 - 51
52-500
2001- 5000
30001-50000
50001-200000
200001- *
Bacteria/100 mL
Costa Rica

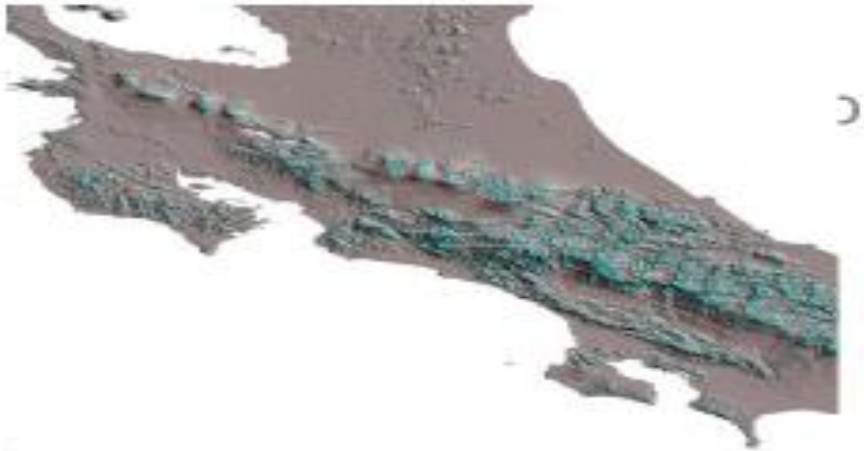
Discussion

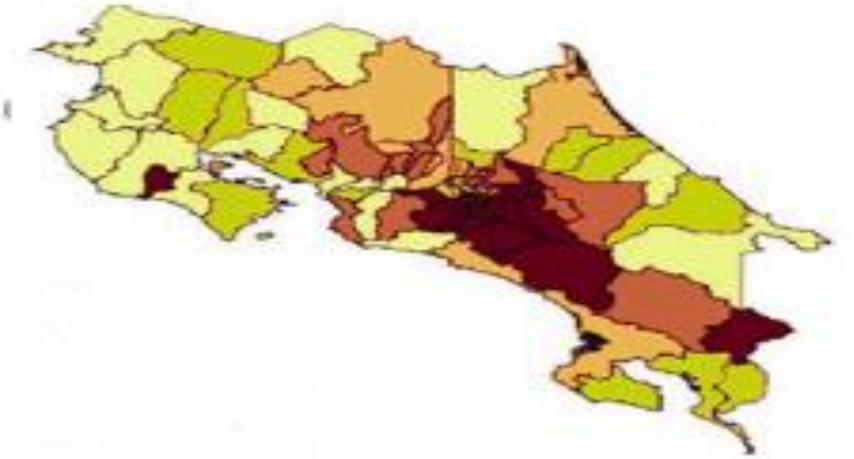
		Incidence1	Height	Temperature	Groundwater
Incidence1	Pearson Correlation	1	.867**	-.853**	.651**
	Sig. (2-tailed)		.000	.000	.002
	N	20	20	20	20
Height	Pearson Correlation	.867**	1	-.980**	.774**
	Sig. (2-tailed)	.000		.000	.000
	N	20	20	20	20
Temperature	Pearson Correlation	-.853**	-.980**	1	-.773**
	Sig. (2-tailed)	.000	.000		.000
	N	20	20	20	20
Groundwater	Pearson Correlation	.651**	.774**	-.773**	1
	Sig. (2-tailed)	.002	.000	.000	
	N	20	20	20	20

