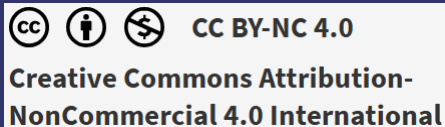
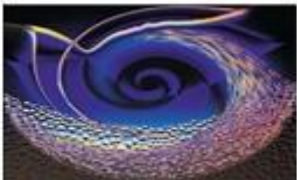


“Quantification by qPCR of the bacterium
Helicobater pylori in drinking water-Environmental
and health public risk significance”

Virginia Montero Campos, Ph.D



Quantification by qPCR of the bacterium Helicobater pylori in drinking water-Environmental and health public risk significance © 2015 by [Virginia Montero Campos](#) is licensed under [CC BY-NC 4.0](#)



**The 8th International Workshop on Contaminant
Bioavailability in the Terrestrial Environment**

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 but a right of every individual

V.Mor

Walley Bay, Costa Rica



Presentación

- Introducción
- Antecedentes del problema
- Metodología
- Resultados
- Conclusiones y Recomendaciones

Costa Rica



Introducción

- *H. pylori*, se descubrió por en estómagos de pacientes con gastritis y úlceras en los años 1982-83, hace casi 35 años, por los Drs. Barry Marshall y Robin Warren de Perth, Australia.
- En reconocimiento a su descubrimiento fueron galardonados con el Premio Nobel 2005 en Medicina y Fisiología.



Introducción

Desde 1994 y a la fecha la OMS lo declara como un cancerígeno en humanos, pertenece al grupo I de IARC.



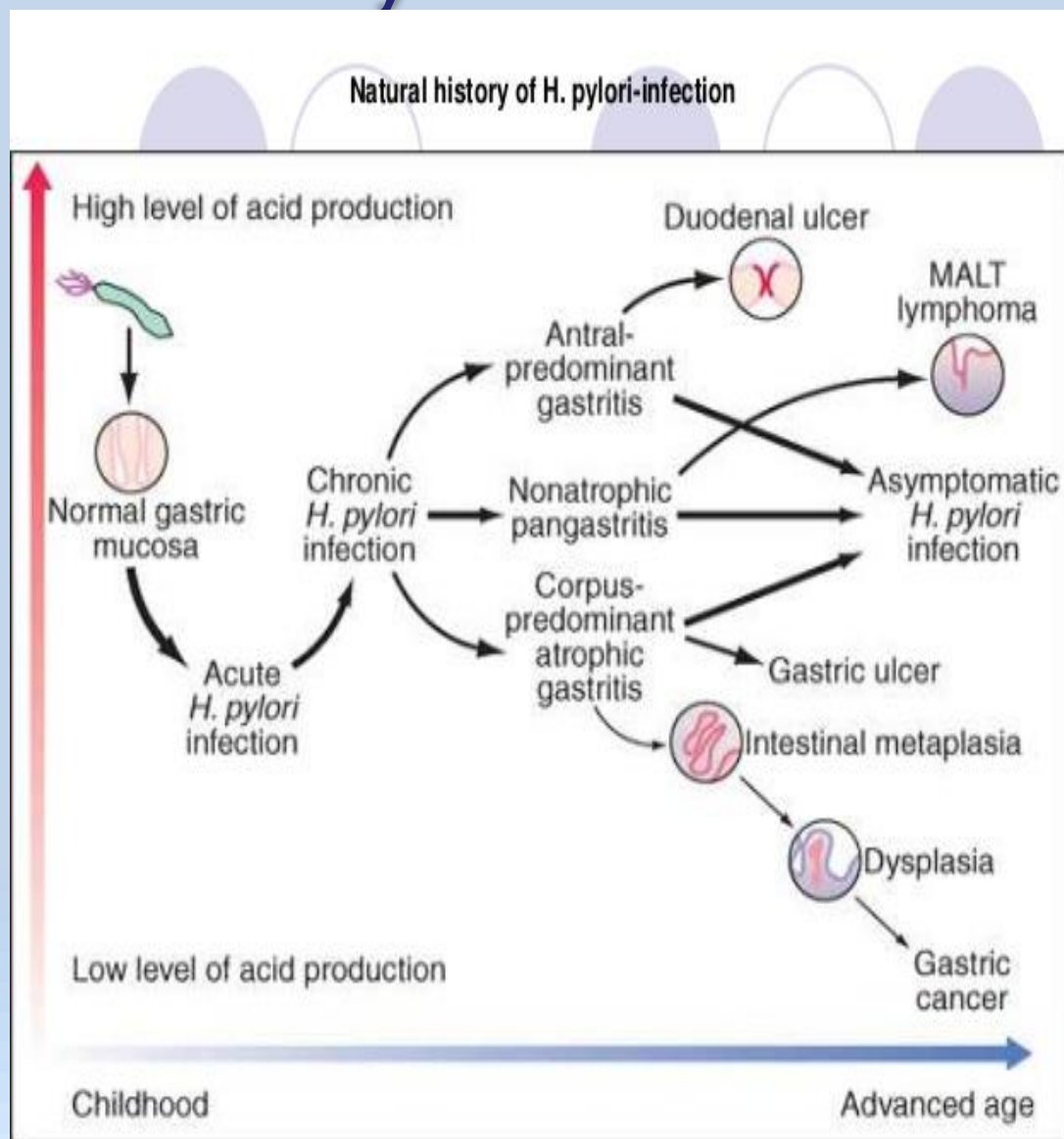
International Agency for Research on Cancer



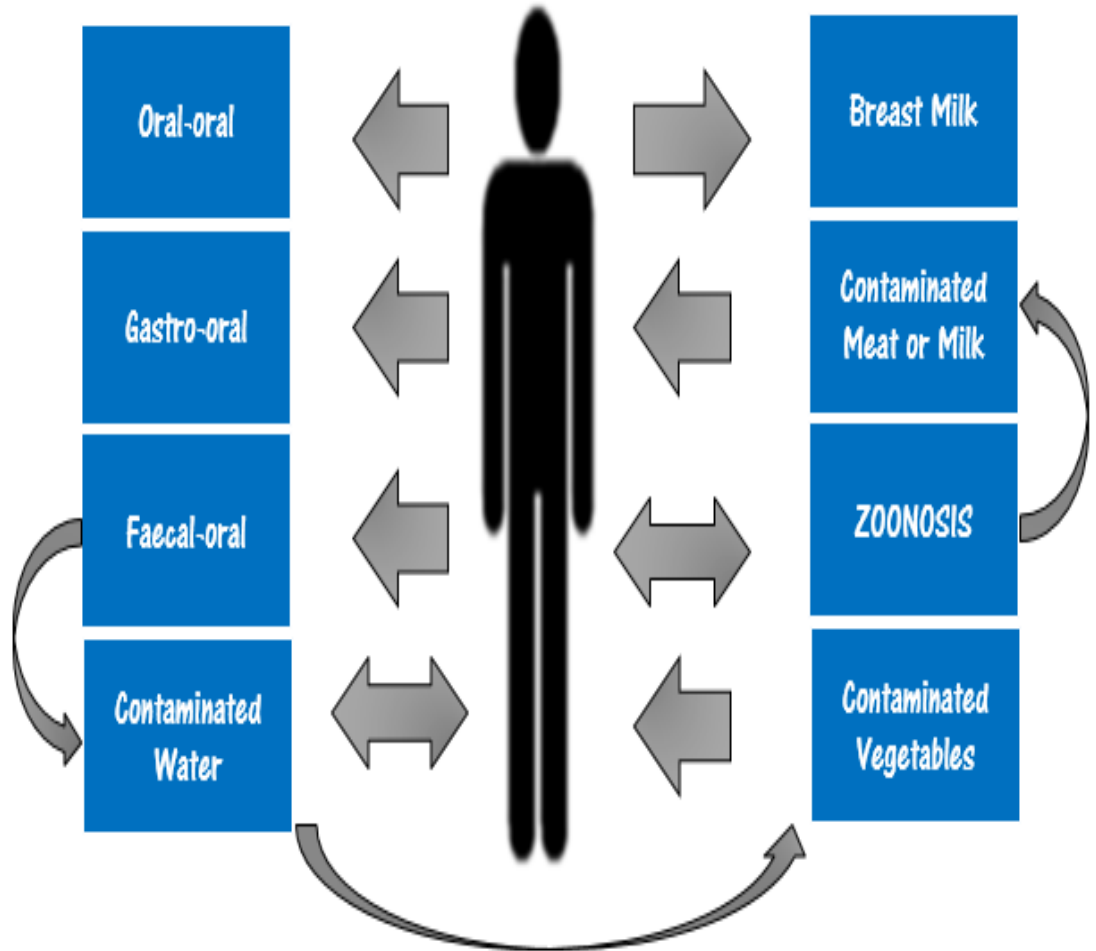
CAS No	Agent	Group	Volume	Year
002871-01-4	HC Red No. 3	3	57	1993
059820-43-8	HC Yellow No. 4	3	57	1993
	<i>Helicobacter pylori</i> (infection with)	1	61, 100B	2012
	Hepatitis B virus (chronic infection with)	1	59, 100B	2012
	Hepatitis C virus (chronic infection with)	1	59, 100B	2012
	Hepatitis D virus	3	59	1994
000076-44-8	Heptachlor	2B	79	2001
000118-74-1	Hexachlorobenzene	2B	79	2001
000087-68-3	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
000058-93-5	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

Mecanismos de acción y variaciones genéticas

- Se dice que *H. pylori* coloniza de forma crónica el estómago de más de la mitad de la población mundial.
- Sin embargo sólo entre el 10-20% de los pacientes infectados desarrollan enfermedades graves, como úlcera péptica, cáncer gástrico y linfoma (el problema de gastritis no ha sido cuantificado).

