Droplet Digital PCR Detects Low-Density Infection in a Significant Proportion of *Helicobacter Pylori*-Negative Gastric Biopsies of Dyspeptic Patients

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INTRODUCTION: **_Helicobacter pylori_-infected individuals may present low-density infection, undetectable by conventional tests such as histology, rapid urease test, or urea breath test. Droplet digital polymerase chain reaction (ddPCR) is more sensitive than other polymerase chain reaction methods. We aimed to evaluate the ability of ddPCR to detect *H. pylori* infection in patients diagnosed as negative by conventional tests.**

METHODS: **Dyspeptic patients (n = 236) were tested for *H. pylori* by histology, urea breath test, and rapid urease test. Patients were classified as having 3 positive (n = 25, control group), 2 positive (n = 12), one positive (n = 41), or zero positive (n = 158) diagnostic tests. DNA was extracted from gastric biopsies. Triplicate ddPCR testing for each of the 16S rDNA, ureA, and vacA(s) genes was performed using a QX200 ddPCR system (Bio-Rad). A gene was considered positive when detected by at least 2 of 3 repeated ddPCRs. *H. pylori* positivity was defined as having 2 or more positive genes.**

RESULTS: **All the biopsies of the control patients were positive for all 3 16S rDNA, ureA, and vacA(s) genes. *H. pylori* infection was detected in 57 (36%), 22 (54%), and 9 (75%) patients with zero, 1, and 2 positive diagnostic tests, respectively. The density of infection was 5, 121, 599, and 3,133 copies of *H. pylori* genome equivalents for patients with zero, 1, and 2 of 3 positive test results and for the control group, respectively.**

DISCUSSION: **ddPCR detected low-density "occult" *H. pylori* infection in a significant proportion (36%) of patients diagnosed as negative by conventional methods. The number of conventional positive tests was related to the density of infection.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A290

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INTRODUCTION

H. pylori infection is associated with dyspeptic symptoms, peptic ulcer, and gastric cancer. Infection is present in more than half of the human population (1). Its mortality is high, mostly related to gastric cancer; in fact, it is responsible for 783,000 deaths each year and is the third cause of cancer death worldwide (2). Adequate diagnosis and treatment of the infection are curative for peptic ulcer and may prevent the development of gastric cancer (3).

Several conventional methods are available for the detection of active *H. pylori* infection. The main noninvasive tests are the urea breath test and the stool *H. pylori* antigen test (4). Invasive tests require endoscopy to obtain tissue biopsies and include rapid urease test, culture, and histological examination (5,6). Among all the available tests, urea breath test and histology are considered the most accurate. The efficacy of all these tests is limited by situations or treatments that decrease bacterial density, such as upper gastrointestinal bleeding or treatment with antibiotics or proton pump inhibitors. Invasive tests are also limited by the possibility of sampling error because of the irregular distribution of bacteria throughout the stomach (7,8).

Many previous studies suggest that subclinical infection with *H. pylori* (i.e., when infection density is below the detection threshold of conventional diagnostic tests [DT]) may be a frequent event. One of the first reports of "occult" infection dates from 2006 when, in a study aimed to describe the bacterial gastric diversity of 23 healthy individuals, Bik et al. found that *H. pylori* was detectable

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by molecular methods in 7 of 11 patients identified as negative by conventional tests (9). Currently, low-density infection has been described in patients on antisecretory or antibiotic treatment (10,11), in patients with extensive atrophy of the gastric mucosa and/or intestinal metaplasia (12), in gastric MALT lymphoma (13,14), and in peptic ulcer bleeding (15).

