



10th World Congress on

Healthcare & Technologies

Conference Venue Hotel: Holiday Inn Lisbon – continental

Healthcare Summit 2017

July 17-18, 2017 | Lisbon, Portugal

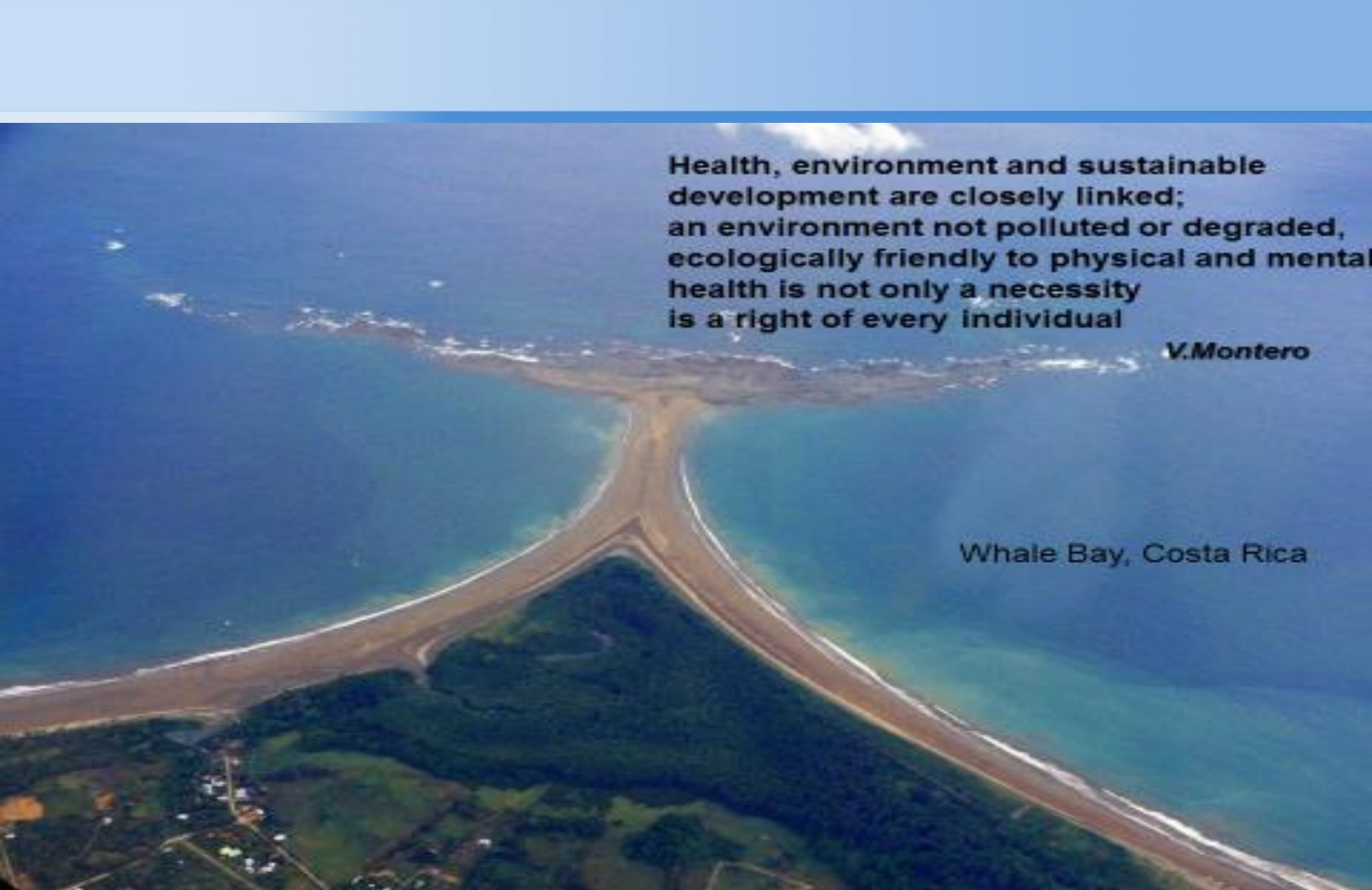
Quantitative Detection of *Helicobacter pylori* by Real Time PCR in Drinking Water—Environmental and Public Health Risk Significance.

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

TEC | Tecnológico
de Costa Rica

Technological Institute of Costa Rica

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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"



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Introduction



International Agency for Research on Cancer



World Health
Organization



Helicobacter pylori
belongs to group 1 IARC



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CAS No	Agent	Group	Volume	Year
002871-01-4	H1C Red No. 3	3	57	1993
059820-43-8	H1C Yellow No. 4	3	57	1993
	<i>Helicobacter pylori</i> (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	Hexachlorocyclopentadiene	3	73	1999
	Hexachlorocyclohexanes	2B	20, Sup 7	1987
000087-72-1	Hexachloroethane	2B	73	1999
000070-30-4	Hexachlorophene	3	20, Sup 7	1997
000142-83-8	2,4-Hexadialal	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, 59, 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	Hydanthone mesylate	3	13, Sup 7	1987
000066-04-4	Hydralazine	3	24, Sup 7	1987
000302-01-2	Hydrazine	2B	4, Sup 7, 71	1999
007647-01-0	Hydrochloric acid	3	54	1992
000058-93-8	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



