Detection of "*Candidatus* Helicobacter suis" in Gastric Samples of Pigs by PCR: Comparison with Other Invasive Diagnostic Techniques

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Recently, a new 16S ribosomal DNA-based PCR assay was developed for the specific detection of "*Candidatus* Helicobacter suis" (former "*Gastrospirillum suis*") in porcine gastric samples. In the present study, this PCR assay was compared to three other invasive diagnostic methods (rapid urease test, immunohistochemistry, histologic analysis by Giemsa staining). Antral stomach samples from 200 slaughterhouse pigs from Belgium and The Netherlands were examined. Bacterial presence was determined in 77% (154 of 200) of the samples by PCR in combination with Southern blot hybridization, 56% (111 of 200) of the samples by immunohistochemistry, 61% (122 of 200) of the samples by urease testing (20 h postinoculation [p.i.]), 36% (71 of 200) of the samples by urease testing (3 h p.i.), and 33% (65 of 200) of the samples by Giemsa staining. The intrinsic specificity of the PCR assay was assessed by Southern blot analysis with an "*Candidatus* H. suis"-specific probe and sequencing of PCR products. Interassay sensitivity and specificity values were assessed for each test by pairwise comparisons between tests. Agreement between tests was evaluated by calculating Cohen's kappa coefficient. From that analysis, the PCR assay was considered the most reliable benchmark. Microscopic detection of immunohistochemically labeled or Giemsa-stained "*Candidatus* H. suis" cells in stomach sections proved to be highly specific (100%) but relatively insensitive (72 and 42%, respectively) compared to the PCR assay. A longer incubation time of the urease test improved its sensitivity considerably (74 versus 55%) but was accompanied by a loss of specificity (72 versus 93%). In conclusion, we found the "*Candidatus* H. suis"-specific PCR assay to be a sensitive and reliable diagnostic method for the detection of "*Candidatus* H. suis" in the stomachs of pigs and could prove to be a valuable tool for further epidemiological studies both for "*Candidatus* H. suis"- and for "*Helicobacter heilmannii*" type 1-related research.

Hyperkeratosis and ulceration of the gastric pars esophagea in pigs are worldwide problems (20). Lesions are characterized by various degrees of damage of the stratified squamous epithelium that range from mild hyperkeratotic changes to severe epithelial desquamation and ulceration. Symptoms of anorexia, chronic anemia, acute gastric hemorrhage, and reduced weight gain have been associated with this disease (2, 9, 20). However, the economic impact of this syndrome is subject to controversy (11, 14). An increase in the incidence of gastric lesions in pigs has been reported in several countries over the years (8, 11; A. Leman, Letter, Int. Pig **11**:44, 1992; S. Roels, R. Ducatelle, L. Willems, and D. H. J. Broeckaert, Abstract, Vet. Pathol. **2**(Suppl.):20, 1996).

The etiologic and pathogenic backgrounds of gastric ulcer disease in pigs remain unclear. In the past, research focused mainly on dietary and stress-related factors (1, 15, 20). More recently, the occurrence of gastric lesions in pigs has been associated with the presence of gastric spiral bacteria (22). These tightly coiled bacteria were described for the first time in 1990 by Queiroz et al. (23) and were referred to as "*Gastrospirillum suis*" (18). Recent phylogenetic analysis, based on 16S ribosomal DNA (rDNA) sequence data, placed "*G. suis*" within the genus *Helicobacter*, and it was renamed "*Candidatus* Helicobacter suis" (6).

In humans, infection with *Helicobacter pylori* is the predominant cause of chronic gastritis and is associated with recurrent peptic ulcer disease and gastric carcinoma (7). However, "*Helicobacter heilmannii*," another helicobacter-like organism sporadically observed in the human stomach and morphologically very similar to "*Candidatus* H. suis," has been associated with human gastric pathology (5, 13; A. Morgner, E. Bayerdörffer, A. Meining, M. Stolte, and G. Kroher, Letter, Lancet **346**:511–512, 1995). Phylogenetic research revealed the existence of at least two different types of this organism in humans (24). A 99.5% 16S rDNA sequence homology between "*H. heilmannii*" type 1 and "*Candidatus* H. suis" has recently been shown, indicating that both sequences are derived from organisms that belong to the same species, which suggests a possible zoonotic role of "*Candidatus* H. suis" (6).

