ORIGINAL RESEARCH PEER REVIEWED

Identification of *Helicobacter suis* in pig-producing regions of the United States

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Summary

Objectives: To develop a non-culture-based method to determine levels of *Helicobacter suis* infection in porcine stomachs and to test the method in a sample of pigs from a variety of regions in the United States.

Materials and methods: A polymerase chain reaction (PCR) assay was developed to quantitate total *Helicobacter* generic DNA and *Helicobacter suis* species-specific DNA in pig stomachs. Primers were derived from 16s ribosomal RNA (rRNA) gene sequences, selected on the basis of relative conservation and divergence of sequences across the various *Helicobacter* species. The assay was standardized using cloned 16s rRNA sequences and was initially tested with DNA isolated from cultured *H suis*. Gastric mucosal scrapings were collected from pigs in three geographic regions of the United States, including the North (Minnesota and Michigan), East Central (Iowa), and South (Oklahoma and North Carolina).

Results: Of a total of 118 pigs tested, approximately half (55.1%; 95% CI, 46.1%-63.8%) were positive for *H suis* DNA. *Helicobacter suis* DNA was detected in pigs from all states tested.

Implications: *Helicobacter suis* is present in US pigs and may be relevant to pig health and production. This quantitative PCR assay will facilitate further study of *H suis* in pigs, including potential therapeutic and prophylactic interventions.

Keywords: swine, *Helicobacter suis*, gastric ulcers

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Resumen - Identificación del *Helicobacter suis* en regiones productoras de cerdo de Los Estados Unidos

Objetivos: Desarrollar un método no basado en cultivo para determinar los niveles de infección de *Helicobacter suis* en estómagos porcinos y para probar el método en una muestra de cerdos de diversas regiones es Los Estados Unidos.

Materiales y métodos: Se desarrolló una prueba de reacción en cadena de polimerasa (PCR por sus siglas en inglés) para cuantificar un DNA genérico de *Helicobacter* total y el DNA específico de las especies *Helicobacter suis* en estómagos de los cerdos. Los primers se derivaron de las secuencias del gen RNA ribosomal (rRNA por sus siglas en inglés) 16s, seleccionadas en base a la conservación relativa y a la divergencia de las secuencias a través de varias especies de *Helicobacter*. La prueba se estandarizó utilizando secuencias de rRNA 16s clonadas y se probó inicialmente con DNA aislado del cultivo de *H suis*. Se recolectaron raspados de muscosa gástrica de cerdos en tres regiones geográficas de los Estados Unidos, incluyendo el Norte (Minesota y Michigan), el Este Central (Iowa), y el Sur (Oklahoma y Carolina del Norte).

Resultados: De un total de 118 cerdos probados, aproximadamente la mitad (55.1%; 95% CI, 46.1%-63.8%) resultaron positivos al DNA del *H suis*. Se detectó el DNA del *Helicobacter suis* en cerdos de todos los estados probados.

Implicaciones: El *Helicobacter suis* está presente en cerdos de Los Estados Unidos y puede ser relevante para la producción y salud porcina. Esta prueba cuantitativa de PCR facilitará estudios adicionales del *H suis* en cerdos, incluyendo intervenciones profilácticas y terapéuticas potenciales.

Résumé - Identification d'*Helicobacter suis* dans des régions de production porcine des États-Unis

Objectifs: Développer une méthode diagnostiques sans culture pour déterminer le degré d'infection par *Helicobacter suis* dans des estomacs de porc et tester la méthode dans un échantillonnage de porcs provenant de régions variées des États-Unis.