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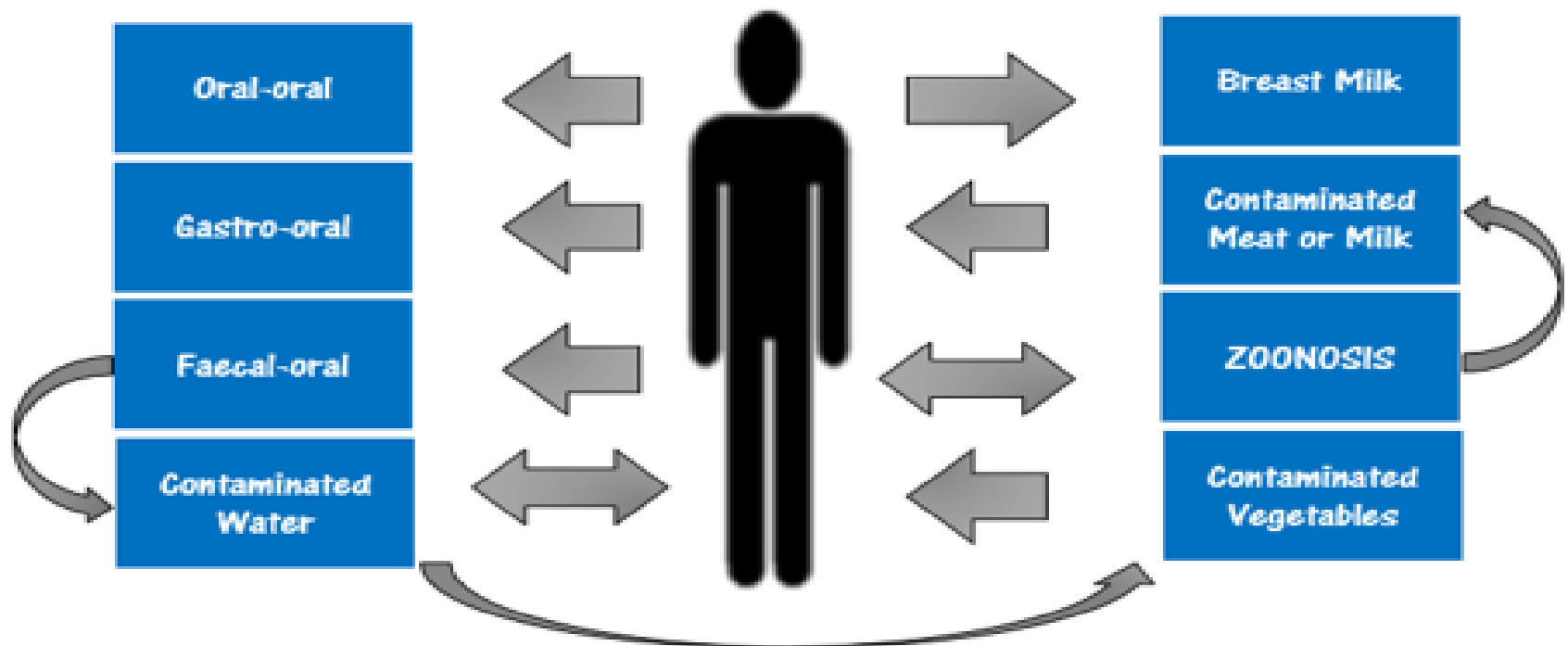
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Based on epidemiological and microbiological evidence several routes of transmission have been proposed



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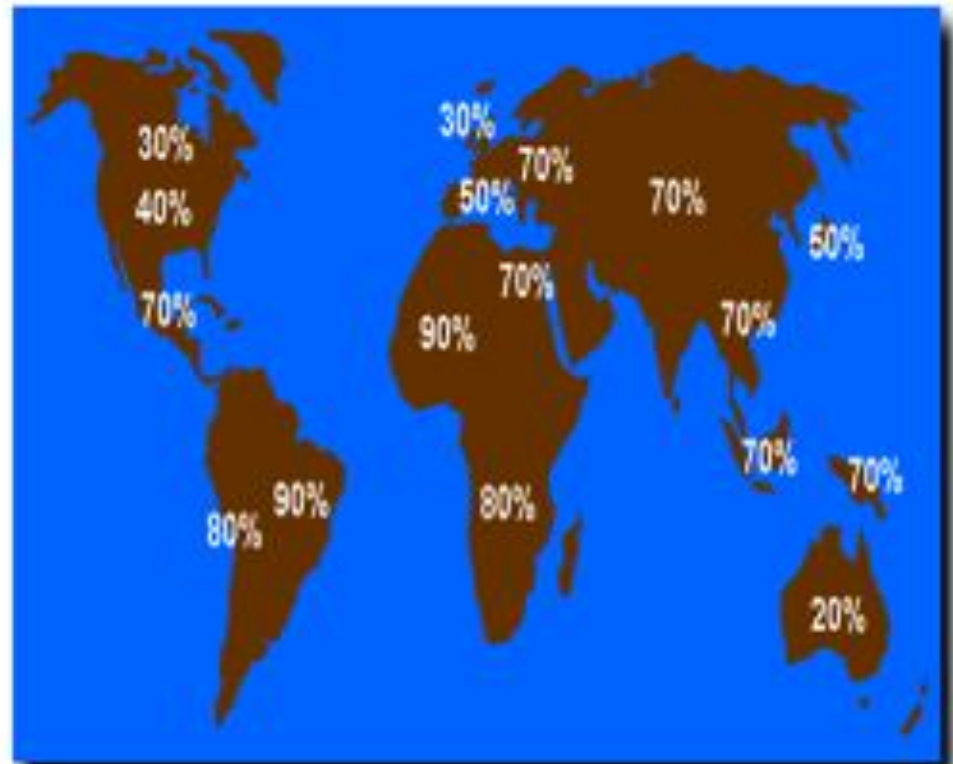
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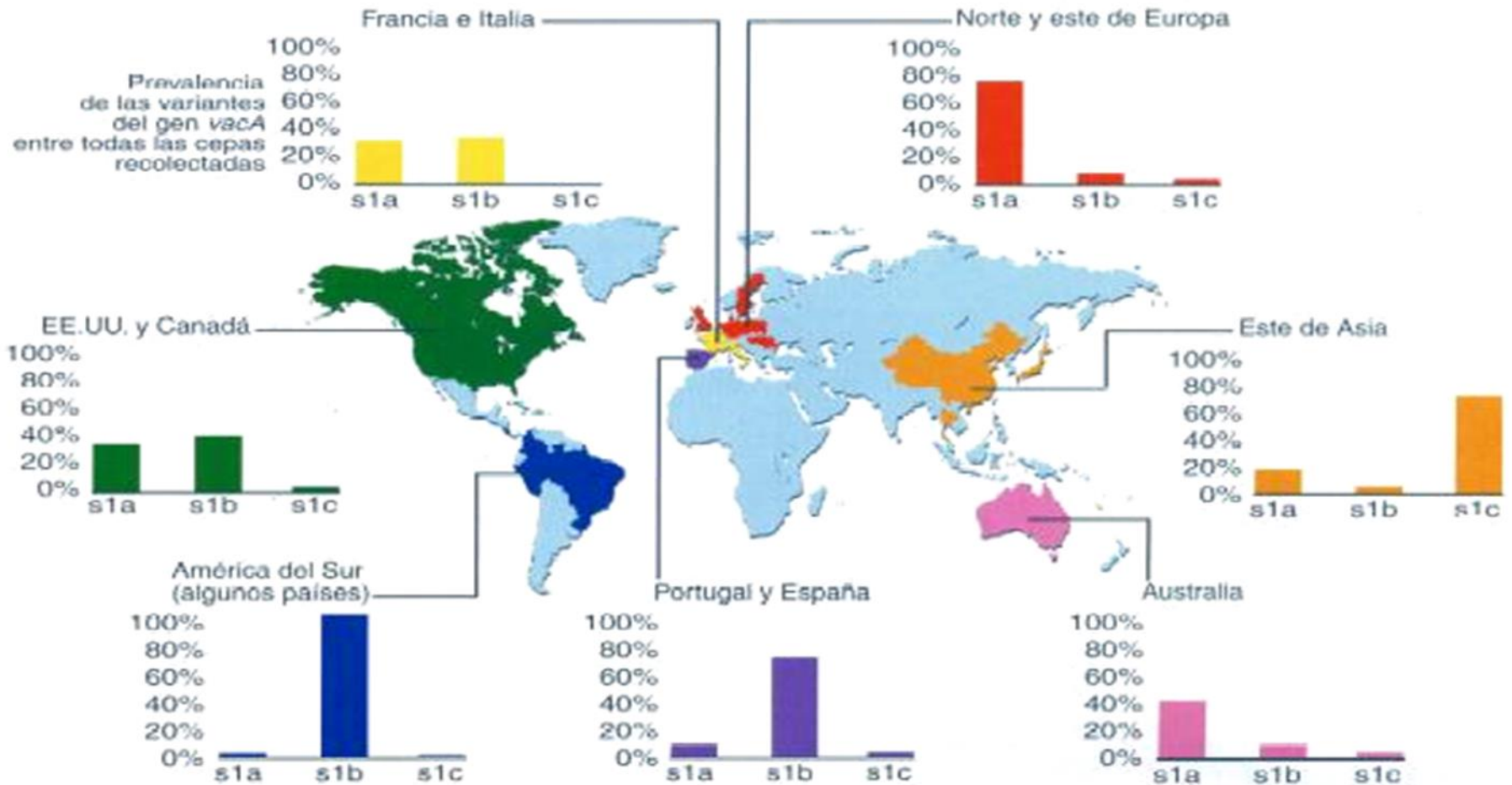
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Background of the Problem