Having accurate DT is fundamental to ensuring quality of care by avoiding unnecessary treatments and/or the underuse of effective therapies. For infectious diseases, molecular DT may help improve the quality of results provided by monoclonal tests, microbiology culture, or histology. Polymerase chain reaction (PCR), for instance, has a sensitivity and specificity close to 100% for the detection of *H. pylori* in patients with chronic gastritis or nonpeptic ulcer bleeding (16,17). Moreover, previous studies suggest that PCR is capable of detecting low-density infection in a significant number of patients with dyspepsia compared with conventional techniques (18,19). More recently, a study of histologically *H. pylori*-negative gastritis showed that 49% of patients with chronic mucosal inflammation were positive by PCR (20). Finally, PCR was able to detect active infection even in a proportion of healthy individuals diagnosed as negative for *H. pylori* by conventional tests (21,22).

Furthermore, highly sensitive molecular techniques can help detecting *H. pylori* in clinical settings such as peptic ulcer bleeding, gastric cancer, or MALT lymphoma in which diagnosis of *H. pylori* is important but difficult to achieve. In this context, we demonstrated that real-time PCR improves the detection of *H. pylori* in paraffin samples obtained during an episode of upper gastrointestinal bleeding due to peptic ulcer (23). In addition, there seems to be an association between the density of infection and the virulence of the strain, with virulent strains having the lowest density (24). This latter finding also suggests that low-density infection may be related to more virulent strains and so may not be innocuous.

The latest commercially available refinement in PCR technology is digital PCR (dPCR) (25). dPCR is a quantitative technology that shows increased sensitivity when compared with conventional or real-time PCR methods while maintaining specificity (26,27). dPCR has proved to be useful for the detection of infectious agents in a variety of sample types and, specifically, in the detection and genotyping of resistance genes in *H. pylori* infection (28–30) and to determine the role of CYP219 polymorphisms in triple therapy efficacy (31).

Droplet digital PCR (ddPCR) is a method for performing dPCR that is based on the generation of water-oil emulsion droplets. In ddPCR, the PCR reaction of an individual sample is fractionated into thousands of droplets (20,000) and subjected to end point PCR. Amplification of the template molecules occurs in each individual droplet, and positive droplets are counted using a fluorescence detector.

In this study, our aim was to evaluate the presence of low-density *H. pylori* infection in dyspeptic patients diagnosed as negative by rapid urease test, urea breath test, and/or histology using highly sensitive ddPCR.

PATIENTS AND METHODS

Study subjects

Outpatients sent to the endoscopy unit of the Hospital Universitari Parc Taulí for dyspeptic symptoms from 2006 to 2014 were prospectively recruited for our study. Patients were contacted before the endoscopy. Those who agreed to participate in the study were instructed to avoid all antisecretory drugs including proton pump inhibitors in the 2 weeks before the endoscopy. Patients who were unable to stop antisecretory drugs, those who had received antibiotics in the 4 weeks before the endoscopy, and those with previous *H. pylori* treatment were excluded. Before the endoscopy, the patient provided signed informed consent, and urea breath test was performed. During endoscopy, 4 antral biopsies were obtained: 2 for histology, one for the urease test and culture, and one for molecular analysis. The study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Hospital Universitari Parc Taulí (Id. number 2015611).

Conventional DT

The DT (histology, rapid urease test, urea breath test, and ddPCR) were performed by technicians who were unaware of the results of the other assessments.

The urea breath test was performed with the UBiTest 100 mg (Otsuka Pharmaceutical Europe, Barcelona, Spain) in accordance with the manufacturer's specifications. A basal breath sample was collected by blowing into a specially designed bag. After this, patients took a pill of 100 mg of ^{13}C-labeled urea in 100 mL of water and filled a second breath bag 20 minutes later. The samples were immediately processed by nondispersive infrared spectrophotometry (POConeTM Infrared Spectrophotometer, Otsuka Pharmaceutical, Tokyo, Japan). In accordance with the manufacturer's specifications, an increase in the proportion $^{13}C/^{12}C$ ($^{13}CO_2$ (%)) of 2.5% or more after urea intake was considered as indicative of *H. pylori* infection.

Rapid urease test was performed after mucosal sampling using the Jatrox-HP test (CHR Heim Arzneimittel GmbH, Darmstadt, Germany) and was read according to the manufacturer's specifications.