To date "*Candidatus* H. suis" has not been cultivated in vitro. Therefore, various alternative diagnostic methods have been used in earlier studies to determine the presence of "*Candidatus* H. suis" in the stomachs of pigs (10, 17, 19, 25; A. Bedel, F. Pichard, N. Wusher, A. Labigne, and M. Huerre, Abstract, Gut **41**(Suppl. 1):A124, 1997). The diversity in prevalences revealed the clear need for a sensitive and specific technique for the detection of "*Candidatus* H. suis." Here we report on a comparative study between a new technique, i.e., a new "*Candidatus* H. suis"-specific PCR assay, and three invasive diagnostic methods, an *H. pylori*-derived immunohistochemical staining method, a urease activity-determining test, and Giemsa staining-based histological detection.

MATERIALS AND METHODS

Sampling. Two hundred stomachs from clinically healthy slaughterhouse pigs were randomly sampled from different slaughterhouses in Belgium and The Netherlands. Ligatures were placed on the esophagus and the proximal duode-

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TABLE 1. Oligonucleotide primers and probe used for PCR amplification and Southern blot hybridization

Primer	Sequence (5′–3′)	Polarity	*Escherichia coli* 16S rRNA position
V832f	TTG GGA GGC TTT GTC TTT CCA	Positive, forward	832–852
V1000f	AGG AAT TCC CTA GAA ATA GGG	Positive, forward	1000–1020
V1261r	GAT TAG CTC TGC CTC GCG GCT	Negative, reverse	1261–1281

num near the junction with the stomach before removing the stomach from the gastrointestinal tract. The stomachs were placed in separate plastic bags and were transported to the laboratory within 3 h after sampling. The stomachs were opened longitudinally along the greater curvatura and were carefully rinsed with tap water. A mucosal biopsy fragment (1 cm^2) was taken from the antral part of the stomach along the curvatura major near the torus pyloricus. Half of this fragment was used for immunohistochemical and histological evaluation and was placed in 4% buffered formalin for 24 h. The remaining fragment was equally divided; one part was used for a urease activity-determining test, while the other part was placed into sterile phosphate-buffered saline (PBS) and frozen in liquid nitrogen for PCR analysis. Each sample was coded and all assays were run without prior knowledge of the results of the other assays. The assays were performed in two different laboratories, laboratory A and laboratory B. To avoid cross-contamination between stomachs during sampling, different sets of scissors and pinchers were used for each stomach and were disinfected by placing them in 95% ethanol, followed by flaming and subsequent decontamination in 0.1 N HCl.

Urease activity. Gastric helicobacters produce urease which hydrolyzes urea into ammonia and carbon dioxide, creating a neutral environment around the bacterium. This change in pH is used in the CUTest (Temmler Pharma, Marburg, Germany) to evaluate the presence of urease-producing helicobacters in a gastric sample. Samples were read at 3 and 20 h postinoculation (p.i.). One small mucosal fragment was taken from each stomach near the torus pyloricus and was placed into a test tube for the urease test (CUTest; Temmler Pharma). The test tubes were placed in an incubator at 37°C and were read at 3 and 20 h. A positive reaction was defined as a color change of the medium from yellow to orange or pink-purple, according to the manufacturer's instructions. As a positive control, a stomach mucosal fragment was taken from a pig in which the presence of "*Candidatus* H. suis" was confirmed by Giemsa staining, PCR, and immunohistochemical evaluation. A porcine myocardial biopsy specimen was used as a negative control.