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*



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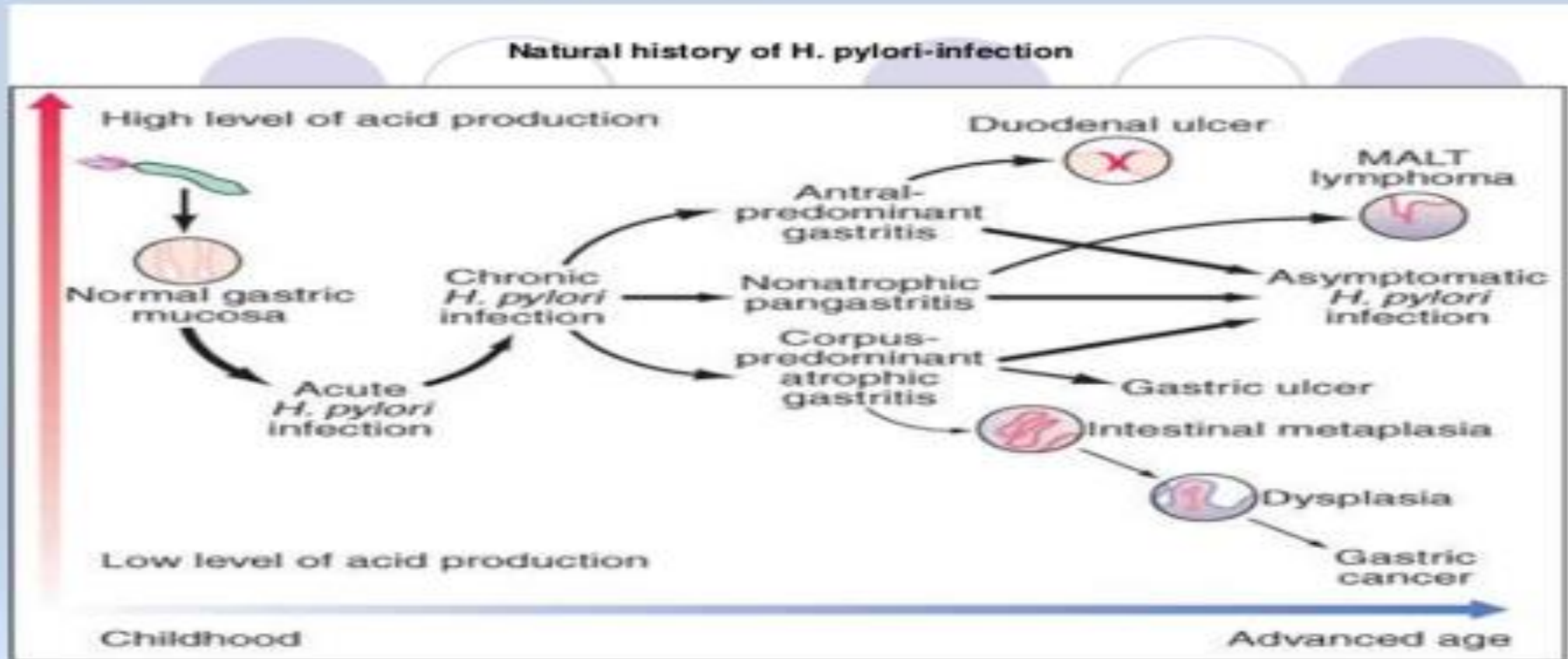
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H. pylori chronically colonizes the stomachs of over half the world's population.



