

Slaughter Pigs Are Commonly Infected by Closely Related but Distinct Gastric Ulcerative Lesion-Inducing Gastrospirilla

ROBERT ROSENDAAL,^{1*} JAN H. VOS,² THIJS ROUMEN,² RENÉ VAN VUGT,¹ GIOVANNI CATTOLI,¹
ALDERT BART,^{1†} HENRICUS L. B. M. KLAASEN,³ ERNST J. KUIPERS,⁴
CHRISTINA M. J. E. VANDENBROUCKE-GRAULS,¹ AND JOHANNES G. KUSTERS¹

Department of Medical Microbiology and Infection Control, Vrije Universiteit, 1081 BT Amsterdam,¹ Department of Gastroenterology, Academic Hospital Vrije Universiteit, 1081 HV Amsterdam,⁴ Animal Health Service, 5282 SC Boxtel,² and Bacteriological R&D, Intervet International BV, 5831 AN Boxmeer,³ The Netherlands

Received 29 October 1999/Returned for modification 26 March 2000/Accepted 30 April 2000

An association between (unculturable) gastrospirillum-like organisms (GLO) and ulcerative lesions in the pars oesophagea in stomachs of swine has been claimed. In dogs GLO detected by microscopy may represent several *Helicobacter* species or subspecies. Therefore we investigated which *Helicobacter* spp. are present in stomachs of swine and their possible association with ulcerative lesions of the pars oesophagea. The presence of *Helicobacter* spp. in the antrum and pars oesophagea in 122 stomachs of slaughter swine was determined by microscopy ($n = 122$), by culture on selective and nonselective media ($n = 112$), and by a genus-specific 16S ribosomal DNA (rDNA) PCR ($n = 80$). GLO could not be cultured. Phylogenetic analysis of 43 16S rDNA fragments (out of 54 PCR-positive biopsy specimens) revealed the presence of *Helicobacter heilmannii* type 1 in 42 of them. This correlated with the presence of bacteria with GLO morphology. *Helicobacter bilis* 16S rDNA was amplified directly from one sample harboring bacteria with *H. bilis* morphology. The association between *Helicobacter* spp. and gastric lesions was investigated with a second group of 41 pigs with ($n = 21$ cases) or without ($n = 20$ controls) gastric lesions. Fifteen of the 21 cases were positive by PCR or microscopy, compared to 7 of 20 of the controls ($P = 0.03$). 16S rDNA sequence analysis of 7 of 14 PCR-positive cases revealed the presence of *H. heilmannii* type 1. Microscopy showed bacteria with GLO morphology. One sample (cases) was culture negative but PCR positive for *Helicobacter pullorum*-related 16S rDNA. In conclusion, our findings indicate that *H. heilmannii* type 1 is the predominant *Helicobacter* spp. in the stomachs of pigs and that its presence is associated with ulcerative lesions in the pars oesophagea.

Gastric ulceration in the pars oesophagea is a well-known problem in swine which can lead to growth retardation, bleeding, and death (6, 20). In The Netherlands ulcerative lesions are observed in up to 25% of slaughterhouse swine (6, 9). Many factors have been considered as possible causes, e.g., nutrition, stress, and infection. However, the etiology of gastric ulcer formation in swine is still unknown.

A spirally shaped bacterium, *Helicobacter pylori*, has been identified as the major cause of peptic ulcer disease in humans (4). It has also been known for some time that spirally shaped gastrospirillum-like organisms (GLO) are present in the stomachs of pigs (15). In view of this knowledge several investigators have studied and confirmed the association between GLO, as detected by microscopy, and ulcerative lesions in the pars oesophagea of stomachs of swine (1, 2, 17). Despite several attempts these GLO could not be grown in vitro, and detection of them was based only on microscopy. In dogs it has been demonstrated that bacteria with GLO morphology can represent several species (5, 10), which may differ in their disease-inducing properties. Determination of whether this also holds true for GLO observed in swine requires a technique that discriminates between these potentially different GLO strains. Cloned 16S ribosomal DNA (rDNA) from GLO derived from

one pig appeared to be 99.5% similar to human *Helicobacter heilmannii* type 1 (13, 18). This enables the detection of *Helicobacter* spp. (including GLO) in pigs based on a 16S rDNA *Helicobacter* genus-specific PCR. Sequence analysis of the amplified fragments can then be used for species or subspecies analysis.