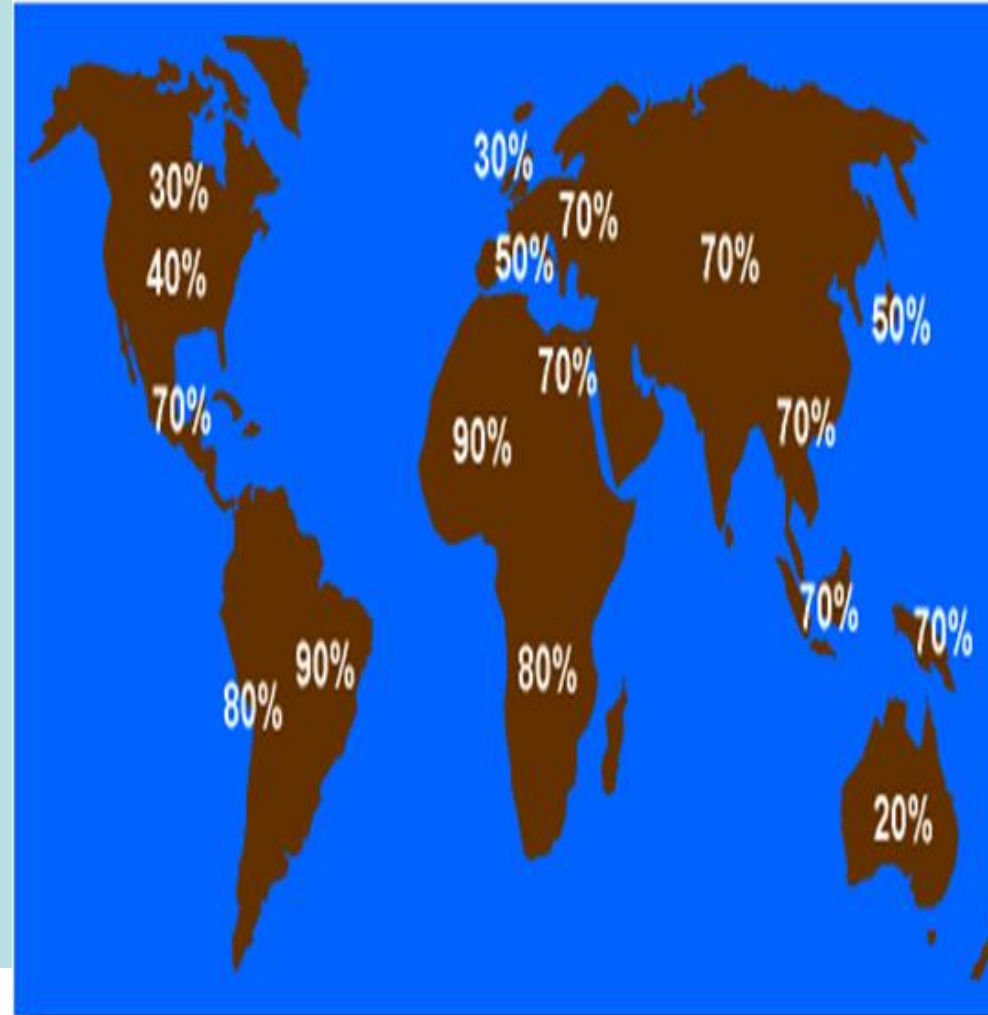


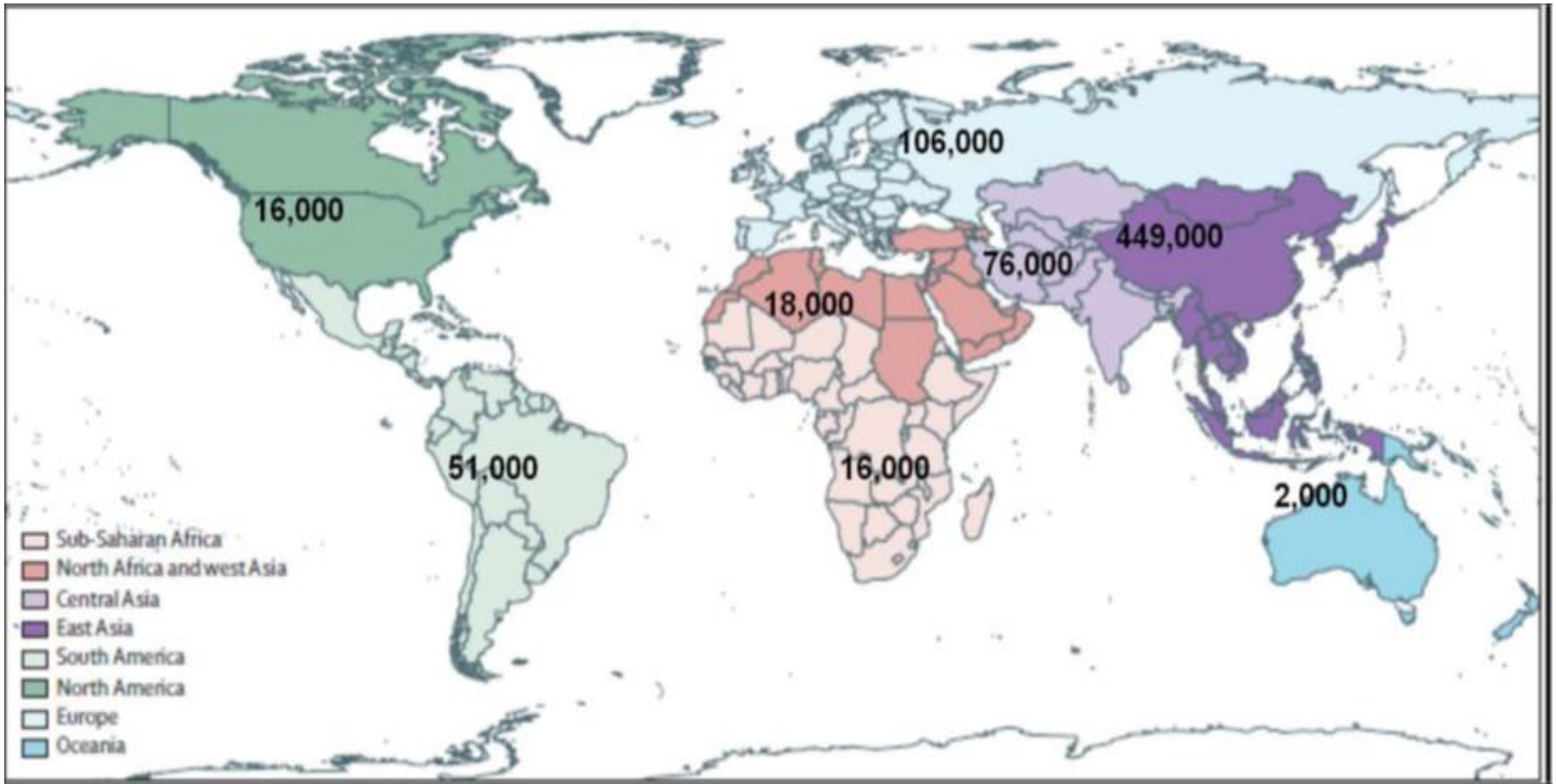
❖ Basado en evidencia epidemiológica y microbiológica varias vías de transmisión han sido propuestas



Antecedentes

La prevalencia de infección del en el mundo es alta, y más en los países de origen tropical, asociados al ingreso per capita bajo.

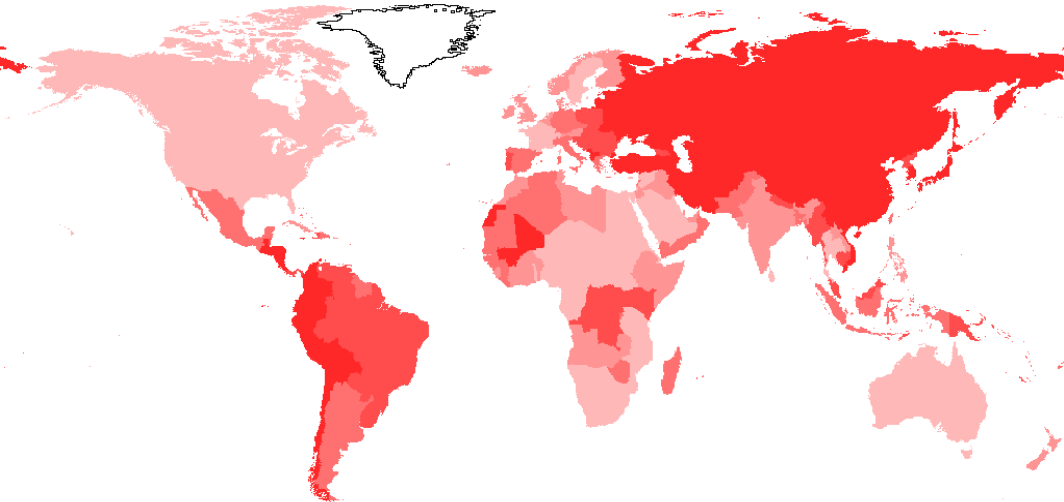




casos de cáncer gástrico ligados con la infección por *H. pylori* al año 2012 en diferentes regiones geográficas (IARC, 2014).