For histology, biopsies were collected in formalin, stained with Giemsa, and then evaluated by 2 pathologists specialized in digestive disease. The pathologists were blinded to the results of the other DT.

H. pylori detection by ddPCR

DNA from the endoscopic biopsies was isolated with MasterPure DNA purification kit (Epicentre, Madison, WI). Isolated DNA was quantified with a QuantiFluor-P Fluorometer Quantus NGS (Promega Corporation, Madison, WI), and the concentration was adjusted to 50 ng/μL with TE Buffer Low EDTA Ultrapure, USB (USB Corporation Cleveland, OH).

TaqMan hydrolysis probe assays were used to amplify the fragments of the 16S ribosomal ribonucleic acid (rRNA) and ureA genes of *H. pylori*. The intercalating EvaGreen assay was used to amplify the vacA gene (s segment) of *H. pylori*. Primers and probes used to amplify the target genes are listed in Table 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A290.

The ddPCR conditions performed using the QX200 Droplet Digital PCR System (Bio-Rad, Pleasanton, CA) are summarized in the supplementary material file, http://links.lww.com/CTG/A290.

The results of ddPCR were generated using QX200 Droplet Reader (Bio-Rad) and analyzed using QuantaSoft software version 1.7.4.0917 (Bio-Rad). Each of the 3 16S rRNA, ureA, and vacA genes were amplified in triplicate by ddPCR. A ddPCR reaction was considered positive if one or more droplets were positive for EvaGreen reactions and/or TaqMan reactions.

To minimize false positive results, we set the restrictive criteria. A biopsy was considered positive for a given gene when at least 2 of the 3 replicates were positive, and *H.pylori* infection was considered present only if 2 or all of the 3 genes tested were positive ddPCRs.

Human β-actin amplification

The quality of the DNA isolated from the biopsy specimens was confirmed by an initial PCR. We used PCR on each DNA extract

to amplify a 83 bp fragment from the β-actin gene region (ACTB gene, Prime Time qPCR Assay, IDT, Coralville, IA).

Bacterial strains

To evaluate the specificity of the technique for vacA (s), ureA, and 16S rRNA, 11 bacterial species were used, obtained either from the clinical isolates of *Staphylococcus epidermidis*, *Corynebacterium sp*., *Proteus sp*., *Candida albicans,* and *Neisseria meningitidis* or from the American Type Culture Collection (ATCC) (Rockville, MD) of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae,* and *Haemophilus influenzae*. Strains J99 and 26695 of *H. pylori* were used as a positive control.

Data and statistical analysis

The mean number of *H. pylori* copies of genome equivalents were compared across all groups with a Kruskal-Wallis test, followed by Dunn post hoc tests. Statistical analyses were performed using GraphPad Prism 6 (La Jolla) and Microsoft Excel 2010 (Microsoft Corp). Significance was set at $P < 0.05$.

RESULTS

Study subjects

A total of 634 dyspeptic patients were initially recruited. Sixty-one patients with some DT not performed were excluded. Of the remaining 573 patients with 3 DT performed, 322 were negative for some or all tests. In this group, 111 patients lacking clinical data (n = 35) or insufficient biopsy sample (n = 34) were excluded as were patients diagnosed with intestinal metaplasia, lymphocytic gastritis, neuroendocrine tumor, or hyperplastic polyps (n = 42). Of the 251 patients with all positive DT, 25 were randomly selected as a positive control group.

Finally, 236 patients with dyspeptic symptoms who had valid results of 3 conventional DT were selected for the molecular analysis. Patients were classified as having 3 positive (n = 25, control group), 0 positive (n = 158), 1 positive (n = 41), or 2 positive results (n = 12) by conventional DT (Figure 1). Patient characteristics, including endoscopic and histological diagnoses, are shown in Table 1.

Validation of PCR assays

All the antral biopsy specimens from the 25 positive controls were positive on all 3 PCR assays. On the other hand, all samples were positive for human β-actin amplification, indicating that no PCR inhibitors were present.

