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Science Letters:

Quantification of *Helicobacter pylori* levels in soil samples from public playgrounds in Spain*

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Abstract: *Helicobacter pylori* are ubiquitous Gram-negative bacteria with a high estimated level of infection in the world populations, but a majority of the infected persons are asymptomatic. This pathogen has been classified by the World Health Organization as a class I carcinogen and recognized as the causal agent of most peptic ulcers and chronic gastritis that might lead to stomach cancer. Although not all the transmission pathways of these bacteria into humans have been properly identified, enough data have suggested that the *oral-oral* or *fecal-oral* ones are the main infection routes. *Helicobacter pylori* have been detected in non-treated water and in drinking water, which suggested that water might be an important infection source. As childhood is the critical period of infection, the aim of the present work was to examine the presence of *Helicobacter pylori* in soil samples from public playing areas of Spanish parks.

Key words: *Helicobacter pylori*, Real time polymerase chain reaction (qPCR), Environmental soil samples
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1 Introduction

Helicobacter pylori are ubiquitous Gram-negative bacteria. It has been estimated that around two-thirds of the world populations are infected, though a majority of people are asymptomatic. This pathogenic microorganism is the cause of most peptic ulcers as well as chronic gastritis, and may also be associated with gastric malignancy. It has been classified by the World Health Organization as a class I carcinogen (van Duynhoven and de Jonge, 2001). The transmission pathways of these bacteria into humans have not all been properly identified, but available data suggest that the *oral-oral* and *fecal-oral* routes are the main ones (Moreira *et al*., 2004). The *fecal-oral* route is related to water-borne diseases and for this reason Hulten *et al*. (1996) suggested that polluted water might be an infection pathway. Also, it is well known that *Helicobacter pylori* is present in non-treated water (Nayak and Rose, 2007) and it has been found in drinking water (Baker and Hegarty, 2001). All this data suggest that water might be an important infection source. Moreover, this pathogen has been detected in food, though there is still no direct evidence that food is involved in transmission (van Duynhoven and de Jonge, 2001). Childhood is the critical period of infection (Moreira *et al*., 2004), and though this might arise from the presence of this pathogen in the family environment, other infection sources should be considered. The aim of the present work is to examine

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the presence of *Helicobacter pylori* in soil samples from public playgrounds.

Taking into account that the detection of *Helicobacter* spp. by growth in a culture media is very difficult, soil samples from different parks in northeastern (NE) Spain were analyzed to find *Helicobacter pylori* DNA by quantitative polymerase chain reaction (qPCR).

2 Materials and methods

2.1 Sample selection

During the course of several weeks, 39 different public parks in urban areas from cities of Barcelona, i.e., NE-Spain (Mataró, L'Hospitalet de Llobregat, Barcelona, Sabadell, and Terrassa), were examined. In some cases, different playgrounds in the same park were sampled. A total of 78 samples of 100 g of surface sand were taken. All playing areas offered ready access to people and animals (dogs, cats, and birds). The samples were collected aseptically in Pyrex bottles and maintained at 4 °C until they reached the laboratory. Subsequently, the samples were re-suspended in 100 ml of 1X phosphate buffered saline (PBS) (pH 7.2) and sonicated for 3 min using an ultrasound bath (Selecta, Barcelona, Spain).

2.2 Real-time PCR quantification

A 2 ml sample was concentrated by centrifugation (5 min, 8000×*g*) to a final volume of 200 μl. DNA extraction and purification were performed using the EZNA® Soil DNA kit (Omega Bio-Tek, Doraville, USA) following the manufacturer's recommendations. The primers and probe used were those described by Kobayashi *et al*. (2002), further adapted to our equipment and reagents. Nine microliters of DNA was analyzed for *Helicobacter pylori* using a LightCycler 1.5 (Roche, Mannheim, Germany) and QuantiTect probe PCR kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

To quantify the soil samples, the turbidity of a cellular suspension from a positive control of *Helicobacter pylori* (corresponding to a clinical sample isolate) was optically adjusted to 0.2 absorbance units at 600 nm, which correlates to approximately 10^8 colony-forming units (UFC)/ml. Serial logarithmic dilutions were then carried out from 10^1 to 10^5 and used as standards. The efficiency of amplification was 2.008±0.0191 according to the estimation by means of the slope calculation method from a calibration dilution curve (Rasmussen, 2001) and the limit of quantification (LOQ) was estimated in 100 cells/g. Once the concentration per ml was predicted using the standard curve, the concentration of the sample by gram of soil was calculated.

3 Results

The detection of *Helicobacter pylori* by qPCR threw out 7 positive samples from a total of 78 (9%). Samples were considered as negative for qPCR if no signal was detected during the assay. These 7 positive samples were coming from different parks, except 2 that came from the same park but different playgrounds. The contamination levels ranged from 10^4 to 10^7 cells/g.

4 Discussion

As far as we known this is the first survey that demonstrates the presence of this pathogen and its pollution level in soil samples from playgrounds. Nevertheless, it still remains necessary to clarify the likelihood of infection by *Helicobacter pylori* through contact with infected soil, and the relative importance of this potential infection pathway compared to those currently recognized and accepted.

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