Matériels et méthodes: Une épreuve d'amplification en chaîne par la polymérase (PCR) a été développée pour quantifier l'ADN générique total d'*Helicobacter* et l'ADN spécifique à l'espèce *Helicobacter suis* dans des estomacs de porc. Des amorces ont été dérivées des séquences de l'ARN ribosomal 16s (ARNr), sélectionnées sur la base de conservation et divergence relative des séquences parmi les différentes espèces d'*Helicobacter*. L'épreuve a été standardisée en utilisant des séquences clonées d'ARNr 16s et fut testée initialement avec de l'ADN isolé d'*H suis* cultivé. Des grattages de la muqueuse gastrique ont été prélevés de porcs dans trois régions géographiques des États-Unis, incluant le nord (Minnesota et Michigan), le centre-est (Iowa), et le sud (Oklahoma et Caroline-du-Nord).

Résultats: Sur un total de 118 porcs éprouvés, environ la moitié (55,1%; IC 95%, 46,1%-63,8%) étaient positifs pour la présence d'ADN d'*H suis*. L'ADN d'*H suis* a été détecté de porcs provenant de tous les états sélectionnés.

Implications: *Helicobacter suis* est présent chez les porcs américains et peut être pertinent en regard de la santé des porcs et de la production porcine. Cette épreuve PCR quantitative facilitera les études futures sur

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H suis chez les porcs, incluant les interventions thérapeutiques et prophylactiques potentielles.

Gastric ulcers are a significant problem in grower and finisher pigs. A 2006 survey of US swine farms identified gastric ulcers as a disease problem in almost half of the medium and large production sites.[1] Gastric ulcers were also reported as a disease problem in sows, especially in large sites (total sow and gilt inventory > 500). The etiology of gastric ulcers is thought to be a complex interplay of environmental factors, including feed, housing, and concurrent infectious diseases.[2] Pathological changes occur in the pars oesophagea, ranging from hyperkeratosis, erosions, and ulceration to frank hemorrhage and death. Gross pathological changes consistent with gastric ulcers are commonly seen at slaughter, where surveys have reported rates ranging from 5% to 100%.[2] The economic impact of gastric ulcers stems both from direct impacts on health and growth and from secondary effects, such as increasing particle size of feed to mitigate ulcer risk, which in turn has a detrimental impact on feed efficiency.[2]

Gastric, *Helicobacter*-like bacteria have been reported in pigs from various countries for over 20 years. Some examples include Brazil,[3,4] Finland,[5] France,[6] Netherlands,[7] Australia,[8] and the United States.[9,10] The *Helicobacter* species found in many of these studies is unclear due to methodology variations, inability to isolate and culture the bacteria described, and evolving understanding and nomenclature of *Helicobacter* species over the time of the studies. It is apparent, however, that *Helicobacter*-like organisms are commonly present in pigs throughout the world.

Helicobacter suis is a spiral bacterium found in the stomachs of swine. It was first identified by molecular methods in pigs from Belgium[11] and has since been successfully cultured in vitro.[12] *Helicobacter suis,* a distinct species of *Helicobacter,* is identical to *Helicobacter heilmannii* type 1 found in humans and is not closely related to *Helicobacter pylori* or the *H pylori*-like organisms previously reported in experimental swine.[13] It is one of the various so-called non-*Helicobacter pylori* helicobacters (NHPHs) and is the most common NHPH found in humans.[14] The possible significance of these NHPHs in human disease has been extensively reviewed.[15] Prevalence of *H suis* appears to be very low prior to weaning, but increases rapidly following weaning and is very high in adult pigs (> 90%)[16] and at slaughter (77%).[17]

The specific causal relationship of *H suis* with gastric ulcers and related lesions remains unclear. There has been a long-standing correlation of *Helicobacter*-like bacteria with gastric lesions in pigs. Several studies have shown that pigs with more severe ulcers tended to have higher numbers of helicobacters or a greater likelihood of being colonized by helicobacters.[4,10] Since gastric ulcers have a complex etiology, it is not surprising that attempts to experimentally induce gastric ulcers with *H suis* have generally been inconclusive.[18] However, two recent experimental infection studies have shown *H suis* infection can reduce daily weight gain by 5%[19] to 10%.[20] Furthermore, *H suis*-infected pigs were more likely to have ulcerative lesions of the stomach and microscopic signs of gastritis than were non-infected pigs.[20]

To date, no comprehensive information on the presence of *H suis* in US pigs has been available. Previous evidence of helicobacters in swine has been noted in the United States,[9,10] but these were not isolated or cultured. Because of the association of *H suis* with the pathogenesis of gastric ulcers and possible zoonotic potential of *H suis*, we have developed and piloted a molecular assay for *H suis* in gastric samples. Using this quantitative polymerase chain reaction (PCR) assay, we found considerable amounts of *H suis* DNA in pigs from several major pig-producing areas of the United States.

