Absence of Helicobacter pylori in Pediatric Adenoid Hyperplasia

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Objectives: To (1) develop a reverse transcription-polymerase chain reaction assay to determine whether Helicobacter pylori and/or other members of the Helicobacteraceae family are detected in hyperplastic adenoids of children and (2) critically analyze published polymerase chain reaction methods to ascertain whether false-positive detection of H pylori has been reported.

Design: Cohort study.

Patients: Adenoid biopsy specimens (78 hyperplastic and 15 normal) were collected from children aged 2 to 10 years.

Methods: Total RNA was extracted before reverse transcription of bacterial RNA using Helicobacteraceae-specific primer. A nested reverse transcription–polymerase chain reaction protocol was designed to detect all species of the Helicobacteraceae family. A piece of each biopsy specimen was examined histologically.

Results: Laryngopharyngeal reflux was suspected in 41% of the children (n=23) on the basis of the Reflux Symptom Index. No evidence of H pylori was found in any adenoid sample. Candidatus Wolinella africanaus was the only Helicobacteraceae family member detected in 1 hyperplastic adenoid. Histologic examination identified very few bacterial organisms. Previous polymerase chain reaction findings may be the result of false-positive H pylori detection.

Conclusions: Inflammation and enlargement of the adenoids is not likely due to ongoing bacterial infection arising from laryngopharyngeal reflux. We conclude that H pylori and other Helicobacteraceae family members are not major contributors to the development of hyperplastic adenoids in children.


Denoid hyperplasia is a cause of upper airway obstruction that is associated with neurocognitive and behavioral problems in children. Adenoidectomy is commonly performed in children to relieve nasal obstruction or as an adjunctive treatment for otitis media. The causes of adenoid hyperplasia are multifactorial; however, there is evidence that gastric contents can reach the middle ear (via the nasopharynx and eustachian tube). Therefore, it is hypothesized that gastroesophageal reflux and laryngopharyngeal reflux (LPR) may cause adenoid hyperplasia.

Recent studies by Harris et al evaluated LPR biomarker expression in hyperplastic adenoids. Markers of reflux that were investigated included pepsin, carbonic anhydrase III, cyclooxygenase 2, and mucin 5AC (MUC5AC). In hyperplastic adenoid tissue, messenger RNA expression of MUC5AC and cyclooxygenase 2 was significantly downregulated compared with controls, there was no significant difference in carbonic anhydrase III expression, and pepsin was not detected immunohistochemically. This study concluded that there was little evidence for a chronic reflux stimulus (LPR) as a potential cause of adenoid hyperplasia. However, in addition to constant reflux contact, there are mechanisms whereby occasional acute reflux episodes may cause inflammatory changes that could eventually lead to adenoid hyperplasia.

Gastric contents contain many pathogenic organisms, including members of the Helicobacteraceae family. Species of the Helicobacteraceae family (including Helicobacter pylori) can colonize various ecologic niches in the body, and some species have been associated with inflammatory diseases. Specifically, H pylori is associated with the development of stomach ulcers and chronic gastritis. The recent finding of decreased MUC5AC expression in hyperplastic adenoids is of interest because MUC5AC is a receptor for...
infection of gastric epithelium with *H pylori* resulted in downregulation of MUC5AC, therefore suggesting that *H pylori* may be present in hyperplastic adenoids. There are reports of *H pylori* detection in hyperplastic adenoids of children; however, the detection rate is highly variable. These conflicting reports are likely the result of different methods involved (Table 1). Histologic examination and the urease/Campylobacter-like organism test appear to give false-positive results when compared with more sensitive polymerase chain reaction (PCR) techniques now available. The objectives of this study were to develop a highly sensitive and specific reverse transcription (RT)–PCR assay to determine whether *H pylori* or other species of the Helicobacteraceae family are present in the hyperplastic adenoids of children with and without suspected LPR and to review published PCR methodology and ascertain whether false-positive detection of *H pylori* has been reported.