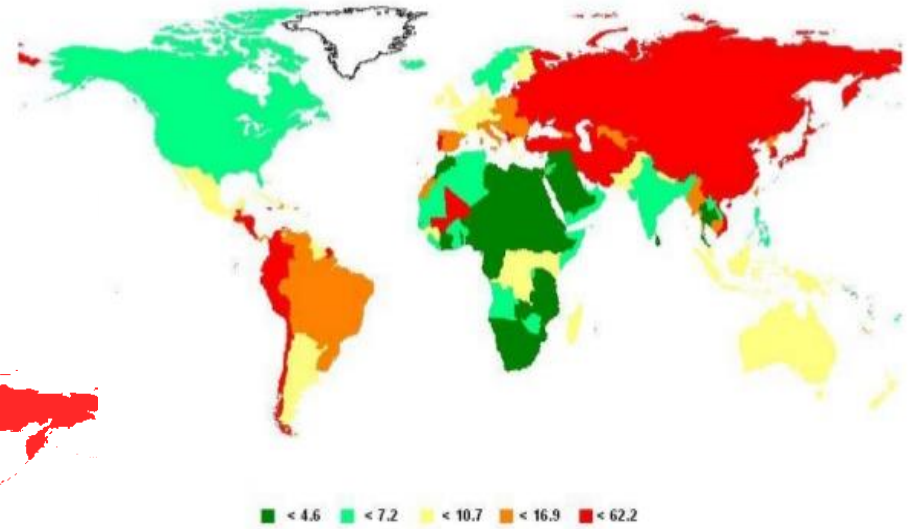
- Costa Rica tiene la novena incidencia de cáncer gástrico (ICG) per capita más alta del mundo y actualmente es la segunda principal causa de muerte por cáncer.

Estimated age-standardised mortality rate per 100,000
Stomach: both sexes, all ages



< 3.2 < 4.9 < 6.8 < 10.5 < 26.5

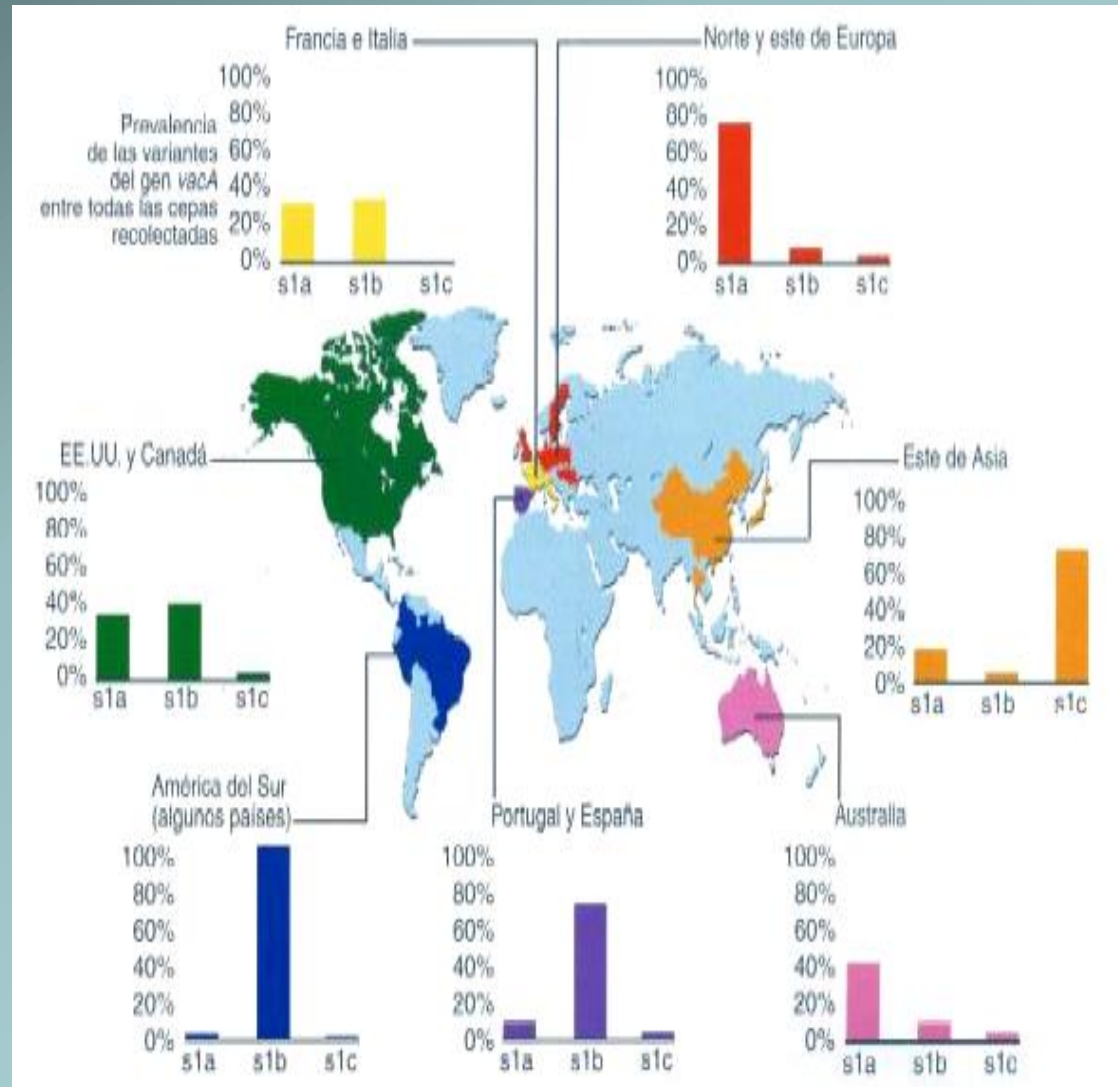
Epidemiology of gastric cancer
Incidence rate /year per 100.000 inhabitants

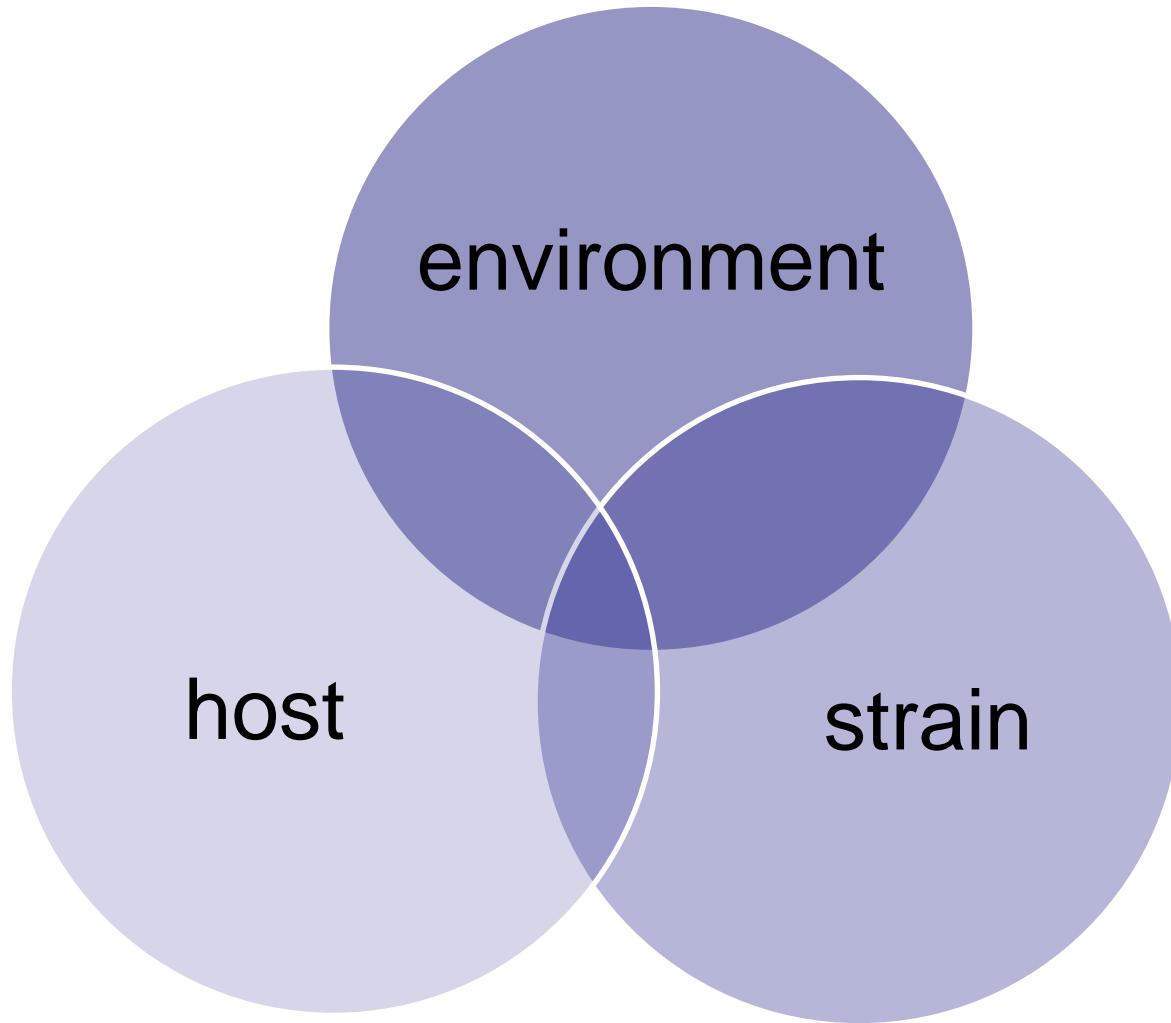


Ferlay et al., IARC Globcan 2008

Prevalencia de la infección por *H. pylori* y su distribución geográfica mundial.