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



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

A circular phylogenetic tree of the phylum Proteobacteria. The tree is rooted at the center and branches outwards. The genera names are written along the branches, with some highlighted in red. The tree is divided into several major clades, including the Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, and the class Rhodospirillum rubrum.

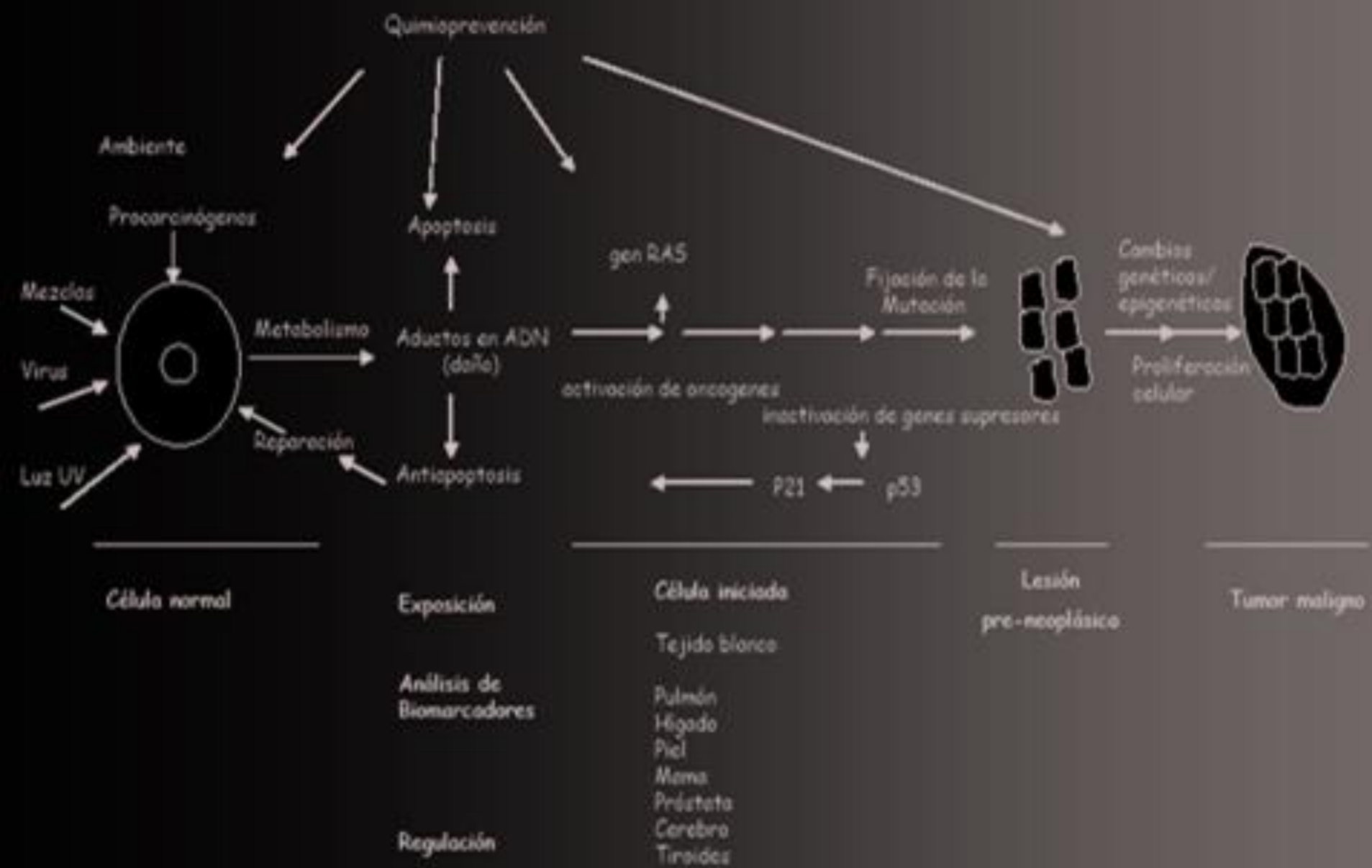
Genera names (clockwise from top):

- Thermomana*
- Hydrogenobacterium*
- Solobacterium*
- Micrococcus*
- Rothia*
- Kocuria*
- Nesterenkonia*
- Brevibacterium*
- Brachybacterium*
- Janibacter*
- Actinomycetes*
- Corynebacterium*
- Propionibacterium*
- Microfumarus*
- Bifidobacterium*
- Atopobium*
- Rubrobacter*
- Gemmatimonas*
- Cellvibrio*
- Pseudomonas*
- Acinetobacter*
- Enhydrobacter*
- Shewanella*
- Enterobacter*
- Serratia*
- Shigella*
- Vibrio*
- Actinobacillus*
- Haemophilus*
- Aggregatibacter*
- Chelonobacter*
- Syntrophomonas*
- Dyella*
- Asticcobacter*
- Paracoccus*
- Bradyrhizobium*
- Methylobacterium*
- Ochrobactrum*
- Sphingomonas*
- Sphingobium*
- Novosphingobium*
- Campylobacter*
- Helicobacter*
- Kingella*
- Urburubella*
- Dugesiella*
- Erythrobacterium*
- Capnocytophaga*
- Prevotella*
- Thermus*
- Melothermus*
- Deinococcus*
- Megasphaera*
- Selenomonas*
- Veillonella*
- Dialister*
- Leuconostoc*
- Streptococcus*
- Lactococcus*
- Enterococcus*
- Tetragenococcus*
- Lactobacillus*
- Abiotrophia*
- Granulicatella*
- Gemella*
- Bacillus*
- Geobacillus*
- Brevitetrastococcus*
- Clostridium*
- Eubacterium*
- Calonella*
- Ornithobacterium*
- Mogibacterium*
- Finogoldia*
- Peptoniphilus*
- Parvimonas*
- Buldisia*

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





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.