Species specificity of the PCR assays

All non-*Helicobacter* species were negative for the 3 genes amplified (vacA, ureA, and 16S). The 2 strains of *H. pylori* were positive for all the 3 genes analyzed.

H. pylori detection frequency and copies of genome equivalents quantified by ddPCR

H. pylori infection was detected by ddPCR in 57 (36%) patients with zero positive DT, in 22 (54%) with one positive DT, and in 9 (75%) with two positive DT (Table 2). The distribution frequency of the amplified genes is shown in Table 2. Individual results of the triplicate ddPCR for each are shown in Figure 2 and Tables 2 and 3, Supplementary Digital Content 1, http://links.lww.com/CTG/A290. All the biopsies of the three positive DT control patients were positive for 16S rDNA, ureA, and vacA(s) genes.

The number of conventional positive tests was related to the density of the infection (Figure 3). The density of the infection in the three positive DT control group was $1{,}88 \times 10^3$, $1{,}11 \times 10^3$, and $6{,}40 \times 10^3$ copies of *H. pylori* genome equivalents for vacA(s), ureA, and 16S rRNA, respectively. The infection density then decreased progressively by 3 orders of magnitude down to ~10 copies in the zero positive DT group (Figure 3 and Table 4, Supplementary Digital Content 1, http://links.lww.com/CTG/A290). These differences in infection density were significant for each of the groups when compared with the zero positive DT samples.

Role of the histological findings for detecting "occult" *H. pylori* infection

Chronic active gastritis (mucosal neutrophil infiltration) was found in 24 of 25 patients with three positive DT (96%) and in only 4 of 158 patients with zero positive DT. As expected, chronic active gastritis increased proportionally with the number of positive DT: 9 of 41 patients with one positive DT

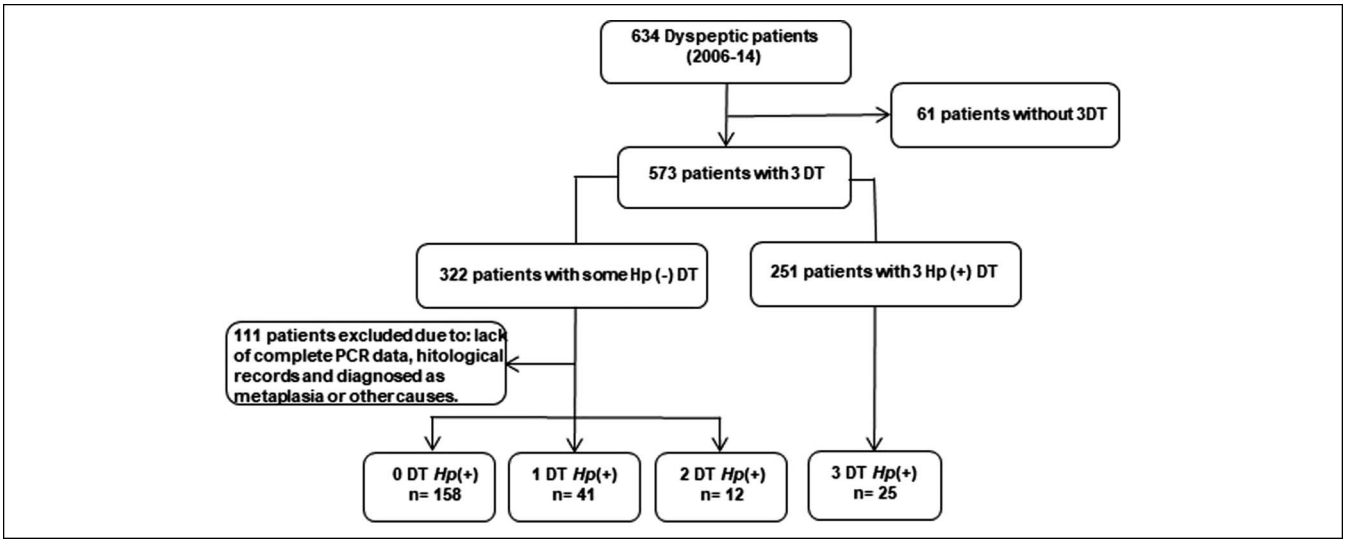

Figure 1. Study patients' flow diagram. DT, diagnostic tests.