Lo que implica que en la colonización por *H. pylori* en el mundo convergen varios factores que determinan, además de la presencia el origen natural, condiciones ambientales y condiciones socioeconómicas que podrían intervenir en la severidad de una infección para una población dada.





environment

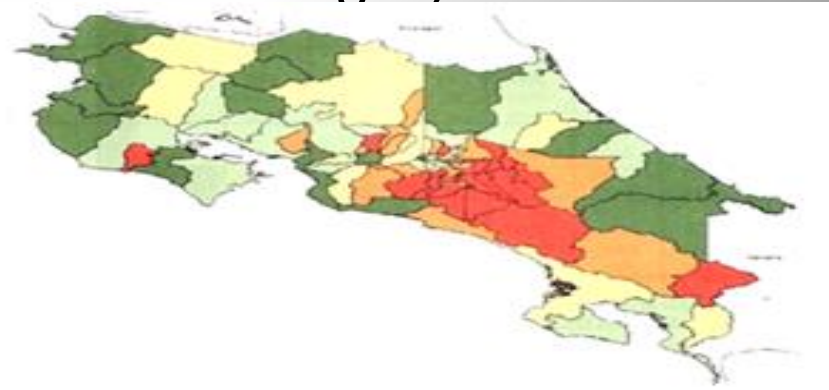
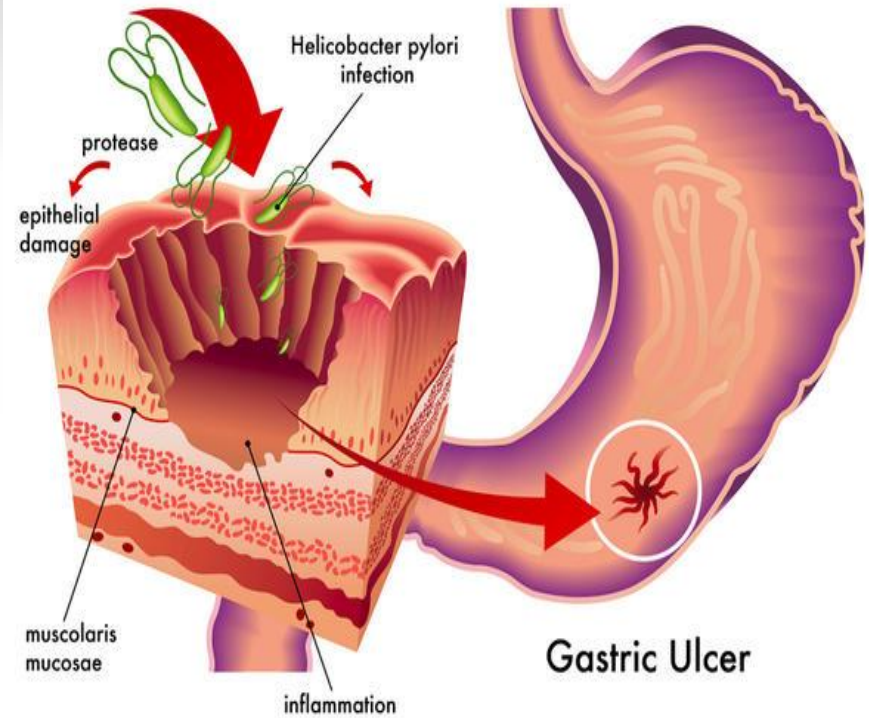
host

strain

En Costa Rica:

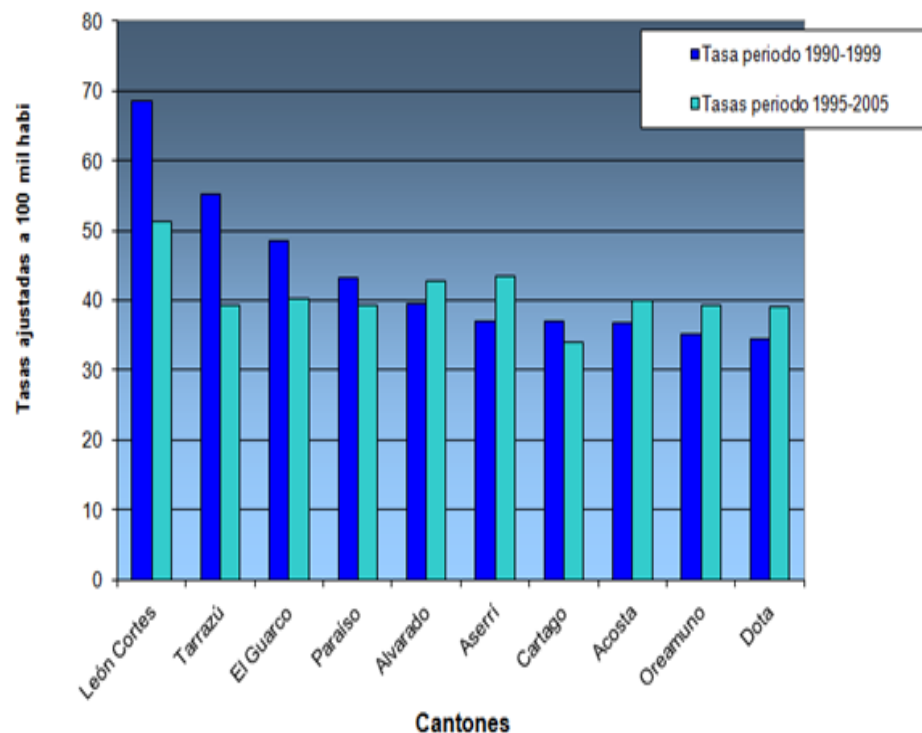
Se le relaciona con gastritis crónica, ulcera duodenal y gastritis atrófica con probabilidades de transformarse en cáncer gástrico especialmente en adenocarcinoma de tipo intestinal.

- Con mayor incidencia en las personas si viven en la parte central en las provincias de Cartago y San José.

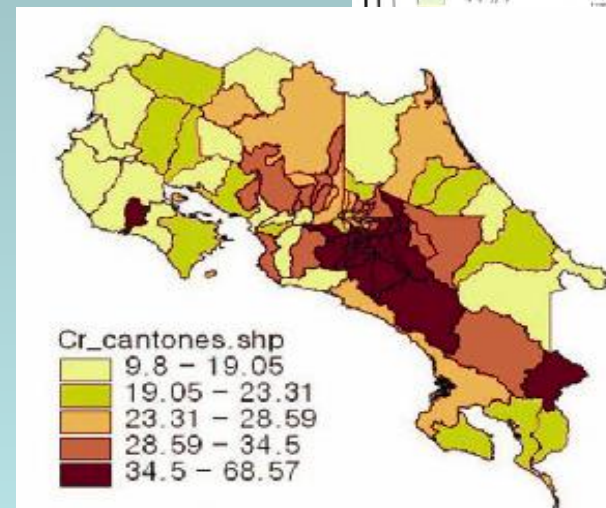
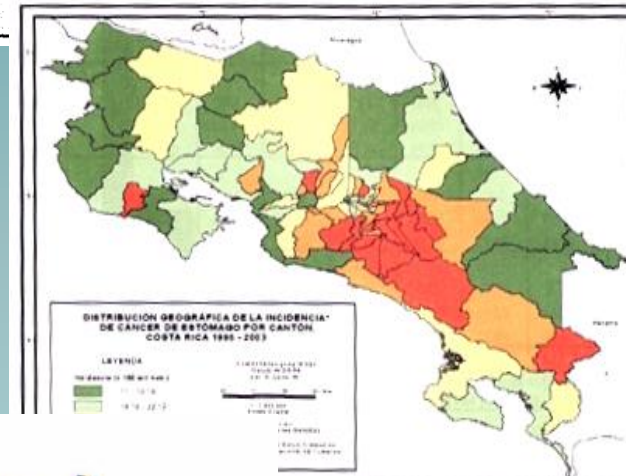
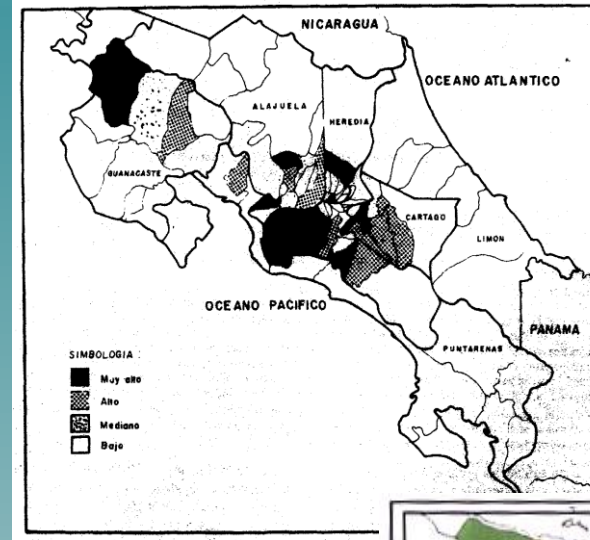


Tasa de los cantones marco del estudio correspondientes al quintil 1 de alta incidencia de cáncer gástrico para los periodos 1999 y 2005.

Cantón	Tasas periodo 1990-1999	Quintil	Tasas periodo 1995-2005	Quintil
León Cortes	68,57	1	51,4	1
Tarrazú	55,12	1	39,32	1
El Guarco	48,48	1	40,3	1
Paraíso	43,12	1	39,42	1
Alvarado	39,59	1	42,77	1
Aserri	36,97	1	43,46	1
Cartago	36,91	1	33,92	2
Acosta	36,68	1	39,95	1
Oreamuno	35,22	1	39,23	1
Dota	34,58	1	39,14	1



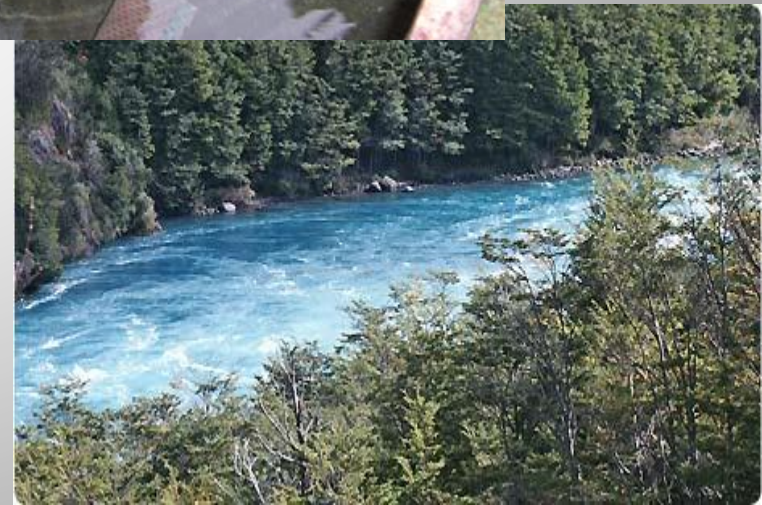
Desde los inicios de la investigación del cáncer de estómago en Costa en la década de 1980, se ha mantenido la misma tendencia de distribución geográfica con respecto a alta y baja incidencia.