### METHODS

**ETHICAL CONSIDERATIONS, PATIENTS, AND TISSUE SAMPLES**

After institutional ethics committee approval and fully informed parental consent, adenoid biopsy specimens were taken from children (aged 2-10 years) at 2 pediatric hospitals in Adelaide, Australia. All patients receiving adenoidectomy (N=78) had confirmed adenoid hyperplasia on direct visualization intraoperatively and were undergoing the procedure to relieve sleep-disordered breathing. Parents of 36 of these children with hyperplastic adenoids were asked to complete the Reflux Symptom Index (RSI) questionnaire to determine the likelihood of LPR occurring in their child. Specimens of normal adenoids (n=15) were collected from children undergoing routine dental surgical procedures who had no history of adenoid or nasal operations, gastroesophageal reflux, LPR, recurrent tonsillitis, otitis media, or evidence of adenoid hyperplasia (eg, severe snoring, sleep apnea, or runny nose more than once a week).

**NESTED RT-PCR FOR DETECTION OF HELICOBACTERACEAE FAMILY MEMBERS**

Following excision, adenoidal biopsy specimens were stored (RNAlater; Ambion, Austin, Texas) at 4°C overnight, then transferred to −20°C until analysis. For analysis, tissue was removed from the storage solution, homogenized (500 µL of TRIzol; Invitrogen Life Technologies, New York, New York), and processed, as previously described. The final RNA solution was stored at −80 °C until required. A 1-µg sample of total RNA was treated (DNA-free kit; Ambion) and then used for complementary DNA (cDNA) synthesis.

Development of the assay started with the use of random hexamers in the RT reaction (SuperScript III; Invitrogen, Carlsbad, California), according to the manufacturer’s protocol. The nested PCR approach used primers designed to amplify bacterial 16S sequences (primers C93 and C94; PCR product size, 1467 base pairs [bp]; Table 2) for the first-round PCR, fol-

### Table 1. Variability in the Reporting of *Helicobacter pylori* in Adenoid Tissue Samples by Different Detection Methods

<table>
<thead>
<tr>
<th>Source</th>
<th>RT-PCR (Method, Primer Specificity)</th>
<th>CLO</th>
<th>RUT</th>
<th>Histology</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirak et al7</td>
<td>3/10 (Nested, 16S rRNA)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Yilmaz et al8</td>
<td>1/38 (Single round, 23S rRNA)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Bitar et al9</td>
<td>0/25 (Nested, 16S rRNA)</td>
<td>ND</td>
<td>21/25</td>
<td>4/25</td>
<td>ND</td>
</tr>
<tr>
<td>Agirdir et al10</td>
<td>20/42 (Nested, 16S rRNA)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>12/42</td>
</tr>
<tr>
<td>Yavasoglu et al11</td>
<td>ND</td>
<td>2/60</td>
<td>2/60</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Eyigor et al12</td>
<td>0/47 (Single, glmM gene)</td>
<td>ND</td>
<td>3/47</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Abbreviations:** CLO, Campylobacter-like organism test; ND, not done; rRNA, ribosomal RNA; RT-PCR, reverse transcription–polymerase chain reaction; RUT, rapid urease test.

### Table 2. Primer and PCR Details for Identification of Species of the Helicobacteraceae Family in Children’s Adenoids

<table>
<thead>
<tr>
<th>Primer Pairs</th>
<th>Primer Sequence</th>
<th>Annealing Temperature, °C</th>
<th>Expected PCR Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>C93a</td>
<td>AGA GTT TGA TYM TGG TGC ATG AG</td>
<td>60.2</td>
<td>1467</td>
</tr>
<tr>
<td>C94a</td>
<td>TAC GGY TAC TTT GGT AGG ACT TC</td>
<td>54.1</td>
<td>397</td>
</tr>
<tr>
<td>C97a</td>
<td>GCT ATG AGG GGT ATC C</td>
<td>65.2</td>
<td>766</td>
</tr>
<tr>
<td>C96a</td>
<td>GAT TTT ACC CCT ACA CCA</td>
<td>61.1</td>
<td>765</td>
</tr>
<tr>
<td>C97-20b</td>
<td>GGC TAT GAG GGG TAT CCG GC</td>
<td>62.1</td>
<td>457</td>
</tr>
<tr>
<td>H3A-20b</td>
<td>GCC GTG CAC CAC GTG TTG C</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C97a</td>
<td>GCC GTG CAG CAC GTG TTG C</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C96a</td>
<td>GCC GTG CAC CAC GTG TTG C</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C97-20DJH</td>
<td>GCT ATG AGG GGT ATG C</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C95DJH</td>
<td>AGC GTC AGT ATT GT TCA CCA GC</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Abbreviations:** bp, base pairs; PCR, polymerase chain reaction.