Materials and methods

Animal work was conducted in accordance with the Zoetis Institutional Animal Care and Use Committee guidelines, in compliance with local, state, and national regulations, and subject to local ethical review.

Source of pigs and sample collection

Experimental samples were obtained from Oklahoma (24 pigs), Iowa (37 pigs), and North Carolina (nine pigs). Additional samples were tested from commercially sourced pigs from Michigan (38 pigs) and Minnesota (10 pigs) as part of unrelated studies conducted at Zoetis, Kalamazoo, Michigan. All pigs were of market age, except for those from Michigan and Minnesota, which were approximately 10 and 4 weeks of age, respectively. Samples, including punch biopsies and gastric mucosal scrapings, were collected at slaughter or euthanasia and were frozen for transport or storage. One limitation of PCR testing methods is the small sample size (100 mg) used for the assays. In order to get a more representative sample of the stomach, we collected a large area of stomach mucosa (approximately 50 cm^2) by scraping, then mixed and sampled from this larger collection area. Initial testing compared punch biopsies with mucosal scrapings from the same stomachs; however, all summary data is from mucosal scrapings.

DNA extraction

Stomach scrapings or biopsy samples (100 mg) were digested with proteinase K at 55°C overnight and stored at -20°C. Total DNA was extracted from the samples using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, California) according to the manufacturer's directions for extraction of genomic bacterial DNA. The DNA was eluted in 100 µL nuclease-free water and stored at -20°C. For isolation of control DNA from *Helicobacter* species, bacteria were pelleted by centrifugation and frozen at -20°C. Bacterial DNA was isolated using the Qiagen DNeasy Blood and Tissue kit according to the manufacturer's directions for isolation of DNA from gram-negative bacteria. Bacterial DNA was stored at -20°C. Initial experiments were conducted using mucosal samples spiked with *Helicobacter* DNA to confirm the absence of inhibitory substances in the purified DNA.

Quantitative PCR

Quantitative PCR (TaqMan; Life Technologies, Grand Island, New York) was conducted using sequences common to *Helicobacter* species (Hb) and specific for *H suis* (Hs) (Table 1). Sequences for the *Helicobacter* genus-specific primers and probe were derived from the *Helicobacter*-common region of the 16s ribosomal RNA (rRNA) gene. *Helicobacter suis*-specific primers were based on *H suis* 16s rRNA gene sequence regions previously identified as unique to *H suis.*[17] The *H suis*-specific TaqMan polymerase chain reaction (TM-PCR) probe was designed using the sequences between the primers. The specific primer and probe sequences were selected from a series of sequences based on the initial assay development and optimization. Primers were obtained from Integrated DNA Technology (Coralville, Iowa) and probes from Life Technologies. The TM-PCR was performed using the described primer and probe sets according to manufacturer's recommendations (25 µL 2× master mix, 0.2 µL of each primer and probe, 5 µL DNA in a 50-µL total volume reaction) using standard

Table 1: Primer and probe sequences used to measure *Helicobacter suis* DNA by quantitative polymerase chain reaction (PCR) in the stomachs of pigs from Iowa, North Carolina, Michigan, Minnesota, and Oklahoma*