Table 1. Patient characteristics

	0 DT *Hp*(+) (n = 158)	1 DT *Hp*(+) (n = 41)	2 DT *Hp*(+) (n = 12)	3 DT *Hp*(+) (n = 25)
Gender (male/female) (n)	55/103	17/24	3/9	14/11
Age (mean ± SD)	47 ± 14	49 ± 14	50 ± 18	47 ± 14
Endoscopic diagnosis (n):				
Normal	128	39	8	15
Duodenal ulcer/erosive duodenitis	21	2	2	6
Gastric ulcer	1	0	1	0
Esophagitis (LA A or B/LA C or D)	2/1	0	0	2/2
Other	5	0	1	0
Histological diagnosis (n):				
Gastritis (no/yes/Nd)	11/144/3	3/38/0	0/12/0	0/25/0
Gastritis activity (no/yes/Nd)	150/4/4	32/9/0	3/9/0	1/24/0
Gastritis severity (0/1/2/Nd)	12/141/2/3	3/35/3/0	2/7/3/0	0/15/10/0
Lymphoid follicle (no/yes/Nd)	120/29/9	24/17/0	4/6/2	8/15/2
Helicobacter pylori	0	4	8	25
Metaplasia/atrophy	0/1	0/3	0/0	0/0

DT, diagnostic tests; LA, Los Angeles esophagitis classification; Nd, no data; mild (A or B) or severe (C or D).

(22%) and 9 of 12 patients with two positive DT (75%) (Table 1). Neutrophil infiltrate was, however, not useful for diagnosing occult infection. Chronic active gastritis was present in only 2 of 57 patients with zero negative DT and *Hp* positive by ddPCR (4%) and in 2 of 101 patients with negative ddPCR (2%) (see Table 4, Supplementary Digital Content 1, http://links.lww.com/CTG/A290).

Among patients with positive ddPCR, lymphoid follicles were present in 11 (19%), 12 (55%), and 6 (67%) patients with zero, one, or two positive DT. Corresponding figures in patients negative by ddPCR were 18 (18%), 5 (26%), and 0, respectively (see Tables 5 and 6, Supplementary Digital Content 1, http://links.lww.com/CTG/A290).

Finally, *H. pylori* detection by ddPCR vs individual DT is summarized in Table 7, Supplementary Digital Content 1, http://links.lww.com/CTG/A290.

DISCUSSION

In this study, we found that ddPCR detects "occult" *H. pylori* in a significant proportion (36%) of dyspeptic patients diagnosed as negative by 3 conventional methods. The prevalence of infection in patients with 1 or 2 positive tests was even higher, at 49% and 75%, respectively. Thus, ddPCR seems to be more sensitive than conventional tests. In addition, because it is a quantitative method, we were able to quantify the density of the infection. Quantification indicated that *H. pylori* escapes conventional DT when the bacterial load is low; in fact, we found a clear linear correlation between the bacterial density and the number of positive conventional tests (Figure 2).

Several authors suggest that PCR-based diagnosis can be considered a gold standard, provided that specific *H. pylori* primers are used to target more than one conserved gene (32–34). In our case, to avoid false positive results, ddPCR positivity was determined on the amplification of 3 specific *H. pylori* genes, each of them in triplicate.

