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# Molecular evidence of *Helicobacter suis* infection in pigs in Nigeria

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***Helicobacter suis*** **mainly colonizes the stomach of pigs and occasionally infects humans. It is a prevalent cause of gastritis and gastric ulcers in both species. This study was designed to determine the colonization rate of *H. suis* in the stomach of pigs in Nigeria. Pig slaughter house surveys were conducted and stomach mucosa samples were collected from the fundus of the stomach of a total of 160 pigs in four locations in Nigeria (Lagos, Delta, Enugu and Plateau States). In each location, 50% of the samples were collected from stomachs with ulceration in the fundus, while the rest were from those with no gross lesions. DNA was extracted and PCR assay was conducted using standard primers. Data was analyzed by descriptive statistics and Chi-square test (p< 0.05). *H. suis* was detected in 8.75% of the samples across the four locations at a frequency of 15, 7.5, 10 and 2.5% in Lagos, Delta, Enugu and Plateau states, respectively. *H suis* was detected in 12.5 and 5% of the stomachs with ulcers and without ulcers, respectively. *H suis* colonizes pigs in Nigeria at a relatively low rate with its colonization rate being higher in stomachs with ulcers. There is need for characterization of the strains of the organism in Nigeria for a better understanding of its possible role in gastric ulceration in pigs.**

**Key words:** Gastric ulcer, *Helicobacter suis,* pigs, stomach.

## INTRODUCTION

Numerous members of the *Helicobacter* species are adapted to the harsh acidic environment of the stomach of humans and many animal species. The colonization of the human stomach by unidentified spiral organisms was reported as early as 1889 (Konturek, 2003), while Bizzozero reported the presence of similar organisms in canine gastric mucosa in 1893 (Danon and Lee, 2001). Interestingly, the role of these bacteria in causation of gastric ulcer disease in man was not established until 1984 (Marshall and Warren, 1984). The discovery stimulated the interest of researchers in this organism, thereby leading to the discovery of increasing numbers of members of this genus and the hosts they are adapted to. *Helicobacter suis* is a zoonotic organism and it has

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**Table 1.** Primers used for detection of *H. suis* in stomach of the pig samples.

| Species | Primers | Target gene | Sequence (5′-3′) | Annealing position | Product length (bp) |
|---|---|---|---|---|---|
| *H. suis* | V832f | 16S rRNA | TTG GGA GGC TTT GTC TTT CCA | 832 to 852 | 433 |
| | V1261r | | GAT TAG CTC TGC CTC GCG GCT | 1261 to 1281 | |

been reported to colonize up to 60% of the stomach of slaughtered pigs (Grasso et al., 1996; Park et al., 2004; Kopta et al., 2010). It is associated with gastric pathologies in these animal species (Haesebrouck et al., 2009; Choi et al., 2001; Roosendaal et al., 2000). It is also the most frequently detected non-*Helicobacter pylori* Helicobacter (NHPH) species in humans with gastric disease (De Groote et al., 2005; Van den Bulck et al., 2005) where it may cause gastritis, peptic ulcer disease and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Flahou et al., 2012; Morgner et al, 2000). The risk of developing MALT lymphoma in fact seems to be higher after infection with NHPH as compared to *H. pylori* infection in humans (Stolte et al., 2002). Predisposing factors to infection may include close contacts and consumption of uncooked or undercooked pork (De Cooman et al., 2014; Pasmans and Haesebrouck, 2014). There is dearth of information on *H. suis* infection in pigs in Nigeria.

This study was therefore designed to provide information on the molecular evidence of infection and the frequency of detection of *H. suis* in pigs in Nigeria and to evaluate a possible association between its colonization and occurrence of gastric ulcers in pigs in Nigeria.

## MATERIALS AND METHODS

### Sampling

Stomach mucosal samples (approximately 2 $cm^2$) were obtained from healthy pigs presented for slaughter at four abattoirs located in Lagos, Delta, Enugu and Plateau states of Nigeria between the months of November 2016 and March 2017. The pigs were of both sexes, between the ages of 12 and 18 months from intensively managed flocks and within the weight range of 50 to 90 kg. The samples were from the fundus of the stomachs of 160 pigs (40 samples from each abattoir). 50% of the stomachs purposively sampled were with ulcers in the fundus while the rest were from stomachs without gross lesions.

### Statistical analysis

Data was explored using descriptive statistics and the association between gastric ulcers and *H. suis* infection was subjected to Chi-square test ($p< 0.05$).