Primer/probe	Sequence	Specificity/use	Position†
HbF	TATGACGGGTATCCGGCCT	*Helicobacter* species	241-260
Hb probe	AGAGGGTGAGCGGACACACTGGAAC	*Helicobacter* species	262-286
HbR	TGCCTCCCGTAGGAGTCTGGA	*Helicobacter* species	297-317
HsF	GGGAGGACAAGTCAGGTGTGAA	*H suis*	526-547
Hs probe	TCCTATGGCTTAACCATAGAACTGCATTTGAA	*H suis*	549-580
HsR	TCTCCCACACTCCAGAAGGATAG	*H suis*	582-604
Hcom1F	GTAAAGGCTCACCAAGGCTAT	Generic control‡	224-243
Hcom2R	CCACCTACCTCTCCCACACTC	Generic control‡	593-612
H205F	TATGACGGGTATCCGGCCT	*H suis*-specific control‡	241-260
V126R	GATTAGCTCTGCCTCGCGGCT	*H suis*-specific control‡	1208-1229

* Stomach samples (mucosal scrapings or biopsies or both) were collected from a total of 118 pigs. The 38 pigs from Michigan were 10 weeks of age, the 10 pigs from Minnesota were 4 weeks of age, and all others were market age. DNA was isolated and the amount of total *Helicobacter* generic and *Helicobacter suis*-specific DNA was determined by quantitative PCR. Quantitation was based on standard curves generated with control plasmid DNA containing selected *Helicobacter* sequences.

† GenBank sequence EF204589.1, source *H suis* strain HS1, 16s ribosomal RNA.

‡ Primers used to generate sequences cloned into a plasmid for generating standard curves.

TM-PCR cycling conditions (40 cycles, denaturing and annealing temperatures of 95°C and 60°C, respectively). *Helicobacter* DNA copy number was calculated (in the TaqMan-based program) using a plasmid DNA standard curve generated with cloned 16s rRNA gene sequences (pCR2.1-TOPO vector; Life Technologies) from *H suis*, using primers H205F and V126R or Hcom1F and Hcom2R (Table 1) for the *H suis*-specific and *Helicobacter* genus-specific TM-PCR, respectively. All samples were tested in triplicate, and all standard curves were generated in duplicate. The cycle threshold (Ct) values for the standard curve template DNA (plasmid DNA containing cloned rRNA sequences) were consistent over time, indicating consistency of amplification and stability of the target DNA. A pure culture of *H suis,* used to determine primer and probe set amplification sensitivity (Figure 1), was created by limiting dilution followed by expansion of the isolated bacterial clone. Purity of the culture was confirmed by culturing for extraneous bacteria. The calculations shown in Figure 1 are based on an assumption of one 16s rRNA gene per bacterial cell as described for the genomic sequence of *H suis.*[21]

Specificities of the primer and probe sets for *Helicobacter* species and *H suis* were confirmed by experiments with several species of bacteria, including several *Campylobacter jejuni* strains, *Lawsonia intracellularis,* and several *Helicobacter* species, including *H pylori*, *Helicobacter cynogastricus, Helicobacter felis,* and *Helicobacter bizzozeronii*. The *Helicobacter* genus-specific TM-PCR detected all of the tested *Helicobacter* species, while the *H suis*-specific TM-PCR detected only *H suis* DNA. Neither primer or probe set detected the non-*Helicobacter* bacteria (data not shown).

Calculations of percent positive pigs were based on the number of samples positive at any detectable level (> 10 DNA copies per 5-mg sample) versus total samples tested (or total samples tested from a given state). Percentages are shown as 95% confidence intervals (modified Wald method; GraphPad Prism 5).