Currently, some researches worldwide have quantified the presence of *H. pylori* naturally in the environment, with diverse water sources, being important reservoirs, such as rivers, lakes, drinking water, municipal and waste water among others.

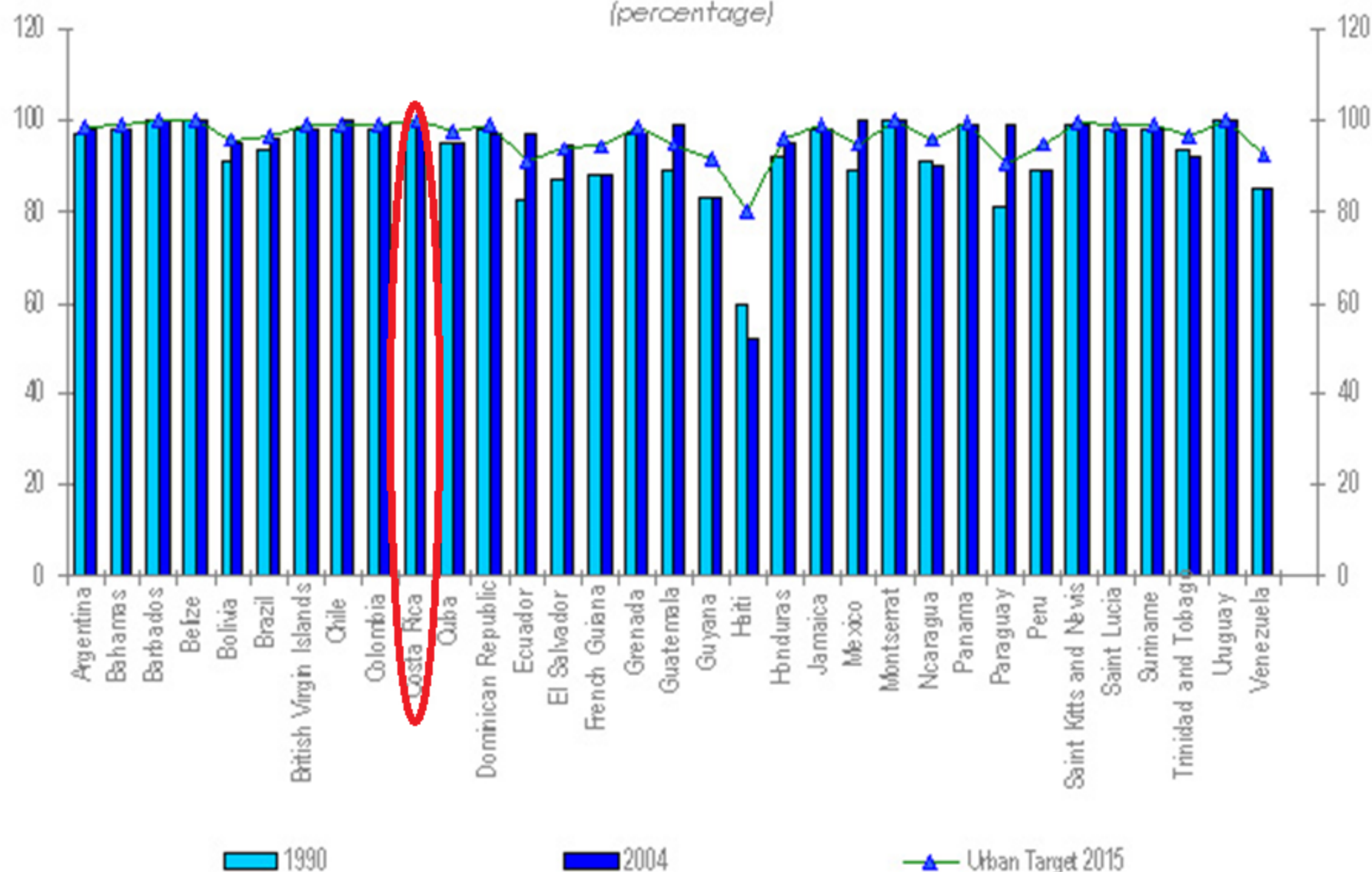
Besides, Santiago et al, have also been recently found in water with residual chlorine intended for human consumption in Spain.

- Quantification has been carried out by means of the Real Time PCR.



Proportion of urban population using an improved drinking water source

Latin American and the Caribbean countries, 1990, 2004
(percentage)



Recently Pérez et al, 2015 determined the presence of *H. pylori* in soil samples from public playing areas of Spanish parks, with contamination levels ranging from 10 000 cells/g to 10 000 000 cells/g.



Necesidad fisiológica de cationes

(Fe^{2+} , Ni^{3+} , Zn^{2+}).

- El hierro tiene dos o mas sistemas de transporte indicando su importancia fisiológica.
- Tiene una alta demanda de níquel como cofactor para su enzima ureasa.
- El zinc, el cual está involucrado en proteólisis de células del huésped y de esta forma contribuye a la severidad de la patología gástrica.

Metodología

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.

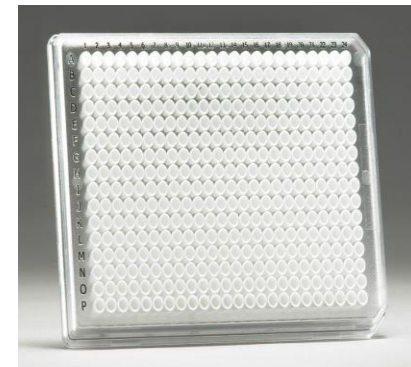
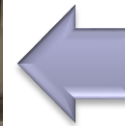
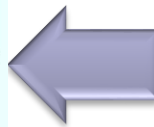
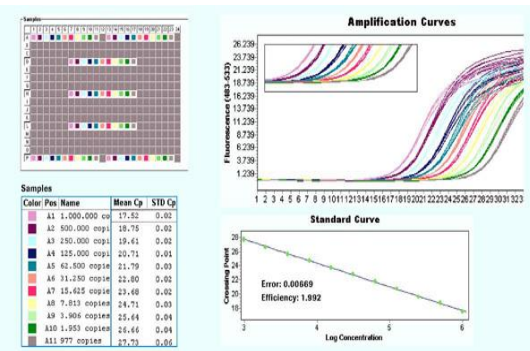
Following, the filter membrane was removed and placed in a tube with beads used for mechanical removal of the microorganisms, after, for the DNA extraction; the steps of the technique were followed as established by the manufacturer.

Metodología

The sampling strategy in Costa Rica included sampling 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).

Metodología

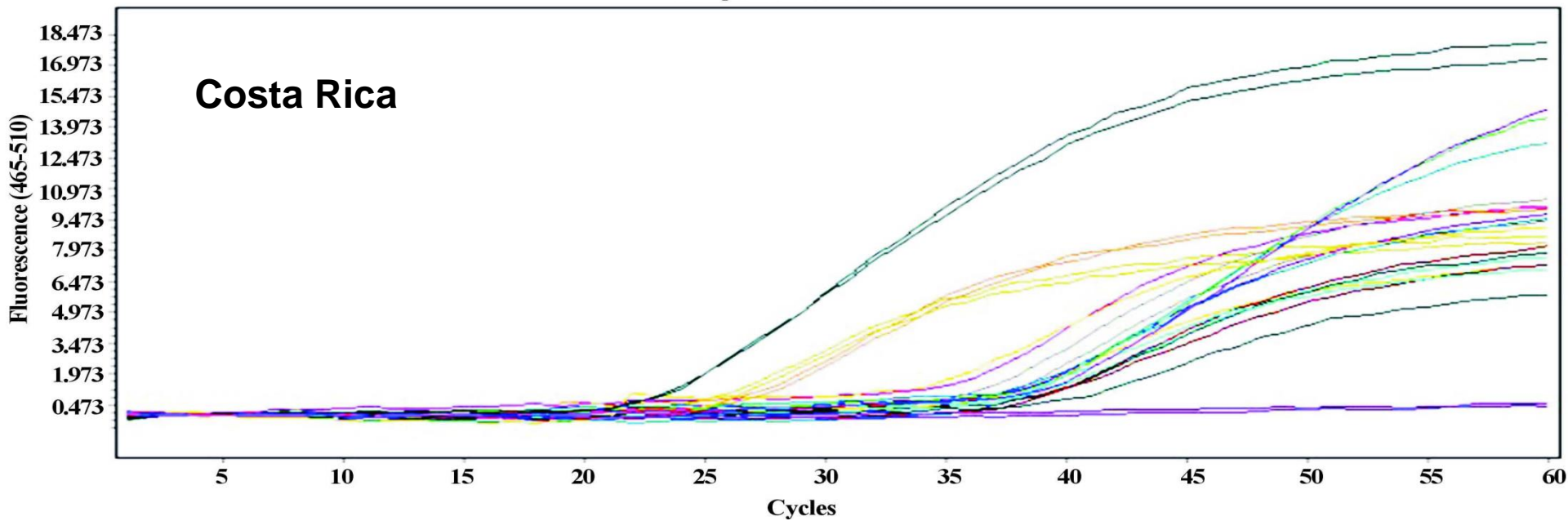


The molecular marker of *H. pylori* glmM was used, and to optimize the qPCR technique, annealing temperature, concentration of primers and probe were standardized