7 See Bulajic et al75
8 See Bohr et al16
lowed by primers specific to Helicobacteraceae sequences (primers C97 and C98; PCR product size, 397 bp; Table 2) as described by Bulajc et al. The PCR was repeated using new second-round primers, which had perfect base pair complementarity to the Candidatus Wolinella africanus sequence (C97-20 and H3A-20; PCR product size, 766 bp; Table 2).

A more sensitive assay was then designed involving reverse transcription of bacterial RNA using 2 pmol of Helicobacteraceae-specific primer (H3A-2040; Table 2) and SuperScript III. After inactivation of the reverse transcriptase, the cDNA was treated with 1 µL of endoribonuclease (RNaseH; Invitrogen Life Technologies). The nested RT-PCR used primers to detect members of the Helicobacteraceae family. For first-round PCR, 3 µL of cDNA was used in a 25-µL reaction consisting of 1× PCR buffer (HotStar Taq; Qiagen GmbH, Hilden, Germany), 25 mM magnesium chloride, 40 mM deoxyribonucleotide triphosphates, 0.5 U of HotStar Taq, and 200 nM of each forward and reverse primer (primers C97 and H3A-20; Table 2). Thirty-five cycles of PCR (Rotor-Gene 6000, Corbett Research; Sydney, Australia) were performed, producing a PCR product of 765 bp. These PCR products were diluted 1000-fold, and 3 µL was used in the second-round PCR. The primers for the second-round PCR were modified to include extra bases to aid stability (C97-2035JH and C95DJH; Table 2) with 35 cycles of PCR, producing a PCR product of 457 bp. Positive control cDNA made from *H pylori* was used in every batch of reactions. For this assay, we established a reproducible sensitivity of 1 genome equivalent (ie, 1 *H pylori* bacterium), and its identity was confirmed by sequence analysis.

All PCR products were run on a 2% agarose gel, stained with ethidium bromide, and photographed under UV light. All PCRs on study and control adenoid samples were performed in triplicate and incorporated negative reverse transcriptase and water controls. Verification of PCR products was attained by sequencing (BigDye terminator v3.1 on an Applied Biosystems 3100 Genetic Analyzer; Applied Biosystems, Foster City, California) at the SouthPath and Flinders Sequencing Facility.

**HISTOLOGY OF ADENOID BIOPSIES**

A small piece of adenoid tissue was formalin fixed and paraffin embedded. Four-micrometer tissue sections were mounted onto glass slides and stained with hematoxylin-eosin, and Diff-Quik/Giemsa, a stain routinely used for identification of Helicobacter in tissue sections. An experienced histopathologist (A.C.T.) masked to the PCR results examined the slides, paying particular attention to the presence of microorganisms.

**RESULTS**

**LPR SYMPTOMS IN CHILDREN WITH HYPERPLASTIC ADENOIDS**

An RSI score higher than 13 is considered indicative of likely LPR in adults. The RSI questionnaire was not developed for use in children; however, a recent study found it to be a useful tool for assessing LPR in children. Some questions could not be adequately answered by the parent because of the limited understanding of children regarding terms such as “heartburn”; in such cases, a 0 score was recorded. For this study, an RSI score higher than 13 was also considered to indicate LPR in children, although we are aware that this may result in underrepresentation of this condition. Of the 56 children, 23 (41%) experienced symptoms consistent with LPR using the RSI score; 24 (43%) had scores lower than 11, consistent with no LPR symptoms; and 9 (16%) had scores of 11 to 13, representing ambiguous LPR symptoms.