Immunohistochemistry. An immunohistochemical staining procedure was performed with the formalin-fixed and paraffin-embedded pyloric sample of each animal to determine the presence of "*Candidatus* H. suis" as described before (6). Five-micrometer-thick sections of formalin-fixed and paraffin-embedded tissue samples were placed on 3-aminopropyltriethoxysilane-coated slides (Sigma-Aldrich NV/SA, Bornem, Belgium) and the slides were dried overnight at 60°C. After dewaxing with xylene and rehydration in series with ethanol and distilled water, sections were placed in 0.1 M citrate buffer supplemented with 2% urea and were boiled (two times for 5 min each time) in an 800-W microwave oven to elicit antigen retrieval. The slides were then incubated with 12% hydrogen peroxide in methanol (30 min) in order to block endogenous peroxidase activity and were subsequently preincubated with 30% normal goat serum in PBS for 30 min to reduce nonspecific antibody binding. A mouse polyclonal antibody directed against *H. pylori* (DAKO, Glostrup, Denmark), diluted 1/20 in PBS, was incubated overnight at 21°C in a moist chamber. The sections were washed and incubated with biotinylated swine anti-rabbit immunoglobulin (21°C, 30 min) and, after rinsing, were covered with peroxidase-conjugated streptavidin-biotin complex. Peroxidase activity was developed with H_2O_2 with diaminobenzidine as a chromogen (Fast DAB Tablet Set; Sigma-Aldrich NV/SA). Finally, the sections were counterstained with Mayer's hematoxylin and mounted. As negative controls, the *H. pylori*-specific polyclonal antibodies were replaced by fetal calf serum in Tris-HCl buffer (pH 7.6) and a section of porcine heart tissue was included. As positive controls, a section of a mouse stomach experimentally infected with *H. pylori* LMG 7539^T and a section of stomach from a colostrum-deprived piglet infected with "*Candidatus* H. suis" were taken. The sections were evaluated by an experienced pathologist.

Giemsa staining. Each formalin-fixed sample was dehydrated in an alcohol-xylene series and was embedded in paraffin wax. Five-micrometer-thick sections were made from each block, and Giemsa staining was performed with each section. The sections were evaluated by an experienced pathologist.

DNA extraction. A single DNA extract was made from each stomach. In a first stage, the superficial cell layers and mucus were scraped from each gastric biopsy specimen with a surgical blade. DNA was extracted from these scrapings by lysis with guanidinium isothiocyanate, and DNA was bound to silica particles by the method of Boom et al. (4).

"*Candidatus* H. suis"-specific PCR. "*Candidatus* H. suis"-specific primers V832f and V1261r (Table 1) were used to amplify a 433-bp fragment of the 16S rRNA-coding gene as described before (6). Each stomach sample was tested twice by performing the PCRs and subsequent Southern blot hybridizations in two different laboratories (laboratories A and B) by using the same DNA extract.

All PCRs were performed in a 50-μl volume. Reaction mixtures contained 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl$_2$, each deoxynucleoside triphosphate at a concentration of 200 μM, 1.5 U of AmpliTaq Gold (Perkin-Elmer Biosystems, Foster City, Calif.), and 25 pmol of both forward and reverse primer (Eurogentec, Seraing, Belgium). PCR amplification was performed under the following conditions: 9 min of preincubation at 95°C to activate AmpliTaq Gold, followed by 40 cycles of 30 s at 94°C, 30 s at 60°C, and 45 s at 72°C. A final extension was performed for 5 min at 72°C. As a positive control, plasmid DNA from a cloned "*Candidatus* H. suis" 16S rDNA fragment was used as described earlier (6). DNA extracts from the stomach of a gnotobiotic piglet and the heart of a clinically healthy adult pig were used as negative controls.

Detection and analysis of amplified DNA products. Twelve microliters of each amplification product was analyzed by gel electrophoresis in 2% agarose gels in TBE buffer (89 mM Tris-HCl [pH 8.0], 89 mM boric acid, 2.5 mM EDTA). PCR products were transferred to Hybond N$^+$ membranes (Amersham, Uppsala, Sweden) for Southern blot analysis. Hybridization was performed with the "*Candidatus* H. suis"-specific [γ-^{32}P]ATP-labeled probe V1000f (Table 1) by standard procedures as described before (6). In order to ensure the specificity of the probe hybridization, blots were washed twice with 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 55°C.

DNA sequencing. Ten PCR-positive stomachs which were negative by all three other tests were selected. A second "*Candidatus* H. suis"-specific PCR was performed with the DNAs extracted from these stomachs. As a positive control, the PCR product amplified from plasmid DNA including a cloned "*Candidatus* H. suis" 16S rDNA fragment was used. Each PCR product was purified with the PCR product presequencing kit (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Subsequently, fluorescent sequencing templates were produced from each PCR product by using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Perkin-Elmer [PE] Biosystems, Foster City, Calif.) in combination with primers V832f and V1261r, according to the manufacturer's instructions. The dye-labeled extension products were precipitated, washed, and dried before being loaded onto the sequencing gel. The samples were suspended in 20 μl of Template Suppression Reagent (PE Biosystems), denatured by heating for 2 min at 95°C, and passed through POP6 gel-filled capillary columns on an ABI PRISM 310 Genetic Analyzer (PE Biosystems). All derived sequences and the complete "*Candidatus* H. suis" 16S rRNA gene sequence (GenBank accession no. AF127028) were aligned and analyzed by using the Genebase software package (Applied Maths, Kortrijk, Belgium).