### DNA extraction and polymerase chain reaction (PCR)

DNA from 50 mg of tissue was isolated using the ZR Fungal/Bacterial DNA MiniPrep™ isolation kit (Zymo research corporation, USA) following the manufacturers instruction. For PCR assay, previously published primers targeting the 16S rRNA-coding gene of *H. suis* (De Groote et al., 2000; Proietti et al., 2010) were used (Table 1). The primers were manufactured by Integrated DNA Technologies, Belgium. Genomic DNA as positive controls was obtained from the Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, University of Ghent, Belgium. Reactions were performed in a 25 µl consisting of 1 µl template DNA, 12.5 µl One Taq Quick-Load 2X Master Mix (20 mM Tris-HCl, 1.8 mM $Mgcl_2$, 22 mM $NH_4Cl$, 22 mM KCl, 0.2 mM dNTPs, 5% glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween® 20, Xylene Cyanol FF, Tartrazine, 25 units/ml One *Taq* DNA Polymerase), 0.2 µM of each primer and 10.5 µl of nuclease free water in a G-Storm GS1 Thermal Cycler. Amplification of the 16S rRNA gene of *H. suis* was done after an initial 2 min denaturation at 95°C in 35 cycles of 94°C for 30 s, 52°C for 30 s, 68°C for 1 min with a final extension step at 68°C for 10 min and held at 4°C. The PCR products were analyzed by electrophoresis in 1.5% agarose gel containing 5 µl of GRGreen (Nucleic Acid Gel Stain, 10,000X in water) and examined by transillumination.

## RESULTS AND DISCUSSION

A 433 bp fragment of the 16S rRNA-coding gene of *H. suis* was amplified in 8.75% (14/160) of the samples from four states in Nigeria (Figure 1). This included 15% of the samples from Lagos State (6/40), 7.5% from Delta State (3/40), 10% from Enugu State (4/40) and 2.5% from Plateau State (1/40). *H. suis* was detected with overall occurrence of 12.5 and 5% in the stomachs with ulceration and without gross ulceration, respectively (Figures 2 and 3). There was no significant association between the occurrence of gastric ulcers and *H. suis* infection in the stomachs (Table 2).

This study provides molecular evidence of infection and the frequency of detection of *H. suis* in pigs in Nigeria. The widespread detection of this organism in pigs calls for further search retrospectively and prospectively into its role in the causation of disease in pigs and man in Nigeria as it is an established pathogenic and zoonotic agent (Haesebrouck et al., 2009; Flahou et al., 2017).

The current findings show that *H. suis* detection rates in pigs in Nigeria are relatively low (8.75%) as compared to other reports from Europe and Asia, where it often exceeds 60% in slaughter-age pigs (Park et al., 2004; Hellemans et al., 2007). This variation in colonization may be due to the influence of climatic conditions and management factors since the influence of environmental factors on H. pylori colonization in humans has been previously documented (Kusters et al., 2006). The ease

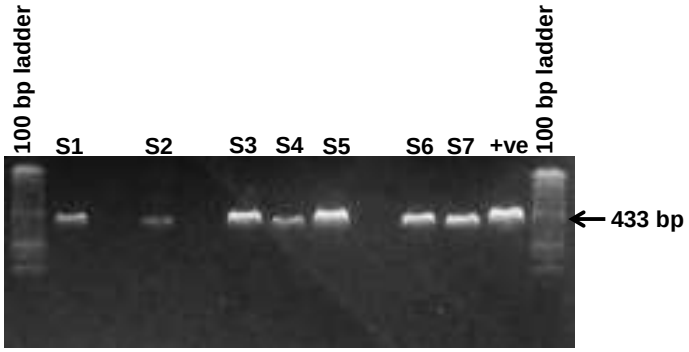


**Figure 1.** Gel electrophoresis image of the amplified 16sRNA gene of *H. suis* as 433 bp bands from pig stomach samples from Nigeria. S1-S6, Positive samples; +ve, positive control.