We analyzed gastric biopsy specimens from swine for the presence of different *Helicobacter* species or subspecies by direct 16S rDNA PCR and sequence analysis. *Helicobacter*-specific primers were selected based on 16S rDNA of different *Helicobacter* spp. and some other bacterial genera. In addition, we attempted to culture GLO directly from stomach samples. Finally, the association between *Helicobacter* spp. and ulcerative lesions in the pars oesophagea was investigated.

MATERIALS AND METHODS

Animals. In the first series 122 swine stomachs collected at three different slaughterhouses in The Netherlands were investigated for the presence of different *Helicobacter* spp. All stomachs were processed within 3 h after collection, mostly at the slaughterhouse in order to avoid loss of viability of bacteria due to delay by transportation. The stomachs were opened along the greater curvature and washed gently with tap water. Mucus from the antrum and/or esophagus was collected for microscopic examination ($n = 122$), culture ($n = 112$), and PCR ($n = 80$). For microscopy, mucus was smeared directly on glass slides and air dried. Mucus was inoculated on different agar media for culture immediately after collection. For PCR analysis, samples were stored in 100 μ l of buffer (10 mM Tris-HCl [pH 8], 1 mM EDTA, 0.5% Tween 20) at -20°C .

The association between *Helicobacter* spp. and ulcerative lesions was investigated with stomachs of a second series of pigs sent in for pathological examination to the Animal Health Service in Boxtel, The Netherlands. Stomachs were scored for the presence of macroscopically visible lesions according to a previously described classification (9). Stomachs were classified on a scale from 0 to 5, reflecting an increasing abnormality of the mucosa of the pars oesophagea. Score 0 means no abnormalities. Scores 1 and 2 imply increased thickening of the

* Corresponding author. Mailing address: Academic Hospital Vrije Universiteit, Department of Medical Microbiology and Infection Control, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands. Phone: 31 20 4440488. Fax: 31 20 4440743. E-mail: r.roosendaal@azvu.nl.

† Present address: Department of Medical Microbiology, Academic Medical Center, 1100 DD Amsterdam, The Netherlands.

TABLE 1. Primers

Primer	Sequence (5'-3')
8FPL2.....	TGC AGA GTT TGA TCC TGG CTC AG
8F.....	AGA GTT TGA TCC TGG CTC AG
H16s6.....	CAC CCT CTC AGG CCG GA
274R.....	TCT CAG GCC GGA TAC CCG TCA TAG CCT
782R.....	GTT TGC TCC CCA CGC TTT C
1492R.....	GGT TAC CTT GTT ACG ACT T

mucosa (hyperkeratosis). Scores 3 to 5 reflect an increasing damage of the mucosa from less than 5 small (length, <2.5-cm) to more than 10 large (length, >5-cm) erosions or frank ulcers. We defined all stomachs with score 3 or higher as stomachs with ulcerative lesions. Biopsy specimens of the pars oesophageae and the antra of 21 stomachs with ulcerative lesions (cases; 4 with score 3, 2 with score 4, and 15 with score 5) and of 20 stomachs without lesions (controls) were collected. Small specimens of the luminal sides of these biopsy specimens were cut out and stored in 200 μ l of buffer (10 mM Tris-HCl [pH 8], 1 mM EDTA, 0.5% Tween 20) at -20°C for PCR analysis.

In addition, tissue specimens from the antrum, cardia, fundus, and pars oesophageae were collected for microscopic examination.

Detection of helicobacter bacteria by microscopy and culture. Mucus samples from the stomachs of the first group of pigs ($n = 122$) were Gram stained and examined by light microscopy.

Culture of mucus was performed in an atmosphere of 85% N_2 -10% CO_2 -5% O_2 on both selective and nonselective media. Selective media were Columbia agar (BBL)-7% lysed horse blood with Dent supplement (Oxoid), brain heart infusion agar (Difco)-10% lysed horse blood with Skirrow supplement (Oxoid), brain heart infusion agar-2% horse serum-0.0001% dextrin with Skirrow supplement, and brucella broth (Difco)-1% newborn calf serum with Dent supplement. As nonselective media Columbia agar-7% lysed horse blood, brain heart infusion agar-7% horse blood, and chocolate agar with 1% IsoVitalX (BBL) were used. Cultures were checked daily for up to 2 weeks.