Sensitivity, specificity and agreement between tests for detection of "*Candidatus* H. suis." Because a "gold standard" has not yet been defined for "*Candidatus* H. suis" detection, the sensitivity and specificity of each test were calculated by using the results of PCR, immunohistochemistry, Giemsa staining, and urease testing as independent benchmarks. The agreement between tests was evaluated by calculating Cohen's kappa coefficient. A value of 1 indicates perfect agreement, while a value of 0 indicates that agreement is no better than chance. In general, a value between 0.4 and 0.5 is considered to represent moderate agreement.

RESULTS

Urease activity. No color change was seen with the heart biopsy specimen, which is in contrast to the strong positive reaction noted with the "*Candidatus* H. suis"-positive control sample. The results for both incubation time intervals are discussed.

Urease activity was observed in 71 (36%) and 122 (61%) of the 200 gastric samples after 3 and 20 h of incubation, respectively. In general, a longer incubation period increased the sensitivity but decreased the specificity of the CUTest compared to those of the other tests (Table 2). A better concurrence of the results of the urease test after 20 h of incubation and the "*Candidatus* H. suis"-specific PCR assay was seen. A total of 94% (3 h p.i.) and 90% (20 h p.i.) of the urease-positive stomachs were also PCR positive. For 10 of the 112 stomachs (9%), a positive CUTest could not be confirmed by PCR or histologic detection.

TABLE 2. Comparison of sensitivity, specificity, and concurrence of different tests for detection of "Candidatus H. suis" used in this study[a]

Test	% Positive pigs	Comparison with the following benchmark tests:											
		PCR			Immuno			Urease (20 h p.i.)			Giemsa staining		
		SE (%)	SP (%)	κ	SE (%)	SP (%)	κ	SE (%)	SP (%)	κ	SE (%)	SP (%)	κ
PCR	77				100	52	0.54	90	44	0.35	100	34	0.25
Immuno	56	72	100	0.54				70	67	0.01	100	66	0.56
Urease (3 h p.i.)	36	44	93	0.03	48	76	0				62	60	0
Urease (20 h p.i.)	61	71	72	0.35	77	56	0.01				83	50	0.25
Giemsa staining	33	42	100	0.25	59	100	0.56	44	86	0.25			

[a] Abbreviations: Immuno, immunohistochemistry; SE, sensitivity; SP, specificity; κ, Cohen's kappa coefficient.

Giemsa staining. Tightly coiled, "Candidatus H. suis"-like organisms were observed in 65 (33%) of the 200 Giemsa-stained stomach sections. They were mostly seen lying separately or in small clusters in the gastric crypts of the antral region. A 100% specificity was calculated by using immunohistochemistry and PCR as a benchmark, indicating that all Giemsa staining-positive stomachs were also immunohistochemically and PCR positive (Table 2). Conversely, compared to PCR and immunohistochemistry, a substantial number of the stomachs were considered false negative, as shown by the low sensitivity values (42 and 59%, respectively). A concurrence of both positive and negative results by Giemsa staining and PCR was noted for 56% of the samples. A relatively high degree of concurrence was seen between immunohistochemistry and Giemsa staining (77%).

Immunohistochemistry. "Candidatus H. suis"-like bacteria were detected in 111 (56%) of the 200 stomach samples by using polyclonal antibodies directed against *H. pylori*. Bacteria were seen as long, brown, tightly spiraled organisms lying separately or in small clusters with a patchy distribution over the sample. These bacteria were often observed in close contact with the mucosal cell lining. Fragments of spiraled bacteria and immunostained coccoid-like bacteria were also seen frequently. A sample was considered positive if multiple morphologically characteristic "Candidatus H. suis"-like bacteria were present. For the positive mouse control only *H. pylori*-like bacteria were stained, while for the positive pig control only "Candidatus H. suis"-like bacteria were detected. For the negative controls no staining was observed.