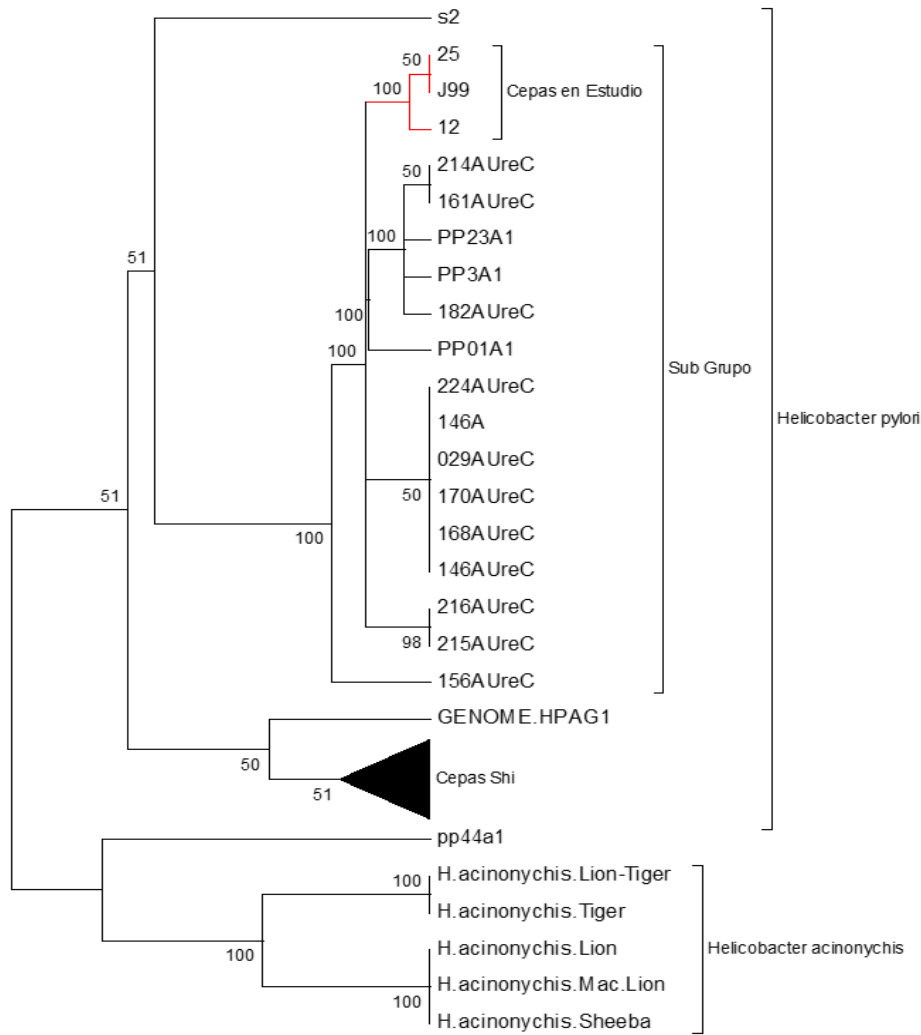
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- Phylogenetic origin of strains *Helicobacter pylori* isolated in drinking water in Costa Rica, 2010.

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).



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Methodology

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

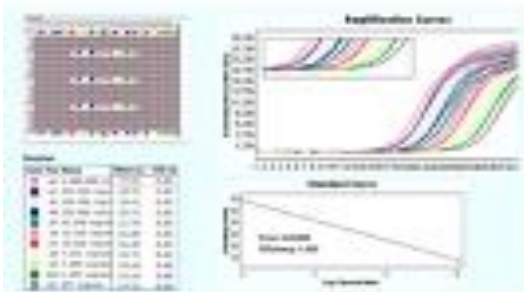
The sample was filtered in the lab using the membrane filtration system Biosart® (0.45 µm filter).

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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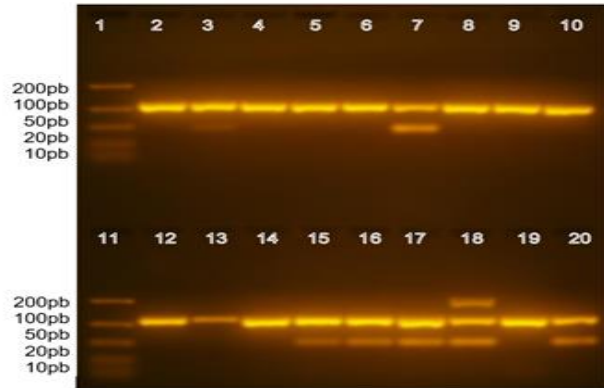
Methodology

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

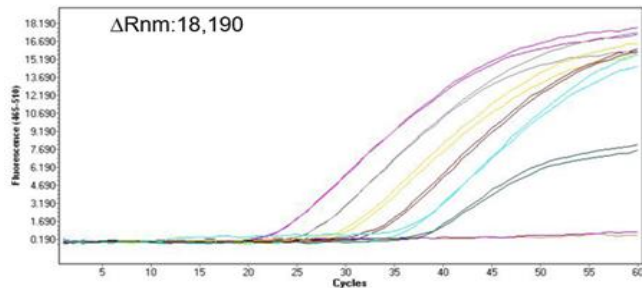
- The molecular marker of *H. pylori* *glmM* was used, and to optimize the qPCR technique, annealing temperature, the concentration of primers and probe were standardized.