PCR analysis. After the gastric specimens were thawed, protease (2 mg/ml) was added, and samples were heated for 60 min at 37°C and subsequently boiled for 10 min. The samples were centrifuged for 5 min at $12,000 \times g$, and PCR was performed on 2 μ l of the supernatant in a final volume of 50 μ l. PCR was performed in a Perkin-Elmer 9700 thermocycler, and the primers used for these PCRs (25 pmol per reaction) are listed in Table 1. Each PCR (final MgCl_2 concentration, 1.5 mM) consisted of a denaturation step of 3 min at 94°C followed by 40 cycles of 1 min of denaturation at 94°C , 1 min of annealing at 65°C for the first cycle reduced by 1°C per cycle to 55°C , and 2 min of extension at 72°C . Finally, elongation was completed at 72°C for 10 min.

Two positive-control samples with *H. pylori* DNA equivalent to 100 or 1 CFU were always included with each run of PCRs. For each 10 samples a negative control consisting of 100 μ l of distilled water that was processed in the same way as the biopsy specimens was included.

Primer combinations 8FPL2-274R and 8F-H16s6 were used to amplify fragments of approximately 280 bp of *Helicobacter* 16S rDNA directly from biopsy specimen lysates. As an inhibition control we spiked PCRs with a double-stranded DNA fragment of 339 bp flanked by sequences of primers 8F and H16s6; we used an amount that gave a clear signal on gel but that did not affect the sensitivity of the PCR.

Both the combinations 8F-782R and 715F-1492R were used to amplify the 16S rDNA of cultured *Helicobacter bilis*-like bacteria for sequence analysis. Amplified samples (10 μ l) were analyzed by electrophoresis in a 1% agarose gel and visualized by ethidium bromide staining and long-wavelength UV light.

Cloning. All DNA manipulations were performed according to standard techniques unless stated otherwise. PCR fragments were ligated into the pGEM-T vector according to the manufacturer's instructions (Promega) and introduced into competent DH5 α F cells. Transformants were selected on Luria-Bertani plates supplemented with ampicillin at 100 $\mu\text{g}/\text{ml}$ and isopropylthio- β -galactoside and 5-bromo-4-chloro-3-indolyl- β -D-galactoside. Several transformants were picked, and plasmids were isolated with a QIAprep plasmid kit. Restriction analysis with *Pst*I and *Nco*I (New England BioLabs, Beverly, Mass.) was performed to confirm the presence and size of the insert.

Sequencing. The DNA sequences of the pGEM-T plasmids containing the amplified 16S rDNA fragment were determined with the Thermo Sequenase premixed cycle sequencing kit and standard Texas red-labeled M13 forward and reverse primers on a Vistra system 725 DNA sequencer (Amersham Pharmacia Biotech, Essex, United Kingdom).

Sequences were aligned with ClustalX (21). The alignments were used to calculate a distance matrix from which the most likely phylogenetic tree was inferred with the programs of J. Felsenstein distributed as part of the PHILYP package (7).

Histopathological examination. Gastric biopsy specimens of the second group of 41 animals were fixed in 10% buffered formalin, embedded in paraffin wax, cut at a thickness of 4 μm , and stained with hematoxylin and eosin and by the Warthin-Starry staining procedure. Gastritis was classified semiquantitatively as

absent (only a few scattered mononuclear cells), mild, moderate, or severe, reflecting the presence of an increasing number of mononuclear cells.

Statistical analysis. To compare proportions the Fisher exact test was used.

Nucleotide sequence accession numbers. Sequences determined have been deposited in GenBank under the following accession numbers: AF252624 (*H. pullorum*), AF252625 (*H. heilmannii* type 1), AF252626 (*H. bilis*).

RESULTS

Primer selection. In a pilot study primers 8F and 274R were used to detect *Helicobacter* spp. in stomach samples of pigs by PCR. However, our data (not shown) revealed that 247R was not as *Helicobacter* sp. specific as claimed (18). In order to select *Helicobacter* spp.-specific primers, alignments were performed with the following 16S rDNA sequences (GenBank accession numbers are in parentheses): *Helicobacter pullorum* (L36141), *Helicobacter muridarum* (M80205), *Helicobacter cinaedi* (M88150), *Flexispira rappinii* (M88137), *H. pylori* (U01330), *Helicobacter acinonyx* (M88148), *Helicobacter felis* (M57398), *Gastrospillum hominis* type 1 (L10079), *G. hominis* type 2 (L10080), *Wolinella succinogenes* (M88159), *Campylobacter coli* (L04312), *Campylobacter jejuni* (L04315), *Campylobacter lari* (L04316), *Escherichia coli* (M24828), *Pseudomonas aeruginosa* (X06684), and *Proteus vulgaris* (X07652).