Immunohistochemical detection showed good sensitivity (72%) and excellent specificity (100%) values when the results were compared to the results of the "Candidatus H. suis"-specific PCR assay.

"Candidatus H. suis"-specific PCR and Southern blot analysis. A 16S rDNA fragment specific for "Candidatus H. suis" was amplified by PCR by using primers V832f and V1261r, with a 433-bp band found to be produced by electrophoresis for 72% (144 of 200) of the stomach DNA extracts and the positive control. All DNA bands hybridized with the V1000f probe after Southern blot hybridization. In addition, 10 stomach samples negative by electrophoresis were confirmed to be positive by this technique, bringing the total rate of PCR positivity for stomachs to 77% (154 of 200). No PCR product was obtained with the negative control DNA extracts from stomachs. Interlaboratory reproducibility was 100%, since both laboratories produced identical PCR results.

Sequencing of 16S rDNA fragments. By the "Candidatus H. suis"-specific PCR assay, an ~0.4-kbp band was obtained on electrophoresis for the selected stomachs (n = 10) and the positive control. A 433-bp 16S rRNA gene fragment was determined from all 10 stomachs and the positive control by

direct sequencing. Comparison of the derived positive control sequence amplified from plasmid DNA enclosing the cloned "Candidatus H. suis" 16S rRNA gene with the complete "Candidatus H. suis" 16S rRNA gene sequence (Genbank accession no. AF127028) showed a perfect match between this sequence and the corresponding zone in the complete 16S rRNA gene sequence. Pairwise comparisons between the sequences derived from the stomachs with the positive control sequence revealed a maximum homology divergence of less then 0.5%, which corresponds to a maximum difference of 2 nucleotides.

Sensitivity, specificity, and agreement. Benchmarking of the "Candidatus H. suis"-specific PCR assay results to those obtained by immunohistochemistry, urease testing (20 h p.i.), and Giemsa staining revealed that the PCR is highly sensitive, with values of 100, 90, and 100%, respectively. Specificity, however, was remarkably lower, with calculated values of 52, 44, and 34%, respectively. These apparently low specificity values were effected by 43, 44, and 89 stomachs which were PCR positive but which remained negative by immunohistochemistry, urease testing (20 h p.i.), and Giemsa staining-based detection, respectively. For 18 stomachs, the PCR assay was the only method that was able to detect "Candidatus H. suis." On the basis of Cohen's kappa coefficient, the PCR assay showed the best agreement with the immunohistochemical detection method (Table 2). A fairly good agreement was also seen between both histological detection methods. A very poor agreement was observed between the urease test after 3 h of incubation and the other tests.

DISCUSSION

In 1990, helicobacter-like organisms were described for the first time in the stomachs of pigs by Queiroz et al. (23) on the basis of a histologic study. Since then, several nonspecific diagnostic methods that have detected "Candidatus H. suis" at various frequencies have been used (10, 16, 17, 19, 25; Bedel et al., Abstract, Gut **41**(Suppl. 1):A124, 1997). However, it is unclear whether these differences in prevalence are caused by epidemiological variation or by an incongruity between diagnostic methods.

In the present study, we compared a new "Candidatus H. suis"-specific PCR assay with three other nonspecific tests, i.e., immunohistochemistry, urease testing, and Giemsa staining-based detection. The lack of comparative data between diagnostic methods for the detection of "Candidatus H. suis" in porcine gastric samples did not allow us to use a gold standard. For this reason, each method was benchmarked to each other in this study to assess sensitivity and specificity, while agreement between tests was evaluated by using Cohen's kappa coefficient. Histologic detection of "Candidatus H. suis" was clearly more sensitive by immunohistochemical analysis than

classical Giemsa staining. The immunohistochemical labeling of "*Candidatus* H. suis" cells with *H. pylori*-derived antibodies enabled us to positively identify stomach sections with few "*Candidatus* H. suis" cells, whereas Giemsa staining was unable to detect bacteria in sections with so few "*Candidatus* H. suis" cells.

Indirect detection of "*Candidatus* H. suis" by evaluation of the urease activity of a stomach sample was influenced by the incubation time. When the results were read after 3 h, a significantly lower level of detection was seen compared to that seen after a 20-h incubation period. However, this was accompanied by a loss of specificity in all benchmark tests, which was probably affected by the false-positive results induced by other urease-producing microorganisms.