Table 2. Distribution frequency of the amplified genes by ddPCR

		ddPCR positive for *Hp* (n(%))		
No. of *Hp*(+) genes	*Hp* gene	0 DT *Hp*(+) (158 patients)	1 DT *Hp*(+) (41 patients)	2 DT *Hp*(+) (12 patients)
1	vacA (s)	54 (34%)	30 (73%)	9 (75%)
	ureA	76 (48%)	23 (56%)	9 (75%)
	16S	59 (37%)	22 (54%)	11 (92%)
2	vacA(s)-ureA	38 (24%)	20 (49%)	9 (75%)
	vacA(s)-16S	38 (24%)	20 (49%)	9 (75%)
	ureA-16S	47 (30%)	20 (49%)	9 (75%)
3	vacA-ureA-16S	33 (21%)	19 (46%)	9 (75%)
2 or 3	2 or 3 ddPCR *Hp* (+)	57 (36%)	22 (49%)	9 (75%)

ddPCR, droplet digital polymerase chain reaction; DT, diagnostic tests.

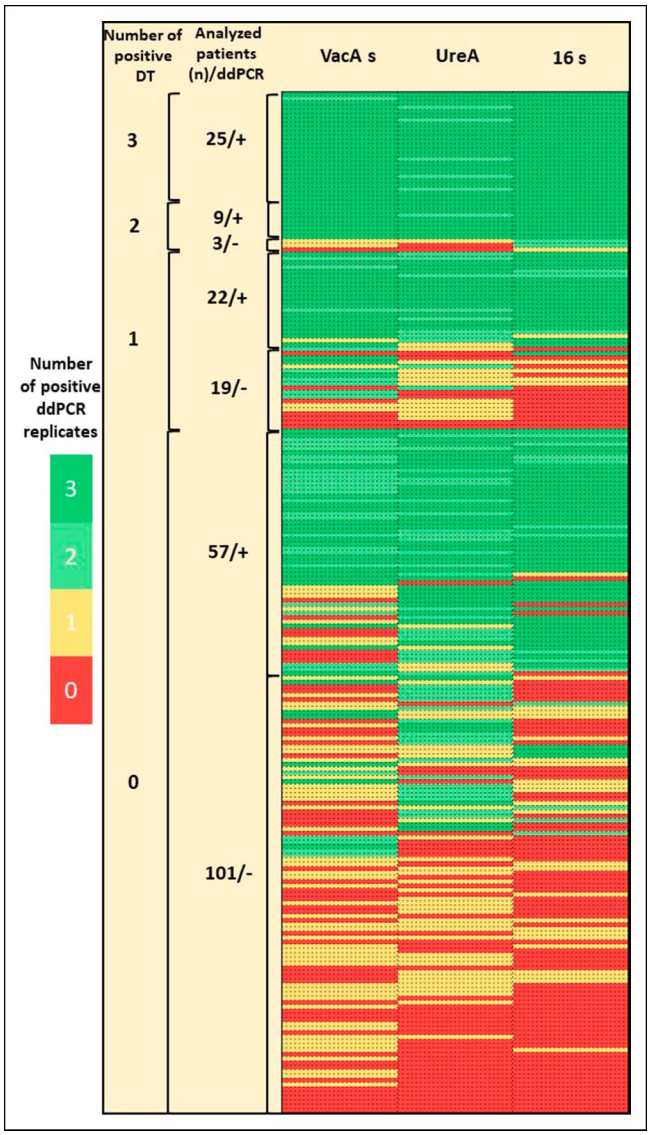

Figure 2. Individual results of triplicate ddPCR for each amplified gene. Each line in the figure represents a patient. Each gene amplification was performed in triplicate. Color was assigned to number of positive ddPCR replicates for *Helicobacter*: Dark green color (3 positive replicates), light green color (2 positive replicates), yellow color (one positive replicate), and red color (zero positive replicates). DT, diagnostic tests; ddPCR, droplet digital polymerase chain reaction.