Criteria for the selection of primers were as follows: at least a two-base difference at the 3' end between helicobacter 16S rDNA (including *Gastrospillum* and *W. succinogenes*) and nonhelicobacter 16S rDNA, amplification of a relatively short 16S rDNA fragment of 200 to 300 bp to minimize the risk of missing positive samples due to DNA degradation, and amplification of a 16S rDNA region that was sufficiently variable to enable phylogenetic analysis of generated fragments.

H16s6 fulfills our specificity criteria. In combination with 8F (eubacterial primer) H16s6 amplifies a fragment of 279 bp in a highly variable region of the 16S rDNA.

With serial dilution of *H. pylori* DNA and *C. jejuni* DNA the primer combination 8F and H16s6 proved to be highly sensitive and specific. *H. pylori* DNA was detected at an equivalent of 1 CFU, whereas a 10^6 excess of *C. jejuni* DNA was not amplified.

The usefulness of the 16S rDNA fragments generated by 8F and H16s6 for phylogenetic analysis was investigated by comparing phylogenetic trees generated by analysis of the 279-bp highly variable fragments and complete 16S rDNA of the different *Helicobacter* species and related genera. No major differences between the phylogenetic trees generated by both approaches were observed (data not shown).

Screening for the presence of *Helicobacter* species or sub-species. Fifty-four of the 80 stomachs investigated (first series) were PCR positive. Sequence analysis of 43 fragments revealed the presence of *H. heilmannii* type 1 in 42 of them (Fig. 1). The corresponding positive smears revealed the presence of bacteria with GLO morphology. In one other (GLO-negative) biopsy sample bacteria with *H. bilis* morphology were observed but not cultured. 16S rDNA analysis of the amplified fragment revealed the presence of *H. bilis*-related bacteria (strain from pig 69). Isolation attempts were negative, except in one case where an *H. bilis*-related species (as determined by 16S rDNA analysis) was cultured (isolate from pig 63). In one case bacteria with GLO morphology were observed after 1 week of culture on brain heart infusion agar-7% horse blood with Skirrow supplement and amphotericin B. However, they were lost upon subsequent subculture.

Association of *Helicobacter* spp. with ulcerative lesions. *Helicobacter* spp. were found in the stomachs of 15 of the 21 cases, whereas 7 out of the 20 controls were positive ($P = 0.03$). PCR was inhibited in eight samples, but never in both gastric spec-

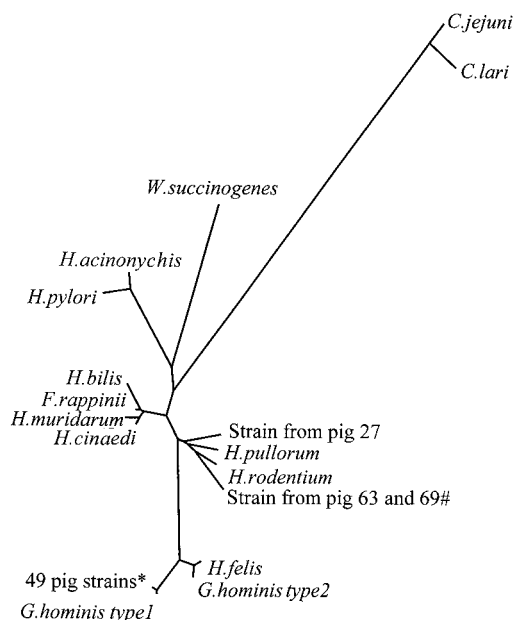


FIG. 1. Inferred evolutionary distances of selected 16S rDNA gene sequences. The following 16S rDNA sequences were selected directly from GenBank (accession numbers are in parentheses): *C. jejuni* (Z29326), *C. lari* (L04316), *W. succinogenes* (M26636), *H. acinonychis* (M88148), *H. pylori* (U01330), *H. cinaedi* (M88150), *H. muridarum* (AF013464), *H. pullorum* (L36146), *F. rappinii* (AF034135), *H. rodentium* (U96300), *H. bilis* (AF04784), *H. felis* (U51870), *G. hominis* type 1, (L10079), *G. hominis* type 2 (L10080). An alignment was made from these sequences using the ClustalX programs (21). This alignment was then used to calculate a distance matrix from which an unrooted phylogenetic tree was inferred by the neighbor-joining method with the help of the programs of J. Felsenstein (University of Seattle) that are distributed as part of the PHYLIP package (7). *, 42 strains are from series 1, and 7 strains are from series 2; #, the sequence for the strain from pig 63 is from an isolate; all other pig strain sequences are from direct PCR on gastric biopsies containing unculturable GLO.