In this study we found that the "*Candidatus* H. suis"-specific PCR assay in combination with Southern blot hybridization was the most sensitive method, detecting "*Candidatus* H. suis" in 77% of the stomachs examined. However, low specificity values were noted when the PCR assay was compared to the other tests, and this was caused by the large number of samples with positive results by PCR. Nevertheless, we considered the "*Candidatus* H. suis"-specific PCR assay to be highly accurate for the following reasons. First, all PCR products that were positive by electrophoresis were also confirmed to be positive by subsequent Southern blot hybridization with an "*Candidatus* H. suis"-specific probe under stringent conditions. Second, a positive result established by either the immunohistological or the Giemsa staining-based detection method was in agreement with a positive result established by PCR for 100% of the stomach sections. Furthermore, sequencing of the 16S rRNA fragment amplified from stomachs which were positive by PCR but negative by all three other tests revealed that these sequences were derived from "*Candidatus* H. suis" cells and not from other helicobacters. In the same sense, no amplimers were produced from stomachs harboring other helicobacter species. Cross-contamination during sampling was carefully avoided and would be very unlikely to account for the larger number of PCR-positive samples. Finally, all PCR results could be reproduced by using the facilities of a different laboratory, thus validating the repeatability of the PCR assay.

A fairly good agreement was observed between the PCR assay and the immunohistochemical detection method, in contrast to the agreement observed between PCR and the other tests. From the results of the statistical analysis by use of Cohen's kappa coefficient, it was also concluded that urease activity testing is rather unreliable, especially when the results are read after 3 h.

The high prevalence of "*Candidatus* H. suis" in the stomachs of pigs, as observed in the present study, is in agreement with the results of studies performed in other countries; such studies have reported prevalences as high as 88% (16). This indicates that "*Candidatus* H. suis" is a common organism in the pig stomach worldwide. In epidemiological studies with humans, similarly high prevalences of *H. pylori* have been observed in association with poor hygienic conditions (3, 12, 21). Recently, Melnichouk et al. (16) reported that a specific-pathogen-free-managed farm was free of "*Candidatus* H. suis"-like organisms, whereas three other conventional farms were not. This raises questions about the possible impact of housing and management factors in industrial pig farming on the transmission of "*Candidatus* H. suis." Interfarm and intrafarm epidemiological studies, using the PCR assay as a diagnostic tool, could further clarify this issue.

The association of "*Candidatus* H. suis"-like organisms with the occurrence of porcine gastric ulcer disease (10, 19, 22) is still the subject of debate, as some reports have provided information that has stated the opposite (15, 16). In *H. pylori*, it has been shown that the presence of genes that code for proinflammatory substances is significantly linked to a higher degree of gastric pathology. Such genes have not yet been clearly identified in "*Candidatus* H. suis." Therefore, associating "*Candidatus* H. suis"-like organisms with porcine gastric pathology merely by establishing their presence without any further characterization could prove erroneous. Future research must determine whether *H. pylori*-like or other virulence genes occur in "*Candidatus* H. suis." Epidemiological studies based on new diagnostic tools and experimental studies must establish the exact role of "*Candidatus* H. suis" in porcine gastric ulcer disease. In the same sense, the involvement of "*Candidatus* H. suis" in the onset of human gastric disease must still be established. On the basis of 16S rRNA sequence data, it is suggested that "*Candidatus* H. suis" and "*H. heilmannii*" type 1 could be the same species, indicating a possible zoonosis (6). However, "*H. heilmannii*" is an unofficial name for a group of morphologically similar bacteria that occur in the human stomach and that consist of different *Helicobacter* species that originate from different sources. It is unclear if each species is equally capable of causing gastric disease. Useful epidemiological data could be collected by the "*Candidatus* H. suis"-specific PCR assay and could help clarify the confusion about this subject.

In conclusion, we found the "*Candidatus* H. suis"-specific PCR assay to be a highly sensitive diagnostic method for the specific detection of "*Candidatus* H. suis" in gastric samples when it was compared to histological detection and urease activity testing. The method could have a potential value for further epidemiological studies both for "*Candidatus* H. suis"- and for "*H. heilmannii*" type 1-related research.

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