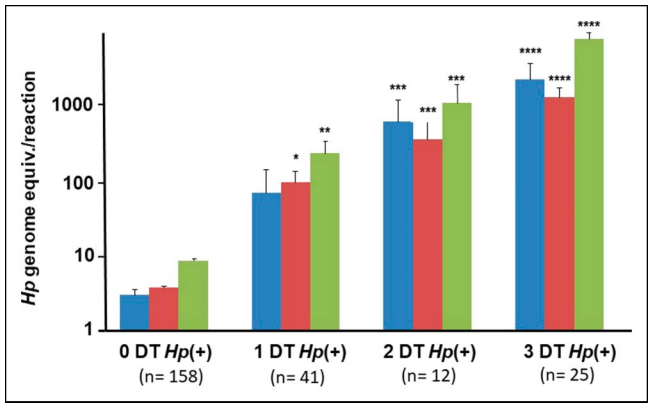

Figure 3. *H. pylori* copies of genome equivalents detected by ddPCR in dyspeptic patients. vacA s (blue), ureA (red), and 16S (green) bars. The results are expressed as means ± SEM: $*P < 0.05/**P < 0.01/***P < 0.005/****P < 0.001$ vs. 0 DT *Hp(+)*. DT, diagnostic tests; ddPCR, droplet digital polymerase chain reaction.

In addition, we selected primers that showed a very high specificity in previous publications (23,35). Therefore, our study suggests that ddPCR may be a useful alternative for detecting *H. pylori* infection, especially for patients with very low-degree, "occult" infection. In fact, the results suggest that persistent low-density infection may remain undetected in a proportion of patients submitted to endoscopy for dyspepsia. The potential causal role of the infection in these symptoms, however, remains to be determined.

Standard tests are suboptimal in certain clinical situations such as MALT lymphoma, peptic ulcer bleeding, or extensive atrophy. Several reports have indicated the presence of *H. pylori* at low levels in these clinical settings. For example, Raderer et al. (14) reported 6 patients with gastric MALT lymphoma with negative results for all *H. pylori* conventional tests who presented complete resolution of the disease after *H. pylori* treatment; the authors attributed this finding to "occult" infection. In addition, Guell et al. (15) reported that 79% of the patients with peptic ulcer bleeding who tested negative for *H. pylori* during the bleeding episode had active infection, which was only detected when the tests were repeated a few weeks after the episode. Our group previously reported that real-time PCR detects *H. pylori* in more than two-thirds of histology-negative samples from biopsies obtained during an episode of bleeding (23) and in 27% of histology-negative samples from dyspeptic patients with a "false positive" urea breath test (36). The prevalence and relevance of "occult" *H. pylori* infection in dyspeptic patients or in those with ulcer are currently unknown. It is possible, for example, that "occult" infection may account for a proportion of idiopathic peptic ulcers, an entity that seems to be increasingly frequent worldwide (37). It may be worth investigating the prevalence of "occult" infection in these cases. ddPCR examinations in the long-term studies after eradication treatment would also be of interest.

Apart from MALT lymphoma, peptic ulcer bleeding, and gastric atrophy, a growing body of evidence indicates that occult infection may pass undetected after one or more of the conventional tests in dyspeptic patients without these conditions, and even in healthy volunteers. In a study of the gastric microbiota of 23 healthy individuals, Bik et al. (21) analyzed *H. pylori* infection by rapid urease test, histology, culture, serology, and 16S rRNA sequencing and detected *H. pylori* infection in 12 individuals by conventional tests (52%) and in 19 of the 23 by molecular tests (83%). Kiss et al. (20) showed that approximately 50% of histological gastritis and *H. pylori*-negative are PCR positive. Some authors suggest that patients with chronic active gastritis in the absence of visible *H. pylori* should be considered as infected individuals in whom *H. pylori* has temporarily disappeared from the mucosa because of PPI or antibiotic use (38). This topic remains, however, unclear. Analyzing epidemiological data, the same authors also suggested that chronic active gastritis in the absence of *H. pylori* may be a distinct entity with an as yet unknown etiologic agent (39). As an alternative, occult *H. pylori* infection may help explain this situation. Conversely, even in the absence of active gastritis, *H. pylori* has been detected by immunohistochemistry (40) or histology (41). Our study reports similar findings using a far