imens of the same animal. Of the 22 positive animals, 14 were positive by PCR. 16S rDNA fragments of 8 of the 14 PCR-positive animals were sequenced. After phylogenetic analysis seven 16S rDNA fragments were identified as belonging to bacteria most related to *H. heilmannii* type 1 (Fig. 1). The corresponding microscopy samples showed bacteria with GLO morphology when positive. From one sample a fragment from a bacterium with 16S rDNA most closely related to *H. pullorum* 16S rDNA was generated (Fig. 1), but no bacteria were observed in the microscopic preparation from this animal. In the 22 *H. heilmannii* type 1-positive animals, these bacteria were found in various locations of the stomach and often at multiple sites (15, 9, 8, and 11 cases in the antrum, fundus, cardia, and pars oesophagea, respectively).

In all cases gastritis was present as indicated by the presence of mononuclear cells. Strikingly, there was no significant association between gastritis and the presence of GLO ($P > 0.05$).

DISCUSSION

Spirally shaped bacteria with GLO morphology have been detected in the stomachs of pigs by light microscopy (15). However, bacteria with GLO morphology can represent different *Helicobacter* species (5, 10). This emphasizes the need for classification of these bacteria by criteria other than strictly morphological characteristics. Thus far culture of GLO from pigs has not been successful. In this study also GLO could not be cultured on artificial media which support the growth of

various *Helicobacter* species. Therefore, comparison of GLO from pigs based on biochemical criteria is not possible. The phylogenetic relationship between bacteria is often established by sequence analysis of the 16S rDNA. 16S rDNA sequencing of DNA obtained from pig GLO has been performed once previously (13). In that study the authors claimed 99.5% homology with *H. heilmannii* type 1 (formerly *G. hominis* type 1) (13, 18). However, their sequence data are not publicly available, and the data were derived from GLO obtained from only one animal. We examined a large number of pigs from three distinct geographic locations for the presence of different *Helicobacter* species by direct PCR and sequence analysis of a highly variable part of the 16S rDNA. Our data demonstrate that *H. heilmannii* type 1 is the predominant *Helicobacter* species in stomachs of pigs. The detection of *H. heilmannii* type 1 by PCR and sequence analysis corresponded with the presence of bacteria with GLO morphology. Therefore, it is highly likely that the GLO associated with gastric ulcerative lesions found by others (1, 17) are *H. heilmannii* type 1. We also confirmed the association of *H. heilmannii* type 1 with the presence of ulcerative lesions in the pars oesophagea of the stomach. Not all animals with gastric ulceration were *H. heilmannii* type 1 positive, which correlates with the findings with respect to GLO of Barbosa et al. (1). This may be due to the detection technique used because a highly sensitive mouse inoculation test showed that all animals with ulcers were GLO positive (14, 17). Strikingly, *H. heilmannii* type 1 was mainly found in the glandular region of the stomach and to a lesser extent in the pars oesophagea, while ulcerative lesions were mostly observed in the nonglandular pars oesophagea. Should there be a role for *H. heilmannii* type 1 in the induction of ulcerative lesions, this finding suggests a direct or indirect influence on the glandular part of the stomach. This could be G-cell hyperfunction or a direct influence on parietal cells, both of which influence acid production. In addition, *H. heilmannii* type 1 might affect mucus production altering the protective capability of the mucosa.