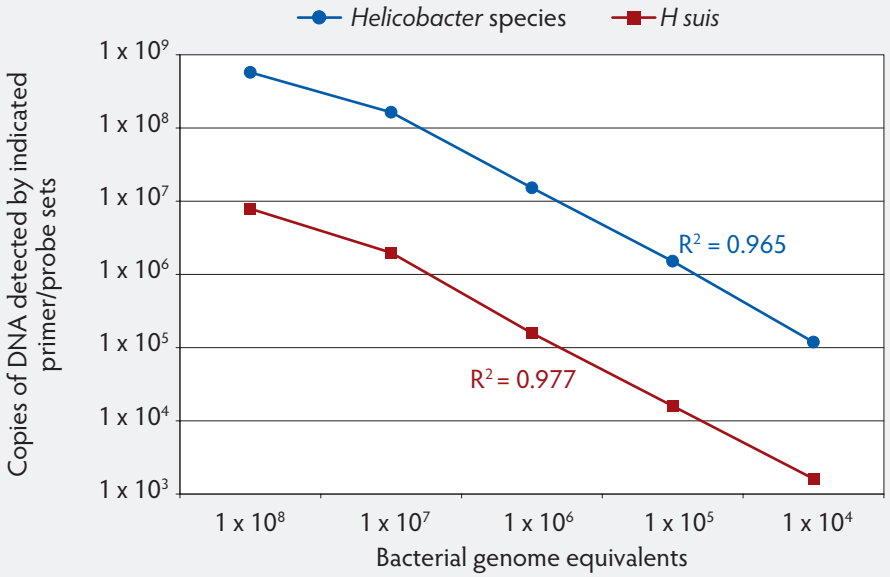
Results

Standard curves were generated for both the *Helicobacter* species and *H suis* primer and probe sets using the respective DNA control plasmids (data not shown). These standard curves were then used to test mock samples that had been spiked with varying amounts of *H suis* DNA isolated from pure cultures of *H suis* and corresponding to the indicated number of bacteria (Figure 1). In general, the Hb TM-PCR resulted in higher relative values than the Hs TM-PCR. The differences in relative efficiency of amplification between the standard curve templates (plasmid DNA) and samples (genomic DNA) can be corrected for by using a correlation such as that described in Figure 1, or by using isolated bacterial genomic DNA as the standard curve template. Thus, the Hb TM-PCR is expected to be more sensitive than the Hs TM-PCR; however, the latter would be more specific for *H suis* and would not detect non-*suis Helicobacter* species that might be present in test samples.

Initially, both scrapings and punch biopsy samples were tested (samples from Oklahoma and Iowa pigs). We found that the scraping method was more sensitive in detecting *Helicobacter* DNA. For example, 45.3% (95% CI, 31.0%-61.6%) of the Iowa pig biopsies were positive in the Hb TM-PCR, while 100% (95% CI, 88.8%-100%) of the scraping-derived samples were positive in the same assay. Therefore, all further samples and all data presented here were obtained with the stomach-scraping method.

Helicobacter suis was detected in pigs from all regions of the United States tested. The results are shown as a histogram of all pigs tested having the indicated number of copies of *Helicobacter* DNA in the stomach mucosal scraping (Figure 2). *Helicobacter* DNA was detected by the Hb TM-PCR in virtually all samples (from all five states), with some samples having as many as 10^8 bacteria (DNA equivalents) per 5 mg of gastric mucosal sample (Figure 2A). In the more specific (less sensitive) Hs TM-PCR assay, approximately half of the samples (55.1%;

Figure 1: Comparison of primer and probe set amplification with bacterial genome equivalents shows that the Hb TaqMan polymerase chain reaction (TM-PCR) is more sensitive than the Hs TM-PCR. TM-PCRs with either the *Helicobacter* genus-specific (Hb) or *Helicobacter suis*-specific (Hs) primers and probes were spiked with total genomic DNA isolated from the indicated number of *H suis* bacteria (based on a visual count of bacteria from a pure culture of *H suis*). All samples were tested in triplicate. A standard curve, based on cloned *Helicobacter* 16s rRNA gene sequences, was used to calculate the genome copy number. R^2 values (Pearson correlation; GraphPad Prism 5; GraphPad Software, Inc, La Jolla, California) for the correlations between spiked bacteria DNA and calculated rRNA DNA numbers are indicated for each assay. Axes are graphed in $\log_{10}$.