more sensitive test such as ddPCR and adds support to the various reports in the literature suggesting that undetectable infection is not exceptional. For example, it is well-known that antisecretory drugs or antibiotics may reduce the density of the infection to levels undetectable by conventional tests; for this reason, it is recommended that these drugs should be stopped in the weeks before the DT are carried out (11). However, the use of PPIs does not seem to explain the occurrence of "occult" infection in this study. Patients were interviewed by telephone before inclusion in the study and instructed to avoid PPIs and antibiotics. On the day of endoscopy, patients were asked again about PPI and antibiotic use before the urea breath test and the endoscopy, and those who had not stopped the drugs were excluded.

A limitation of our study is that the molecular tests were performed on a single antral biopsy. It is well-known that *H. pylori* can show a patchy distribution in the stomach, and the more biopsies are performed, the higher the diagnostic accuracy (42). However, the current cost of ddPCR limited the number of biopsies that could be analyzed for each patient. Another limitation is that because the samples we analyzed were negative by conventional tests, we were unable to create a control group of patients who were unequivocally negative.

At present, we still do not know the relevance of the "occult" *H. pylori* infection. Conceivably, the low levels of infection may be an advantage for these patients, protecting them from disease by modulating the adjacent microbiome or the immune system. Furthermore, the absence of active gastritis may suggest that occult infection is associated with a lower risk of severe clinical manifestations. However, other data suggest that patients with occult *H. pylori* infection may present especially virulent or resistant strains different from those found in high-density infections (24). If so, it would be important to detect and treat this unnoticed occult infection to prevent transmission, peptic ulcer, and gastric cancer. Further studies are needed to characterize low-density infection and to determine the factors that modulate the bacterial load to prevent antibiotic resistances and new infections.

In conclusion, ddPCR detected low-density "occult" *H. pylori* infection in a significant proportion of dyspeptic patients diagnosed as negative by conventional methods. Digital PCR seems to be more sensitive than histology and may be a very useful tool for detecting *Helicobacter* in a subgroup of patients in whom the infection is not identified by conventional methods.

CONFLICTS OF INTEREST

Guarantor of the article: Xavier Calvet, MD.

Specific author contributions: María José Ramírez-Lázaro, PhD, and Sergio Lario, PhD, contributed equally to this work. X.C., M.J.R.L. and S.L. designed the study, performed the statistical analysis, evaluated the results and drafted the article. The remaining authors (M.E.Q., A.M., M.R.B., F.Q., L.G.M., A.C. and T.P.), collected data and critically reviewed the manuscript. All authors approved the final version of the manuscript.

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Potential competing interests: None to report.

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Study Highlights

WHAT IS KNOWN

- ✓ Several conventional methods are available for the detection of active *H. pylori* infection; the efficacy of all these tests, however, is limited by specific situations or treatments that decrease bacterial density.
- ✓ Subclinical infection with *H. pylori* may be a frequent event in dyspeptic patients, and low Helicobacter density may be associated with virulent strains.
- ✓ Highly sensitive molecular techniques can help to detect *H. pylori* in clinical settings in which diagnosis of *H. pylori* is important but difficult to achieve.
- ✓ ddPCR is a quantitative technology that shows increased sensitivity compared with conventional or real-time PCR methods while maintaining the levels of specificity.

WHAT IS NEW HERE

- ✓ We found that ddPCR detected low-density "occult" *H. pylori* infection in a significant proportion of dyspeptic patients diagnosed as negative by three conventional methods.
- ✓ Quantification indicated that *H. pylori* escapes conventional diagnostic tests when the bacterial load is low. We found a clear linear correlation between the bacterial density and the number of positive conventional tests.

TRANSLATIONAL IMPACT

- ✓ ddPCR may be a useful alternative for detecting *H. pylori* infection, especially for patients with very low-degree, "occult" infection.
- ✓ It is important to detect and treat this occult infection to prevent transmission, peptic ulcer, and gastric cancer.

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