Helicobacter species other than *H. heilmannii* type 1 were found in only three animals and seem not to be of major significance. In one animal we detected an *H. pullorum*-related strain. In two other pigs (pigs 63 and 69) *H. bilis*-related strains were found, one of which could be cultured (strain from pig 63). This *H. bilis* isolate was from an *H. heilmannii* type 1, GLO-positive biopsy specimen. This emphasizes the need to apply multiple techniques to study the diversity of *Helicobacter* species in pigs. In Fig. 1 both *H. bilis*-like strains (pigs 63 and 69) are clustered with *H. pullorum* and *Helicobacter rodentium*, whereas individual 16S rDNA sequences clearly classified both strains as being *H. bilis* because the amplified fragments of these strains contain a 170-bp insertion fragment that is present in *H. bilis* and absent in all other helicobacters. In the phylogenetic analysis, however, this 170-bp insertion/deletion is only scored as a single difference (and not as 170), because in the evolution of this bacterium it probably represented a single insertion/deletion event. Strikingly the sequence outside this 170-bp *H. bilis*-specific insertion/deletion is more similar to that of *H. pullorum*, and therefore in the evolutionary tree of Fig. 1 this isolate is clustered more closely to *H. pullorum* than to *H. bilis*.

Gastritis in pigs consisted predominantly of mononuclear cell infiltrates, as observed by others (1, 2, 17). This is in contrast with the chronic active gastritis found in association with *H. heilmannii* in humans (19). *H. pylori* induces active gastritis in humans but only a lymphocytic gastritis in gnotobiotic swine (11). Thus it seems that different hosts present different histopathological responses to the same *Helicobacter*

spp. Conflicting data about the association between GLO and gastritis in pigs have been presented (1, 2, 11, 17). In our study GLO were not associated with the presence of gastritis. This corresponds with the findings of Barbosa et al. (1). However, the same group has demonstrated an association between GLO and antrum gastritis in other investigations (17), a finding also confirmed by others (2). In one study there was even a 100% association, in which antrum gastritis was present in all GLO-positive animals and absent from histologically normal stomachs (17). An explanation for these discrepancies may be differences in the sensitivities of detection of GLO and the use of different criteria for the classification of gastritis. Severe inflammation was observed only in the pars oesophagea in the presence of lesions, regardless of the presence of *H. heilmannii* type 1. In all other cases inflammation was mild or moderate. If *H. heilmannii* induces gastritis in pigs, then this gastritis is moderate at most. This corresponds to the relatively mild gastritis observed in humans in the presence of *H. heilmannii*.

H. heilmannii has been found in association with peptic ulcers in humans (3, 8, 19). In a large study seven out of eight ulcers found in 202 *H. heilmannii*-positive patients were from patients who also received NSAIDs (19). In contrast, Debongnie et al. demonstrated that characteristics of patients with ulcers who were *H. heilmannii* positive differed substantially from those of patients with *H. pylori* and those using NSAIDs (3). A recent study describes ulcer healing in patients with duodenal ulcers after eradication of *H. heilmannii* (8). These observations suggest a role for *H. heilmannii* in ulcerogenesis. Data about the role of GLO (which are probably *H. heilmannii* type 1) in ulcerogenesis in pigs are conflicting (12, 16). Whereas Landrace pigs developed ulcers after inoculation with GLO (16), gnotobiotic piglets did not (12). In the latter study ulcers developed only in animals infected with fermentative bacteria (*Lactobacillus* spp.) in combination with a high-carbohydrate diet. This suggests a primary role for locally induced bacterial acid production in ulcerogenesis.

In conclusion, *H. heilmannii* type 1 is the predominant *Helicobacter* species in swine stomachs and is associated with the presence of ulcerative lesions in the pars oesophagea.

ACKNOWLEDGMENT

Part of this work was supported by Intervet International B.V., Boxmeer, The Netherlands.