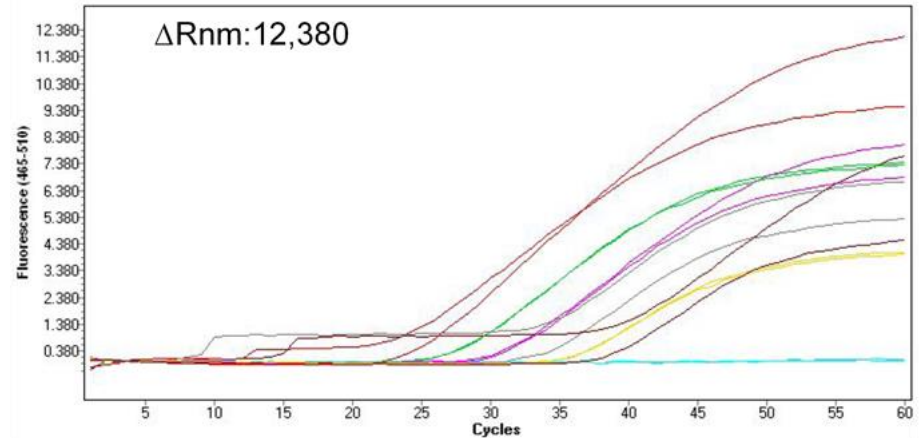
Results



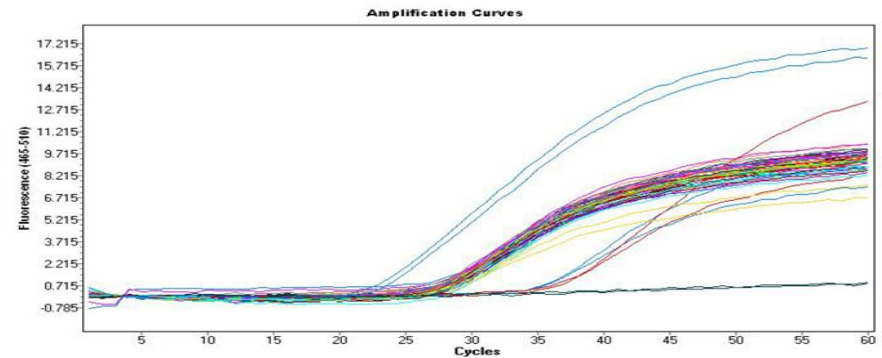
Electrophoresis of the PCR products corresponding to the standards of the three temperatures evaluated.



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



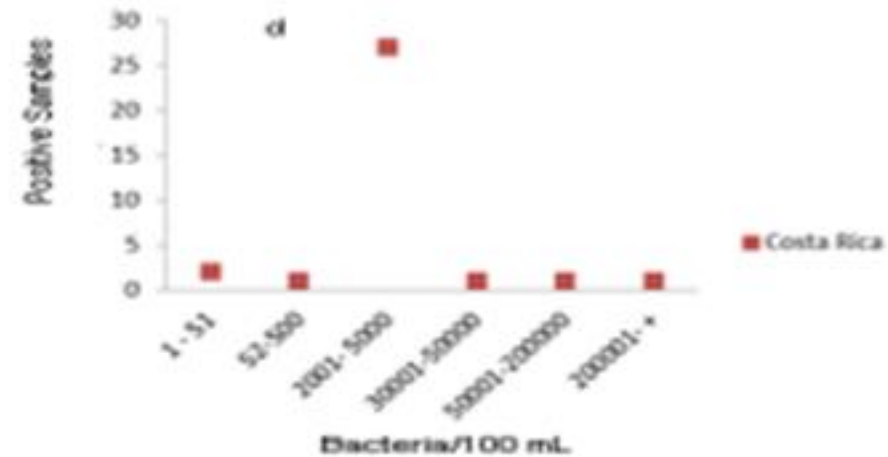
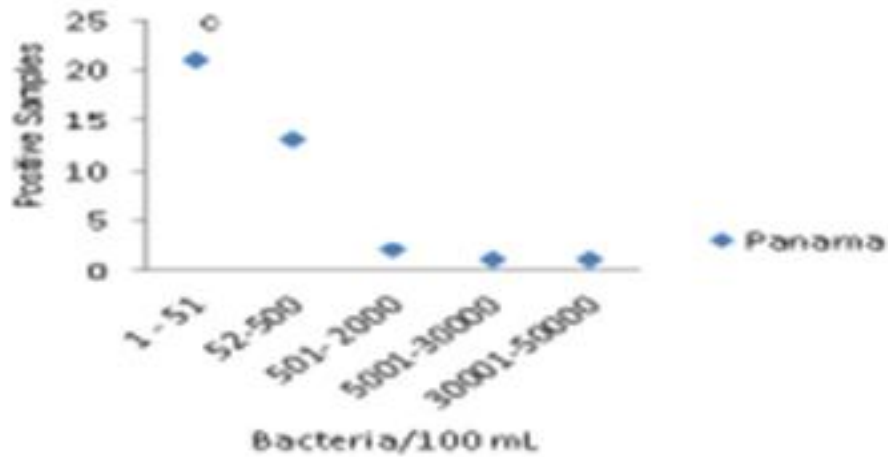
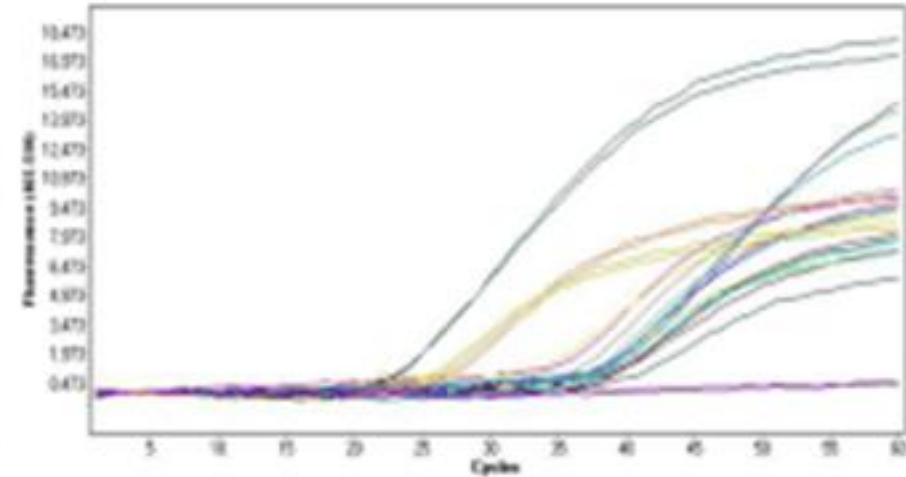
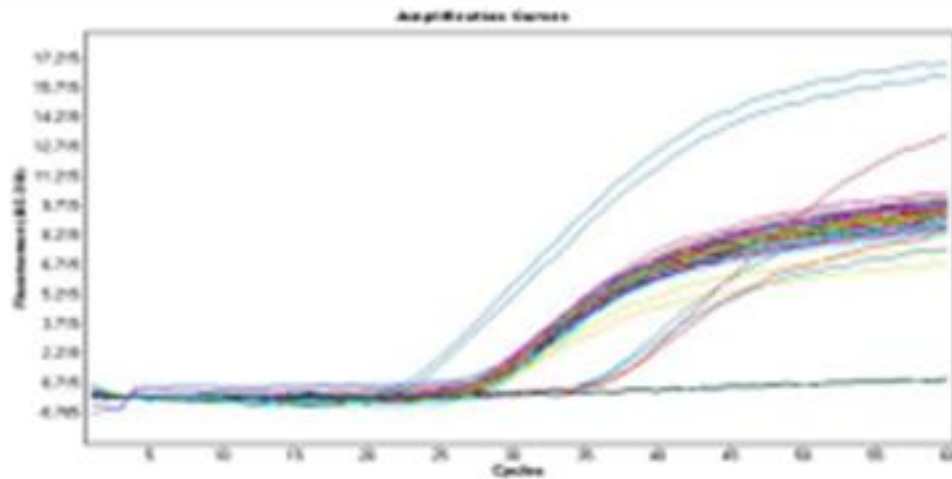
Amplification curves for the development of the standard curve corresponding to the concentration of 1µM of glmMFw and glmMRv primers in the reaction. Y-axis: emitted fluorescence.