95% CI, 46.1% 63.8%) were positive and the overall number of bacteria detected was lower (Figure 2B). In this more specific assay, the percent of pigs positive for *H suis* ranged from 33.4% (95% CI, 21.8%-51.3%) in the Iowa pigs to 100% (95% CI, 67.9%-100%) in the Minnesota pigs, although half of the Minnesota pigs had the lowest detectable numbers of *H suis*. Among the pigs from Michigan, North Carolina, and Oklahoma, 47.4% (95% CI, 32.5%-62.7%), 56% (95% CI, 26.6%-81.2%), and 79% (95% CI, 64.7%-94.2%) were positive, respectively.

Since the currently available *H suis* genomic sequences are derived from European strains,[21] we sequenced several genes from United States-derived *H suis* (Michigan samples) to obtain preliminary information on the similarity of *H suis* strains from the United States and Europe. In addition to the similarity of the 16s rRNA sequences, as evidenced by the results of the TM-PCR, we found that both the *napA* (neutrophil activating protein) and *ureA* (urease) gene sequences of eight US *H suis*-containing samples were identical to the published *H suis* genome (data not shown). Additional methods of analysis (eg, multilocus sequence typing; MLST) could be used to investigate the genetic diversity of *H suis* within the United States and between US and EU strains without the need for isolation and culture.

Discussion

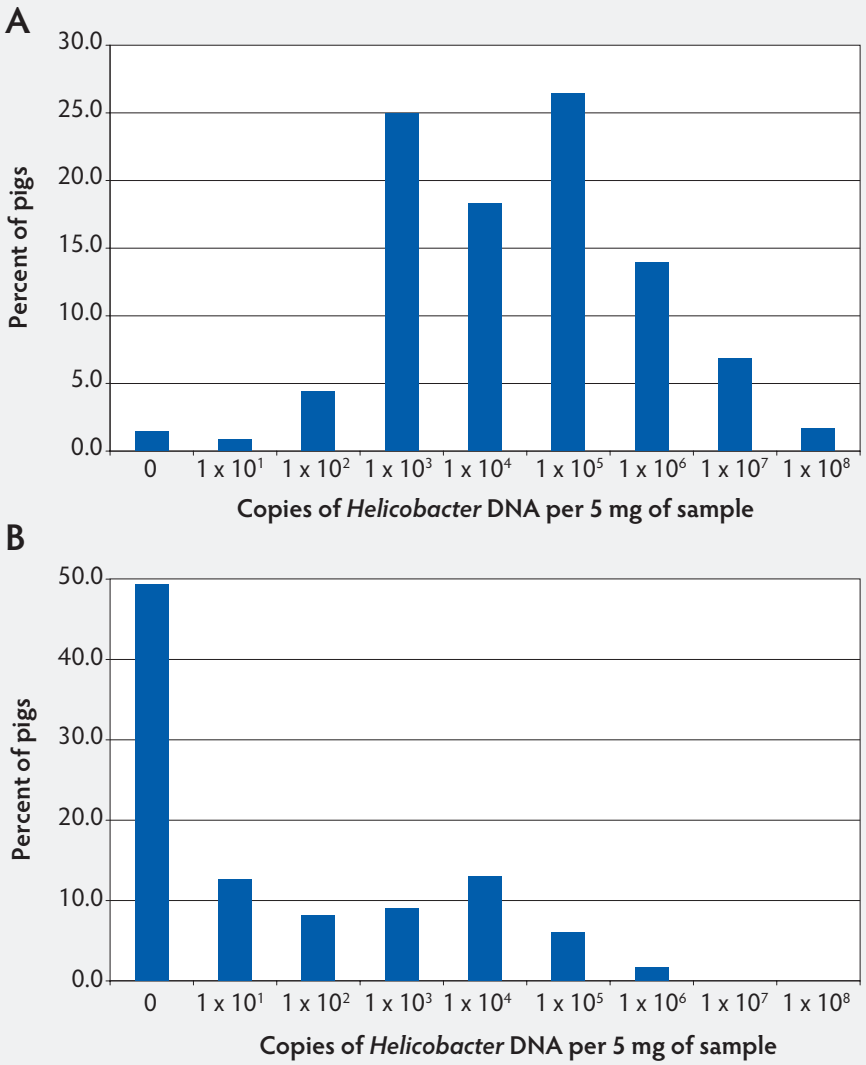
The role of non-culturable or very fastidious bacteria in disease pathogenesis is likely under-appreciated. The classic example is *H pylori*, a bacterium observed in human stomachs since the 1880s, but not cultured and confirmed as the cause of gastric ulcers until the 1980s.[22-24] Likewise, spiral bacteria have been reported in pig stomachs for many years, but *H suis* was not isolated and cultured until 2008.[12] To date, *H suis* has been isolated only in Europe, although PCR methods have previously identified *Helicobacter* sequences consistent with *H suis* in US pigs.[10]