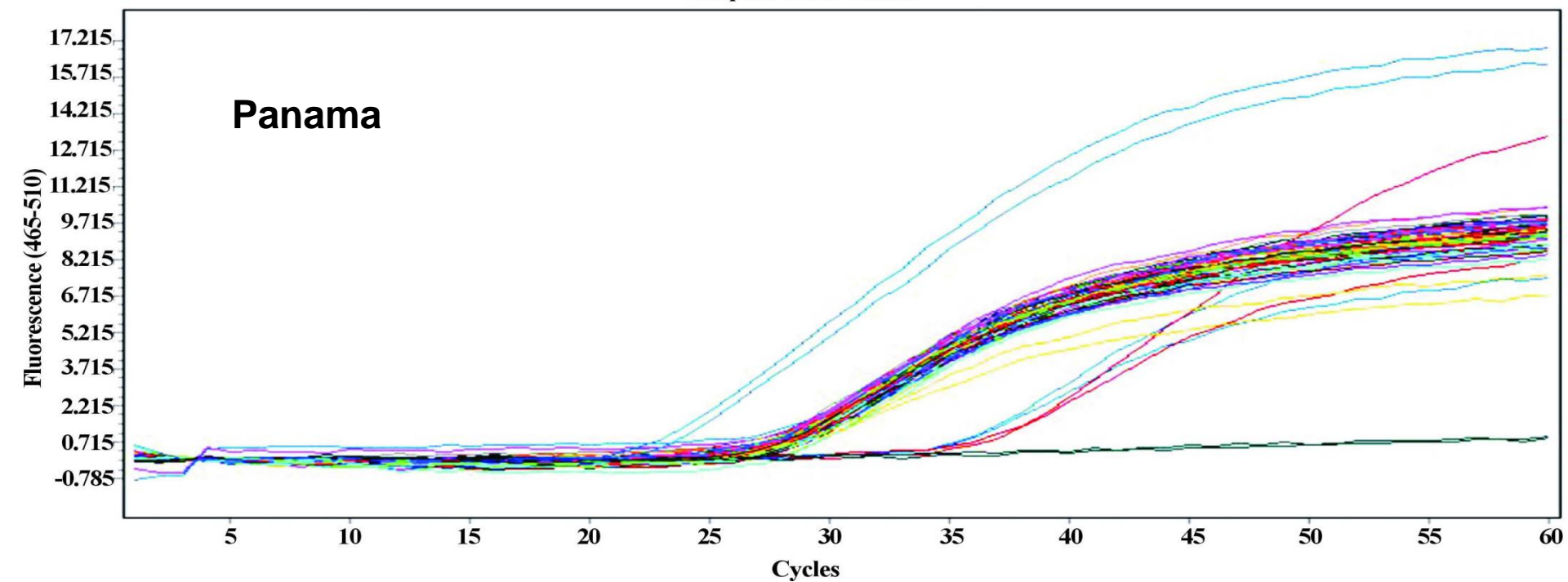
Values of the optimization of qPCR technical processes

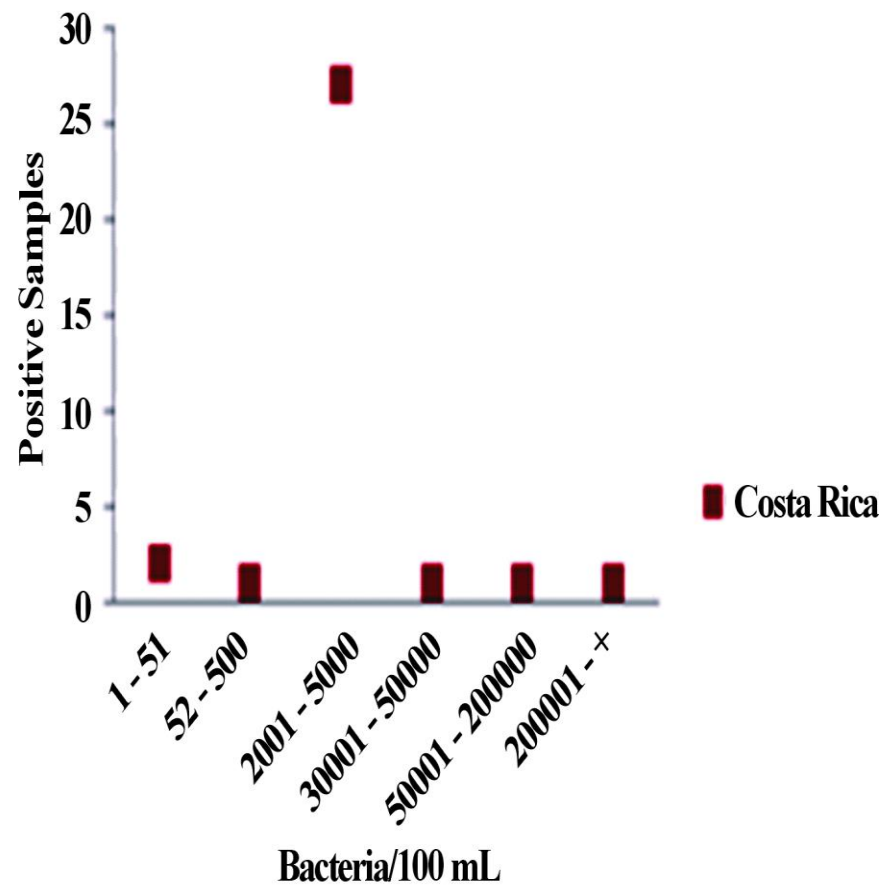
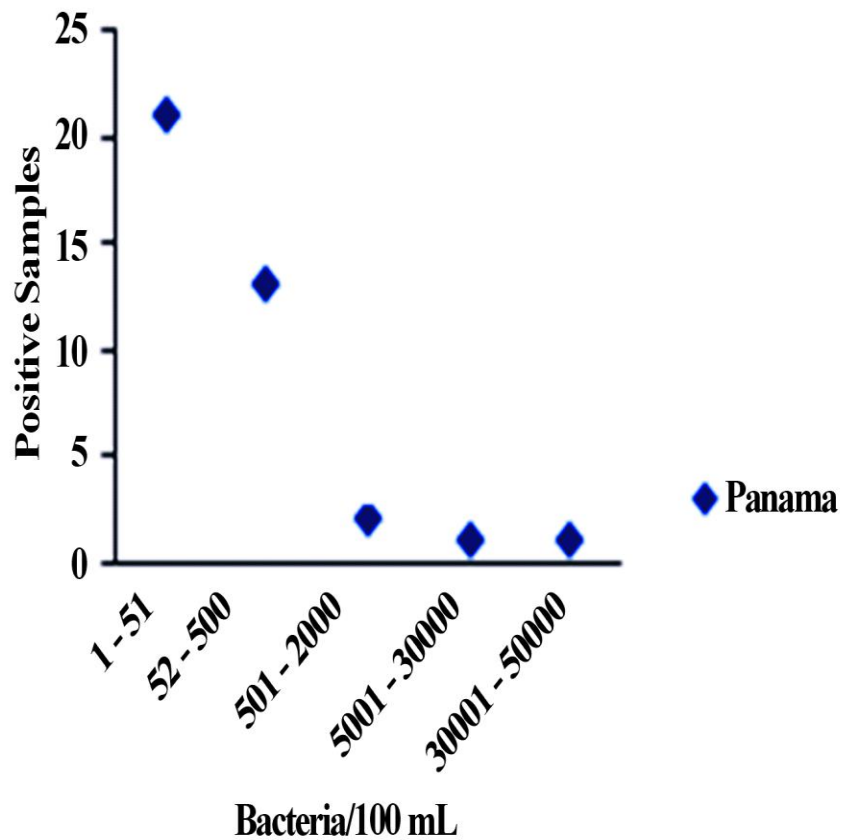
Parameter	Optimum value	Error	Efficiency	Slope	R ²
Annealing temperature for primers	52°C	0.0094	1.963	-3.415	0.9914
glmMRv primer concentration	1µM	0.0374	1.962	-3.416	0.9944
glmMFw primer concentration	1µM	0.0374	1.962	-3.416	0.9944
Probe concentration	1µM	0.0094	1.963	-3.415	0.9914

Amplification Curves



Amplification Curves





Discusión

- En una investigación anterior se determinó diferencias significativas entre el manejo del agua de lugares de alta y baja incidencia de cáncer gástrico en Costa Rica.