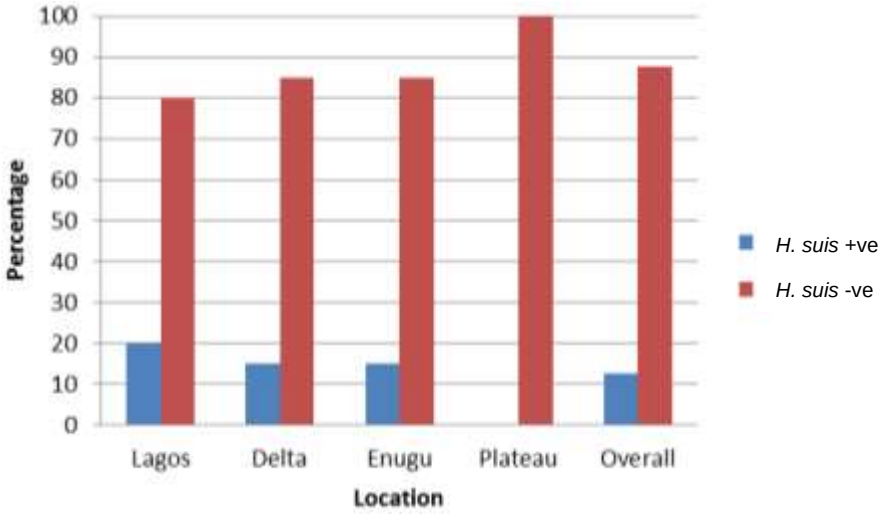


**Figure 2.** Frequency of detection of *H. suis* infection in stomachs of pigs with ulceration in Nigeria.

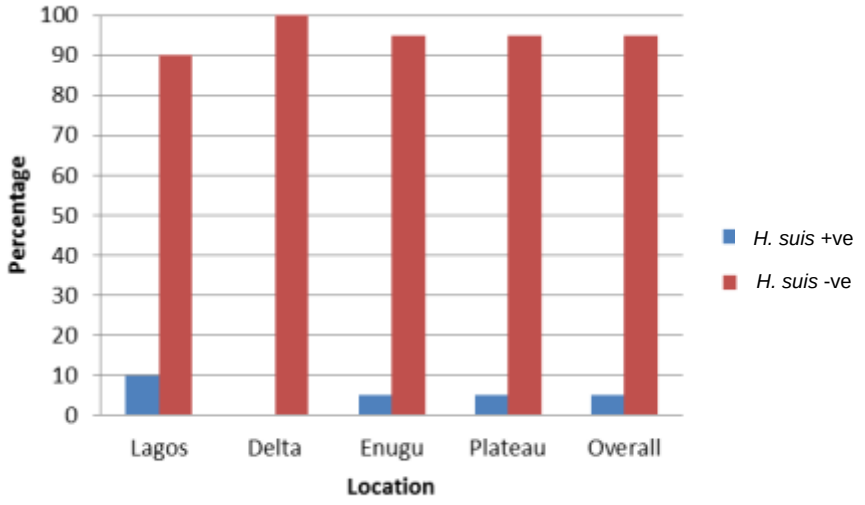


**Figure 3.** Frequency of detection of *H. suis* infection in stomachs of pigs without ulceration in Nigeria.

**Table 2.** Colonization of the stomach mucosa of pigs with *H. suis* in Nigeria.

| Location | | *H. suis* (+) | *H. suis* (-) | Total | Pearson Chi square value | *P*-value |
|---|---|---|---|---|---|---|
| | Ulcer | 4 | 16 | | | |
| Lagos | No Ulcer | 2 | 18 | | | |
| | | 6 | 34 | 40 | | |
| | Ulcer | 3 | 17 | | | |
| Delta | No Ulcer | 0 | 20 | | | |
| | | 3 | 37 | 40 | | |
| | Ulcer | 3 | 17 | | 2.818 | 0.093 |
| Enugu | No Ulcer | 1 | 19 | | | |
| | | 4 | 36 | 40 | | |
| | Ulcer | 0 | 20 | | | |
| Plateau | No Ulcer | 1 | 19 | | | |
| | | 1 | 39 | 40 | | |

of transfer to and adaptation of this organism in man makes it a threat to humans in close contacts with pigs as H. suis is the most prevalent NHPH affecting humans (Haesebrouck et al., 2009). This ease of transfer has been recently attributed to its primate origin (Flahou et al., 2017). H. suis is also known to cause indirect production losses in the swine industry (De Bruyne et al., 2016). In the assessment of the spread of the organism, *H. suis* was detected more frequently in stomachs with gastric ulcers in the fundus (12.5%) but there was no significant association between the occurrence of gastric ulcers and *H. suis* infection. This may infer that although the organism is associated with formation of gastric lesions in pigs, it may not be the sole factor responsible for the ulcerations observed in the fundus of the stomach of pigs in Nigeria. Other studies have conflicting reports on role of the organism in stomach pathologies in pigs (Monteiro, 2011; Queiroz et al., 1996; Roosendaal et al., 2000).

Further studies on this organism in Nigeria should include the characterization of the circulating strains and their associated lesions in pigs. In addition, the frequency of occurrence of *H. suis* in gastric tissues of humans suffering from gastric disease should also be studied as there is previous documented evidence on direct human infection with field strains of *H. suis* from pigs (Joosten et al., 2013).

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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