Amplification curves in the analysis of 30 samples from Costa Rica



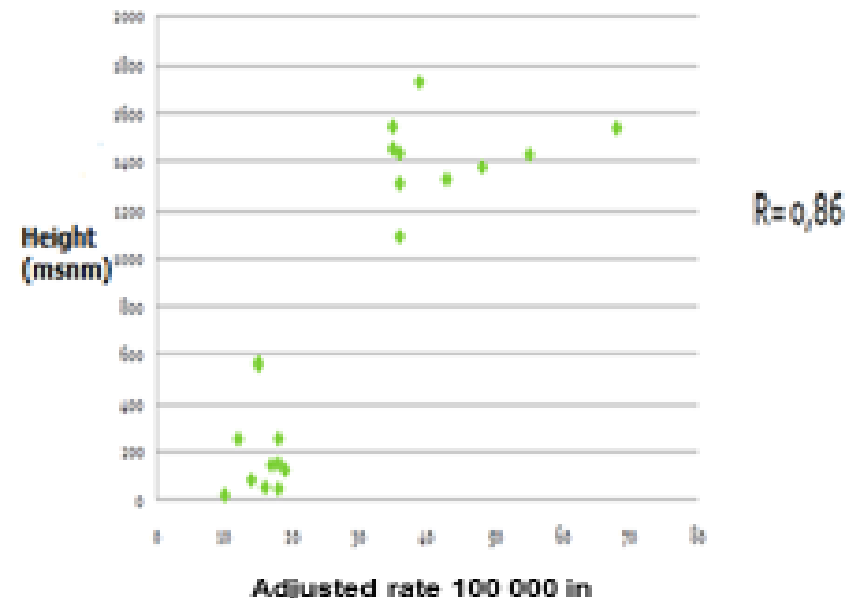
Results



Discussion

		IncidenceI	Height	Temperature	Groundwater
IncidenceI	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.



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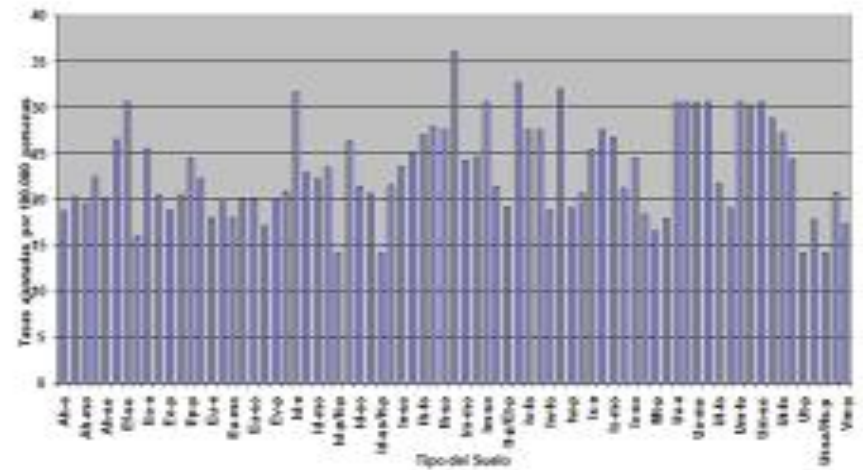
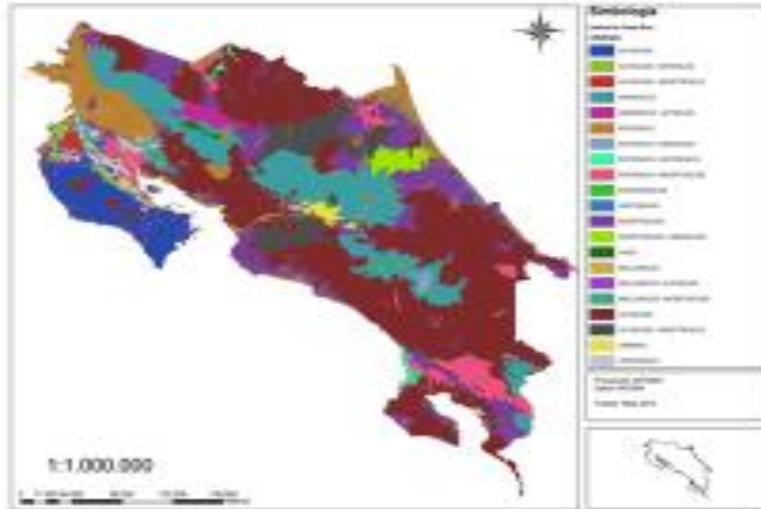
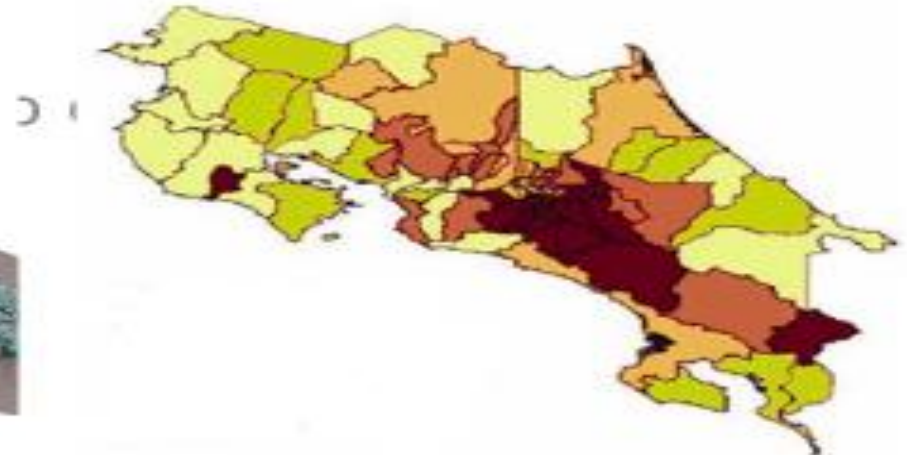
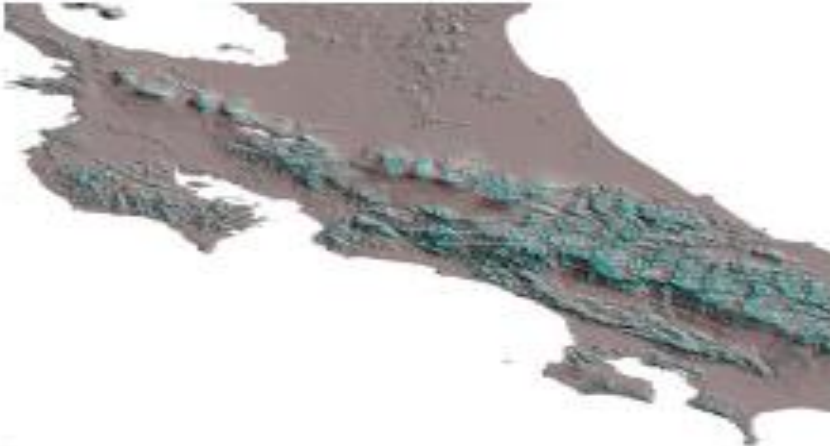
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Discussion