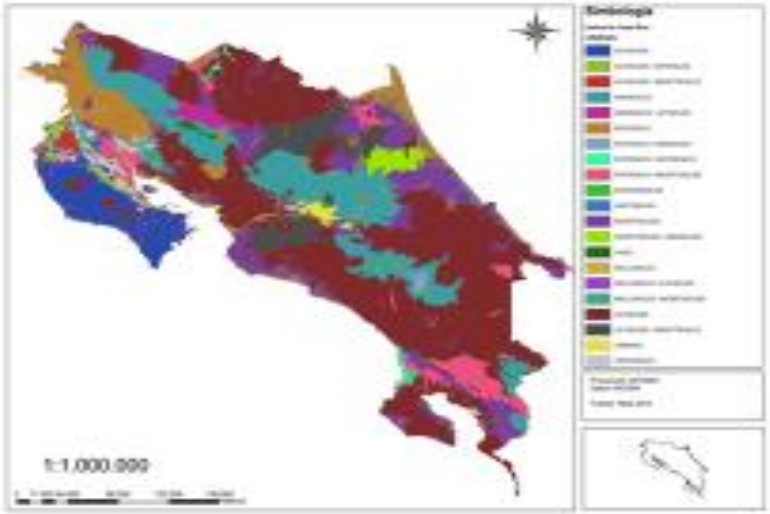
* V Montero, A Hernández, J Camacho. 2014. Culture and molecular identification of Helicobacter pylori in drinking water from areas of high and low incidence of gastric cancer in Costa Rica. Open Journal of Medical Microbiology. 4 (4): 261-269.

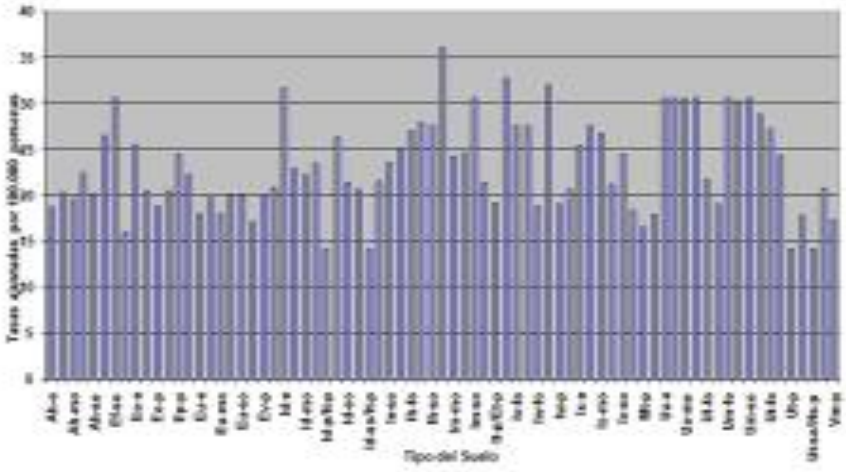


Discussion









Discussion

=> The incidence of CG (intestinal adenocarcinoma), gastritis and ulcers rises rapidly

factors associated with the population (predominance of proinflammatory polymorphisms and Factor Lewis B +)

High water supply managed by municipalities or small associations, especially in areas of high incidence of CG

Socio-economic conditions of the population (not so marked between areas)

favorable environmental conditions for the development of H. pylori such as altitude, climate, air pressure and high organic matter in soil

Presence of pathogenic strains of H pylori in the environment

Discovery of H. pylori in drinking water

Conclusions

- As for the results obtained from the quantification of the bacteria in drinking water, significant differences were observed in water management in both countries, that may result in differences in quality.
- While non-chlorinated water from Chiriquí Panamá presents 330 copies of the bacteria per 100 mL, in Costa Rica chlorinated water shows 3600 copies/100 mL on average.
- Continuous and efficient water chlorination determines non-formation of biofilms in piping conducting water from the sites of chlorination to consumers. According to specific research, the bacterium is capable of resisting in biofilm-like environments, as in the stomach

Conclusions

- Although water in high gastric cancer incidence zones in Costa Rica is dispensed to the population by the Municipalities, it is considered of good quality according to health parameters regarding presence of fecal coliforms. According to findings from this research, water chlorination treatments applied by the Operating Entities have proved to be insufficient in some cases, with important concentrations of viable bacteria still remaining in the water.
- Research should be conducted as to the causes of strong resistance of *H. pylori* to chlorination in the case of CR.
- In addition, marked apparent turbidity exceeding 5 NTU (nephelometric turbidity units), which is the maximum permitted by the Water Quality Regulation in Costa Rica, was determined in the samples analyzed. Solids in suspension, such as soil particles, can serve to hide the bacteria.

Conclusions

- Consequently, for the sake of public health, special relevance is given to treating and dispensing drinking water containing no less than 0.8 mg/L residual chlorine, with apparent turbidity less than 5 NTU, particularly in the case of chlorine-treated superficial waters in areas of high gastric cancer incidence.

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