Characteristics	Counties with higher incidence GC (39%)	Counties with lower incidence GC (7.5%)
Access to water	High	Low
Service Provider	Municipalities	National Entitie
Source of water	100% Groundwater	80% well water 20% Surface water
Ambient temperature	Low	High

		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

Coeficiente de Correlación de Pearson entre:

- Tasa de incidencia de cáncer gástrico y altitud: 0,867
- Tasa de incidencia de cáncer gástrico y temperatura

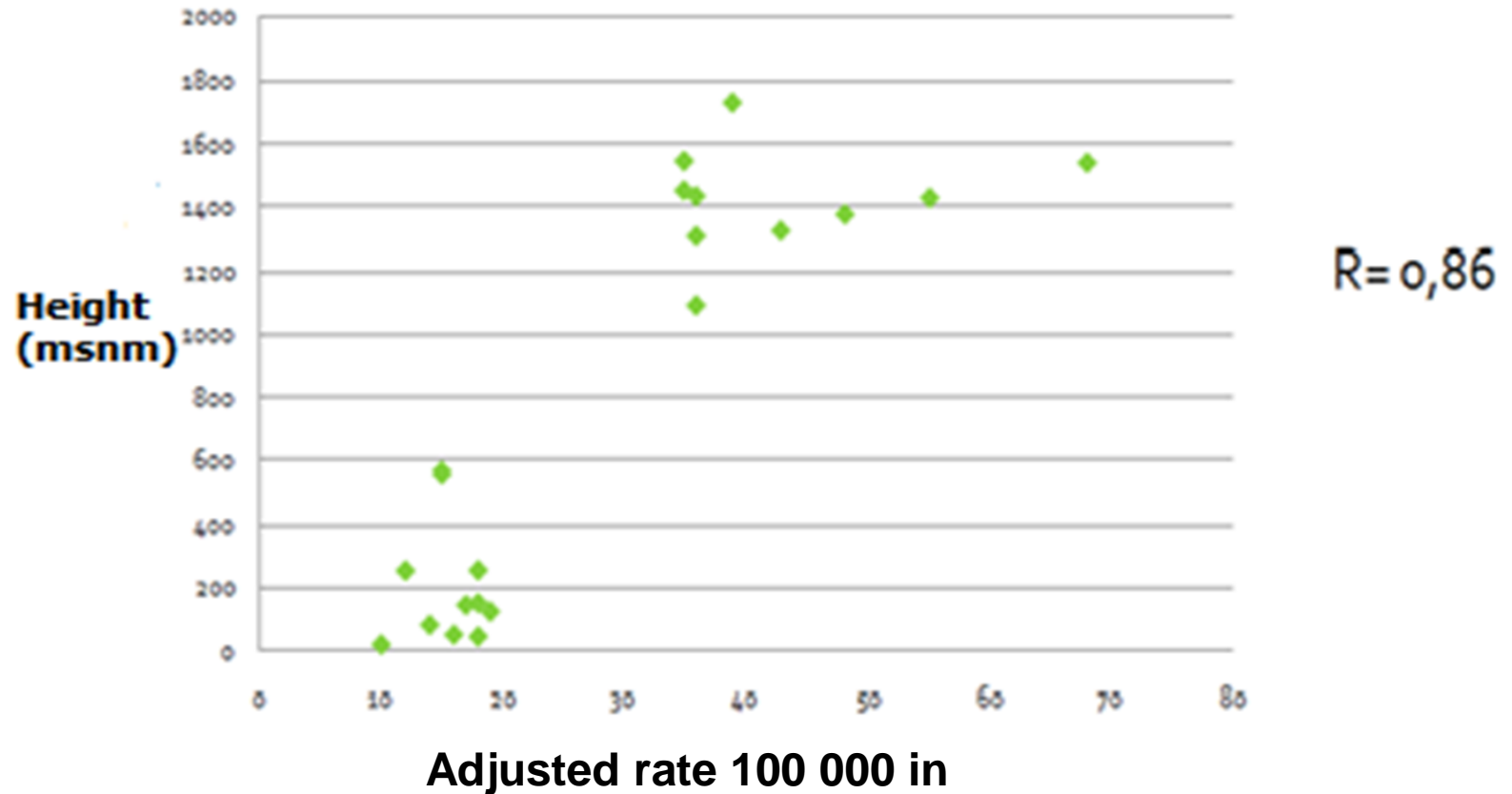
ambiental: - 0,853

- Tasa de incidencia de cáncer gástrico y origen del agua como naciente: 0,651.

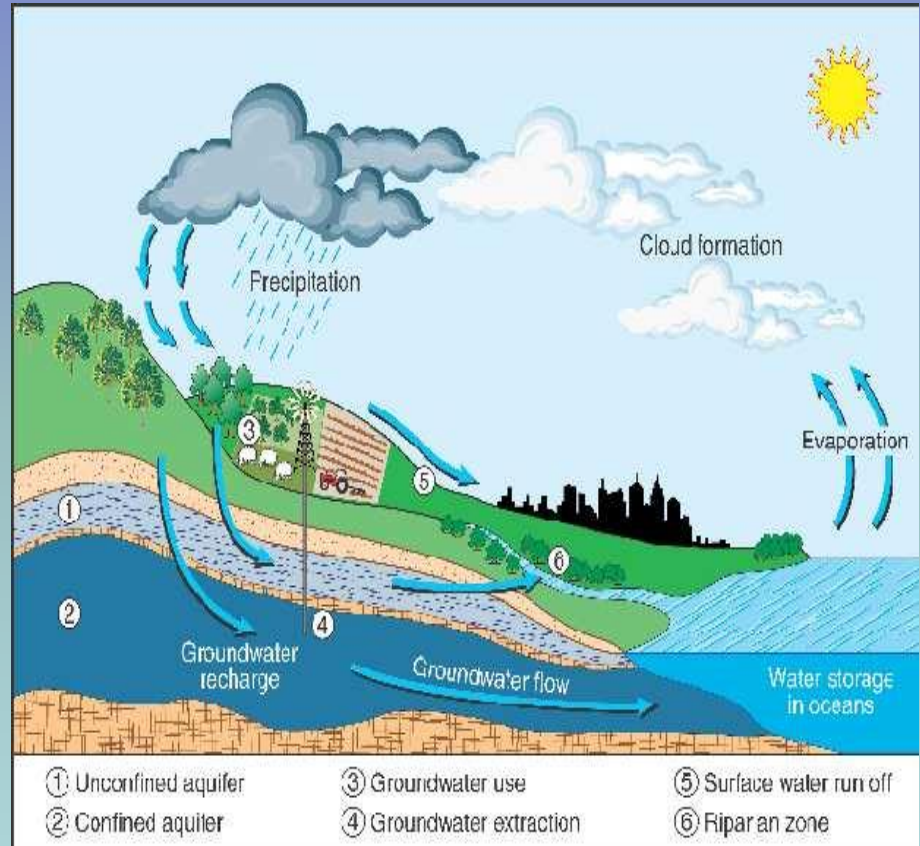
Coeficiente de Regresión Lineal entre incidencia y temperatura: $R^2 = 0,728$.

En la prueba exacta de Fisher para evaluar la independencia entre tasa de incidencia de cáncer gástrico con Ente Operador del agua, se tiene un valor de $p < 0,05$, se concluye que existe relación entre las variables y que esta relación no se debe al azar con un 95% de confianza.