In this study, we readily identified and quantitated *H suis* DNA in pigs from several regions of the United States. Essentially all pigs tested had some detectable *Helicobacter* DNA. However, fewer samples were positive in the *H suis*-specific assay. The difference in the results between the two assays could be due to the relative sensitivities of the two primer sets. Spiking experiments with cultured *H suis* suggests that the *H suis*-specific assay may underestimate the number of *H suis* bacteria present in the samples. It is also possible that other *Helicobacter* species are present in these pigs. Nonetheless, it is clear that some of these pigs had large numbers of *H suis* in their stomachs (as many as 1×10^6 bacteria per 5 mg of gastric mucosal scraping). The applicability of these results to the overall pig population is very limited due to the small number of samples tested, and is likely confounded by several factors, including age of pigs, time of year samples were collected, and variability in sampling a large surface area. Nonetheless, these results suggest that *H suis* may be rather common in US pigs.

Previous studies in Europe have found that the incidence of *H suis* increases with age, starting post weaning and reaching 90% in adult pigs.[16] Our results are consistent with *H suis* being quite common in US pigs; however, this study was not designed to determine estimates of prevalence of *H suis* in specific states or in pigs of different ages. We found no obvious correlation of pig age with being positive on the TM-PCR for *H suis* infection, since the young pigs tested from Michigan and Minnesota were very different in the percent of positive pigs – 47.4% (95% CI, 32.5%-62.7%) and 100% (95% CI, 67.9%-100%), respectively. Further studies are needed to address these specific questions.

The apparently high prevalence of *H suis* raises concerns regarding zoonosis and food safety. *Helicobacter suis* is the most common NHPH found in humans.[14] The potential role of NHPHs from swine (and several other domesticated species) in human disease has been reviewed.[15] Evidence for indirect exposure causing *H suis* infection is lacking, but there has been at least one report of viable *H suis* in pork samples.[25] We have found that *H suis* spreads rapidly from experimentally infected pigs to adjacent groups of pigs and have identified *H suis* DNA in saliva from infected pigs (unpublished data). These observations suggest that *H suis* is highly contagious and easily spread between pigs. Whether *H suis* is also highly contagious to people in direct contact with pigs is not clear, but close contact with pigs has been associated with *H suis* infection (known as *H heilmannii* at the time).[26] While other bacteria certainly have more practical significance as pig-derived zoonoses, *H suis* could well be added to the list. Future studies to confirm transmission of specific, identical *H suis* strains between

Figure 2: *Helicobacter* genus-specific or *Helicobacter suis*-specific DNA in porcine gastric scrapings. TaqMan polymerase chain reactions (TM-PCRs) with either the *Helicobacter* genus-specific (A) or *H suis*-specific (B) primers and probes were used to assay total DNA isolated from porcine stomachs. The results are shown as a histogram of the percent pigs from all states that had the indicated number of *Helicobacter* genome copies per 5 mg of sample. The number of *Helicobacter* genome copies is graphed in $\log_{10}$.