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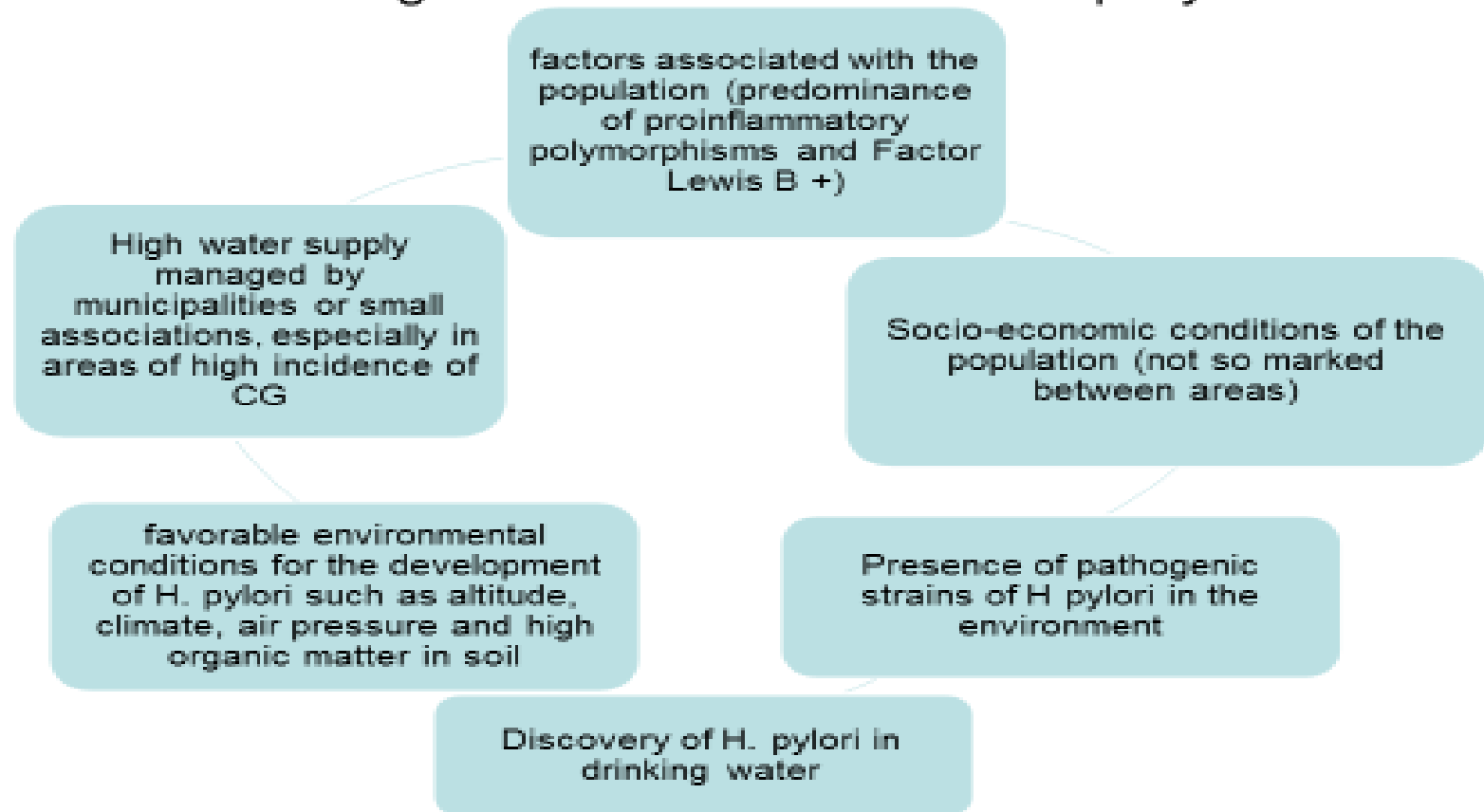
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Discussion

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



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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Special thanks to

- Biotechnology Research Center.
Technological Institute of Costa Rica
- National Laboratory of Water Costa Rica.
- Faculty of Microbiology and Genetic
University of Murcia, Spain.



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Tamarindo Beach, CR

Thank you for your attention



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