REFERENCES

- Barbosa, A. J. A., J. C. P. Silva, A. M. M. F. Nogueira, E. Paulino, and C. R. Miranda. 1995. Higher incidence of *Gastrospirillum* sp. in swine with gastric ulcer of the pars oesophagea. *Vet. Pathol.* **32**:134–139.
- Bedel, A., F. Pichard, N. Wuscher, A. Labigne, and M. Huerre. 1997. Prevalence of helicobacter infection and gastric-associated pathologies in swine originating from pork producers in the west of France. *Gut* **41**(Suppl. 1): A124.
- Debongnie, J. C., M. Donnay, J. Mairesse, V. Lamy, X. Dekoninck, and B. Ramdani. 1998. Gastric ulcers and *Helicobacter heilmannii*. *Eur. J. Gastroenterol. Hepatol.* **10**:251–254.
- Dunn, B. E., H. Cohen, and M. J. Blaser. 1997. *Helicobacter pylori*. *Clin. Microbiol. Rev.* **10**:720–741.
- Eaton, K. A., F. E. Dewhirst, B. J. Paster, N. Tzellas, B. E. Coleman, J. Paola, and R. Sherding. 1996. Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: animal and public health implications. *J. Clin. Microbiol.* **34**:3165–3170.
- Elbers, A. R. W., M. J. C. Hessing, M. J. M. Tielen, and J. H. Vos. 1995. Growth and oesophago-gastric lesions in finishing pigs offered finely pelleted feed *ad libitum*. *Vet. Rec.* **136**:588–590.
- Felsenstein, J. 1989. PHYLIP (phylogeny inference package) version 3.5c. *Evolution* **39**:783–791.
- Goddard, A. F., R. P. H. Logan, J. C. Atherton, D. Jenkins, and R. C. Spiller. 1997. Healing of duodenal ulcer after eradication of *Helicobacter heilmannii*. *Lancet* **349**:1815–1816.
- Hessing, M. J. C., M. J. Geudeke, C. J. M. Scheepens, M. J. M. Tielen, W. G. P. Schouten, and P. R. Wiepkema. 1992. Slijmvliesveranderingen in de pars oesophagea bij varkens: prevalentie en de invloed van stress. *Tijdschr. Diergeneeskd.* **117**:445–450.
- Jalava, K., M. Kaartinen, M. Utriainen, I. Happonen, and M. L. Hänninen. 1997. *Helicobacter salomonis* sp. nov., a canine gastric *Helicobacter* sp. related to *Helicobacter felis* and *Helicobacter bizzozeroni*. *Int. J. Syst. Bacteriol.* **47**: 975–982.
- Krakowka, S., D. R. Morgan, W. G. Kraft, and R. D. Lunk. 1987. Establishment of gastric *Campylobacter pylori* infection in the neonatal gnotobiotic piglet. *Infect. Immun.* **55**:2789–2796.
- Krakowka, S., K. A. Eaton, D. M. Rings, and R. A. Argenzio. 1998. Production of gastroesophageal erosions and ulcers (GEU) in gnotobiotic swine monoinfected with fermentative commensal bacteria and fed high-carbohydrate diet. *Vet. Pathol.* **35**:274–282.
- Mendes, E. N., D. M. M. Queiroz, F. E. Dewhirst, B. J. Paster, G. A. Rocha, and J. G. Fox. 1994. Are pigs a reservoir for human helicobacter infection? *Am. J. Gastroenterol.* **8**:1296.
- Mendes, E. N., D. M. M. Queiroz, G. A. Rocha, S. B. Moura, M. T. Barbosa, S. M. Carvalhaes, M. L. P. Freitas, and A. C. S. Reis. 1992. In vivo culture improves the diagnosis of "Gastrospirillum suis" infection. *Ir. J. Med. Sci.* **61**:78.
- Queiroz, D. M. M., G. A. Rocha, E. N. Mendes, A. P. Lage, A. C. T. Carvalho, and A. J. A. Barbosa. 1990. A spiral organism in the stomach of pigs. *Vet. Microbiol.* **24**:199–204.
- Queiroz, D. M. M., G. A. Rocha, E. N. Mendes, S. B. Moura, A. M. R. Oliveira, and C. A. Oliveira. 1994. Swine experimentally infected with helicobacter as an animal model of ulcerogenesis. *Am. J. Gastroenterol.* **8**:1320.
- Queiroz, D. M. M., G. A. Rocha, E. N. Mendes, S. B. Moura, A. M. R. Oliveira, and D. Miranda. 1996. Association between *Helicobacter* and gastric ulcer disease of the pars oesophagea in swine. *Gastroenterology* **111**:19–27.
- Solnick, J. V., J. O'Rourke, A. Lee, B. J. Paster, F. E. Dewhirst, and L. S. Tompkins. 1993. An uncultured gastric spiral organism is a newly identified *Helicobacter* in humans. *J. Infect. Dis.* **168**:379–385.
- Stolte, M., G. Kroher, A. Meining, A. Morner, E. Bayerdörffer, and B. Bethke. 1997. A comparison of *Helicobacter pylori* and *H. heilmannii* gastritis. *Scand. J. Gastroenterol.* **32**:28–33.
- Taylor, D. J. 1984. Pig diseases, 4th ed. Burlington Press, Cambridge, United Kingdom.
- Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**:4673–4680.