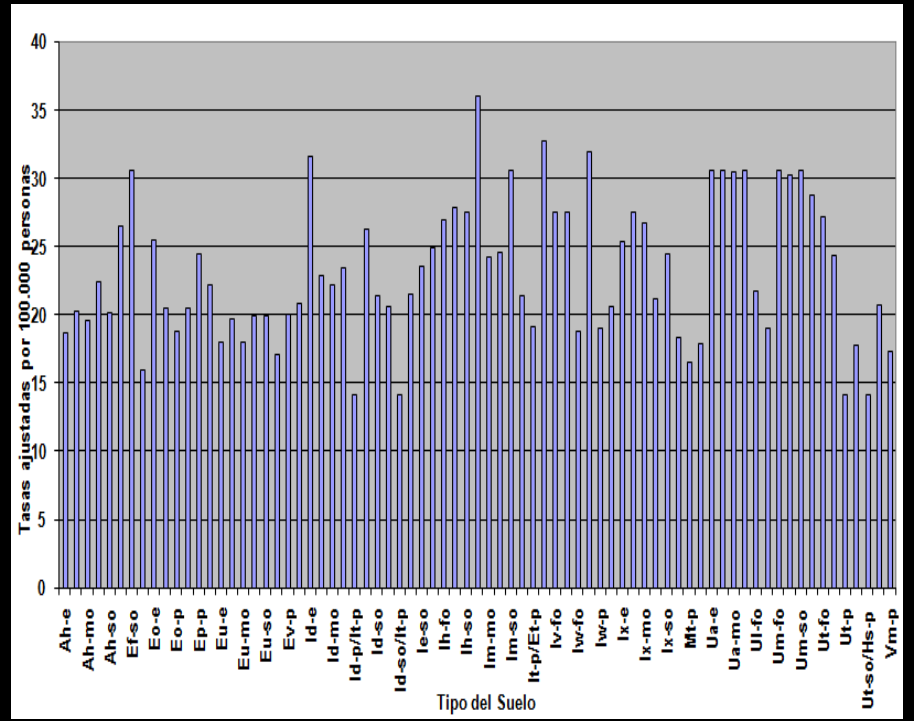
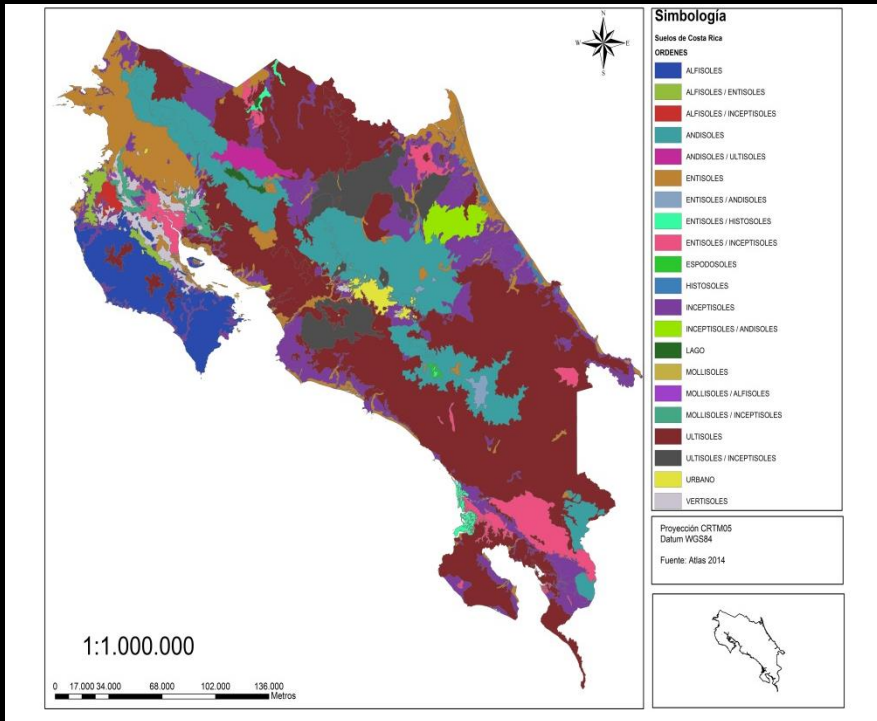
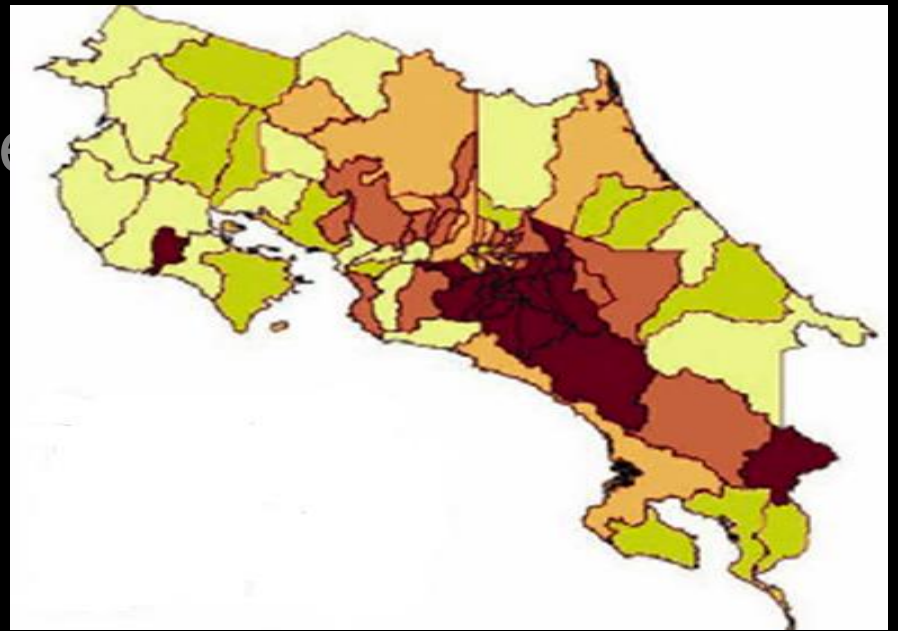
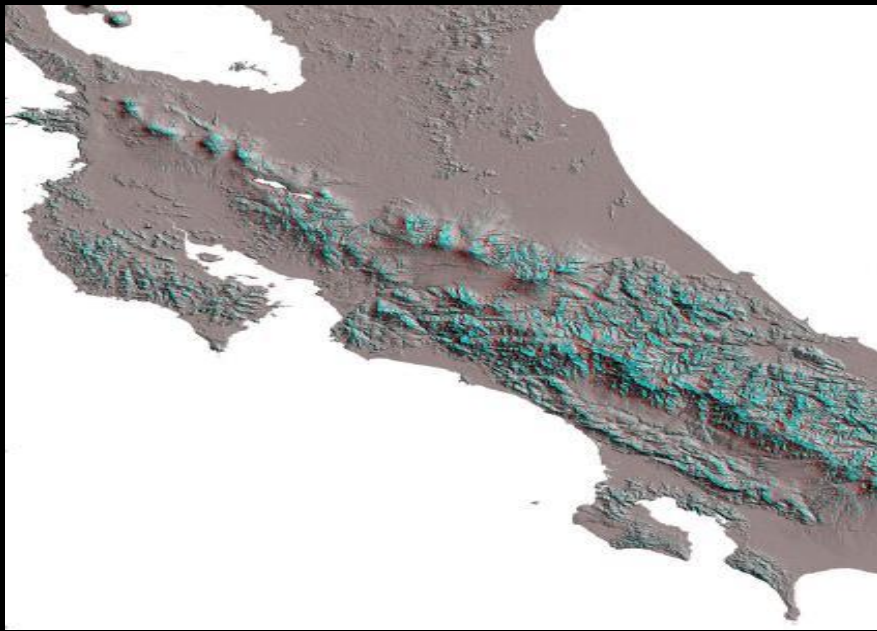
- Correlation between height and incidence rate of gastric cancer in Costa Rica.



- La influencia del suelo es mayor cuando el agua es dispensada a partir de nacientes que cuando es dispensada a partir de pozos profundos a una población específica



Soil condition	Area high incidence (H.I)	Area low incidence (L.I)
Geomofologic, (Denyer y Kussmaul 2000).	Dominated by volcanic material andesitic composition: SiO₂ (54,5%), Fe₂O₃ (3,2%), FeO (3,5%). Presence of rocks that contain higher percentages of iron. Weathering processes that act on the rocks can release higher concentrations of iron in the soil.	Dominated by formations of riolitic composition SiO₂ (70,7%), Fe₂O₃ (1,9%) FeO (0,3%)
Topography and elevation	The higher elevations and lower temperatures favor higher contents of organic matter in soil (MOS). This is availability of easily accessible carbon	Higher temperatures and a slightly undulating relief
Process of soil acidity	Dominated higher concentrations of (MOS), organic matter undergoes a natural process of mineralization that permanently acidified the soil (BERTSCH 1998), al the same time increases the solubility of iron in form Fe²⁺ and Fe³⁺ affected by the changes of the potential oxidation / reduction " in soil	Soils may exhibit greater average soil pH that the regions of high incidence. This implies reduced availability of iron (Barber 1984)



Ecuación de incidencia de cáncer gástrico en la población costarricense:

A + B + C + D + E + F – G => Dispara la evolución a la incidencia
C.G (adenocarcinoma intestinal), gastritis, úlceras

Donde:

A = factores asociados a la población (polimorfismos, Lewis B)

B = Presencia de cepas patógenas de *H pylori* en el ambiente (por los pacientes)

C = Condiciones socioeconómicas de la población (no tan marcado entre las zonas)

D = Acceso de *H. pylori* al agua de consumo

E= Manejo deficiente del agua en las zonas de alta incidencia

F= otro (s)

G = Factor protector

*en **rojo** los factores específicos que podrían aumentar la incidencia.

Conclusiones y recomendaciones

- o Although water in high gastric cancer incidence zones in Costa Rica is dispensed to the population by the Municipalities or ASADAS, 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.
- o Research should be conducted as to the causes of strong resistance of *H. pylori* to chlorination in the case of CR.
- o 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.

Conclusiones y recomendaciones

- Review of updated 2011 information from the Ministry of Health in Panama, shows overall adjusted rates between 9 and 10 cases per 100,000 inhabitants, similar to those of cantons with low gastric cancer incidence in Costa Rica.
- Regarding Costa Rica, gastric cancer high incidence data correspond to people living in the central part of the country, in the provinces of Cartago and San Jose. The last available statistical data from 2003 for men shows results of 31.87 cases per 100,000 inhabitants, and 17.42 cases per 100,000 inhabitants for women in the same year.

Conclusiones y recomendaciones

- o 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.
- o While non-chlorinated water from Chiriquí Panamá presents 3.3×10^2 copies of the bacteria per 100 mL, in Costa Rica chlorinated water shows 3.6×10^3 copies/100 mL on average.
- o 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

Conclusiones y recomendaciones

- With the recent determination and quantification of *H. pylori* in the soil by Pérez and colleagues, water treatment aiming at achieving elimination of apparent turbidity gains special relevance, as turbidity influences survival of the bacteria in the water and its resistance to chlorination.

Conclusiones y recomendaciones

- Consequently, for the sake of public health, special relevance is given to treating and dispensing drinking water containing no less than 0.6 mg/L to 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.

Conclusiones y recomendaciones

- o Further studies to determine augmented resistance to strains of *H. pylori* are deemed to be of immediate importance, given the amounts found in treated waters in Costa Rica.
- o The possibility of modifying the Regulations for Drinking Water Quality must also be explored, allowing for greater residual chlorine if necessary, especially in treated water coming from superficial water systems, as it may possess higher contents of bacteria as well as higher contents of soluble organic matter.
- o Quantitative analyses of the bacterium in water systems intended for the population can be implemented, as well as avoiding dispensation of water with turbidity over 5 NTU.

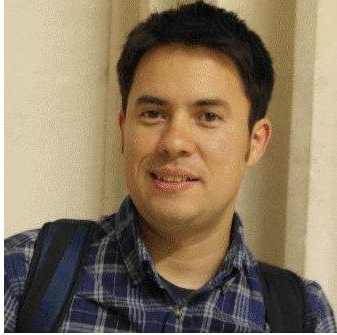
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Special thanks to

- Instituto Tecnológico de Costa Rica.
- Centro de Investigación y de Servicios Químicos y Microbiológicos- CEQIATEC – y al Centro de Investigación en Biotecnología- CIB
- A las 20 Municipalidades que abrieron sus puertas.

- Laboratorio Nacional de Aguas (AyA).
- Universidad de Murcia, España. Facultad de Microbiología y Genética.
- Laboratorio de Bacteriología, Facultad de Microbiología de la UCR. Dr García
- Laboratorio Biotécnica de Kenneth Madriz





Tamarindo Beach, CR

非常感謝您的關注

Thank you !