pigs and humans are required to address the zoonotic potential of *H suis*. Further development of assays such as MLST or other DNA fingerprinting methods would enable these studies. One recent study using MLST methods found a close relationship between porcine *H suis* isolates and a diagnosed human case of *H suis* infection.[27]

The relevance of *H suis* colonization to pig health and production is a matter of ongoing investigation. While it may still be argued that the causal link between *H suis* colonization and gastric ulcers is circumstantial, it is clear that *H suis* has the virulence factors to colonize and cause gastric pathology in pigs. The genomes of two *H suis* strains have been sequenced, and *H suis* contains an array of virulence and colonization factors required for gastric survival and pathogenesis of gastric lesions.[21] Homology of *H suis* genes to genes known to be related to colonization and virulence of *H pylori* shows that *H suis* has numerous genes encoding mechanisms of mucosal adhesion, resistance to low pH, chemotaxis, and motility. Homologs to several *H pylori* genes related to gastric pathology have also been identified, including *vacA* (a cytotoxin of gastric epithelial cells), *napA*, and *ggt* (γ-glutamyl transpeptidase, an apoptosis-inducing protein).[21] Further studies have clearly defined an important and specific role for *H suis* γ-glutamyl transpeptidase in inducing both necrosis and apoptosis of gastric epithelial cells.[28] The pathogenesis of *H suis* has also been studied in laboratory animal models. Infection of mice or gerbils results in gastritis characterized by parietal cell death, proliferation of epithelial cells, lymphocyte infiltration, and formation of lymphoid follicles.[29,30] These lymphoid follicles are sites of an active immune response consisting of B lymphocytes, dendritic cells, and T lymphocytes, mediated in part by secretion of interferon-γ.[31] These potential mechanistic links between *H suis* infection and gastritis leading to ulceration of the pars oesophagea are further supported by recent studies in pigs that linked experimental *H suis* infection to gastritis.[20]

Experimental studies of *H suis* in pigs have begun to ascertain the relevance of *H suis* colonization to pig health and production. Challenge of pigs with mouse-derived *H suis* results in colonization and gastritis (infiltration by lymphocytes and plasma cells), but no significant induction of ulcers in the pars oesophagea.[18] Experimental infection of pigs has been reported to lower daily weight gain of infected groups by 5%[19] to 10%.[20] It is likely that gastric ulcers have a complex etiology that makes an experimental challenge model difficult, and it is not yet known if there are growth or feed-efficiency impacts of *H suis* colonization besides its possible role in causing gastric ulcers. Effective challenge models are expected to be complex and will likely need to include some of the well-known cofactors involved in gastric ulcer formation, such as feed quality and continuity, concurrent disease, and other stressors. Vaccine studies in mice have demonstrated the feasibility of vaccination to prevent *H suis* colonization[32] and disease,[33] and antibiotic susceptibility has been evaluated in mice[34] and in cultured *H suis*.[35] Thus, vaccine or therapeutic trials are feasible and may define the relevance of *H suis* to pig health better than extensive efforts to develop a complex challenge model.

Development of assays not dependent on isolation and culture, as we describe here, will enable further studies into the relevance of *H suis* to pig and human health and the development of strategies for prevention and control of related disease.

Implications

- Detection of *H suis* DNA in pigs from five states in three geographic areas suggests that *H suis* is present in the US pig population.

- This quantitative PCR assay will enable further investigation of the relevance of *H suis* to pig health and production and human health.

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Conflict of interest

Dr Dennis L. Foss, Laurice A. Kopta, Jennifer A. Paquette, Dr Terry L. Bowersock, Dr Leszek J. Choromanski, Dr Jeffery E. Galvin, Traci K. Godbee, and Robert W. Laurinat are employees of Zoetis (formerly Pfizer Animal Health). Margaret Sanchez was an employee of Pfizer Animal Health at the time of the study. Zoetis has a research collaboration and licensing agreement with the University of Ghent, holder of *Helicobacter suis*-related patents.

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