**NESTED RT-PCR ASSAY**

Initial screening of the first 21 of 78 hyperplastic adenoid samples and 6 of 15 control samples gave no PCR products of the expected size. However, the specificity of the reaction was decreased by increasing the number of PCR cycles (45 cycles of PCR amplification), multiple spurious products resulted. A PCR product of the expected size was detected in only 1 hyperplastic adenoid sample. Sequencing and a database search to test for homologic characteristics to known DNA sequences (Basic Local Alignment Search Tool) identified this to be a match with Candidatus *W africanus*, a member of the Helicobacteraceae family, and identified a mismatch between the primer and target sequences. Repeating the PCR using new second-round primers with perfect base pair complementarity to the Candidatus *W africanus* sequence resulted in a PCR product of the expected size observed in triplicate (only in hyperplastic adenoid sample 3). Sequence analysis confirmed a 100% match to Candidatus *W africanus* (Figure 1).

To ensure maximum sensitivity and specificity for detection of all species of the Helicobacteraceae family in our samples, the final assay incorporated a gene-specific primer to Helicobacteraceae for the RT reaction. This was followed by a nested PCR protocol using 2 rounds of PCR, with each round using primers designed with modifications to increase stability/binding within the Helicobacteraceae genome. When all 93 adenoid samples were screened, only 1 sample produced a PCR product of the expected size (Figure 2). This was the hyperplastic adenoid sample, in which the sequence was already confirmed as Candidatus *W africanus* (hyperplastic adenoid sample 3). *Helicobacter pylori* was not detected in any of the hyperplastic or normal adenoid samples, but was readily detected in the positive control.

**HISTOLOGIC ANALYSIS**

The first 6 of 15 normal adenoid samples and 21 of 78 hyperplastic adenoids were analyzed by an experienced histopathologist (A.C.T.). Specimens displayed respiratory and squamous-type epithelia with underlying lymphoid and deep connective tissues, consistent with adenoid tissue with squamous metaplasia. Special attention was paid to the crypts of the epithelium because this is where *H pylori* is often detected in gastric biopsy specimens. Histologically, *H pylori* presents with a characteristic “gull” shape, whereas another Wolinella species, *Wolinella succinogenes*, is believed to have a spiral shape. The morphologic characteristics of Candidatus *W africanus* are unknown; to date, it has not been successfully isolated and cultured.
Structures suggesting the presence of bacterial organisms were identified in only 1 Giemsa-stained hyperplastic adenoid specimen, consisting of 2 rod-like structures, one with a curvilinear shape. This structure is not consistent with identification as *H pylori*. Bacterial organisms were not detected in any of the other adenoid specimens. In the PCR-positive biopsy (hyperplastic adenoid sample 3), no bacteria were observed. Because of the lack of identification of Helicobacteraceae family members using the specific and sensitive nested PCR protocol, histologic analysis of the remaining adenoid samples was not performed.

**Figure 1.** Basic Local Alignment Search Tool search identifies polymerase chain reaction (PCR) product as Candidatus *Wolinella africanus*, a member of the Helicobacteraceae family. Sequence alignment of PCR product (hyperplastic adenoid sample 3) with GenBank sequence of Candidatus *W africanus* shows a perfect match. Primers used for PCR were C97-20 and H3A-20. Sbjct indicates subject.

It has been hypothesized that reflux may be the cause of adenoid hyperplasia in children, especially with increasing rates of diagnosis of gastroesophageal reflux and LPR. Refluxate can contain pathogenic organisms (eg, *H pylori*) that may be capable of colonizing the upper aerodigestive tract and causing inflammation. Detection rates of *H pylori* in hyperplastic adenoids are variable, depending on the methods used. We have designed a nested RT-PCR protocol with high sensitivity...
and specificity for the detection of all members of the Helicobacteraceae family. Using this assay, we failed to identify *H pylori* in all hyperplastic adenoid samples. In addition, histologic examination did not detect *H pylori* in the adenoid samples.

There are many diagnostic tests to determine the presence of *H pylori* in tissue samples, and these have been reviewed recently with respect to sensitivity and specificity for use in children. Tissue samples are formalin fixed, paraffin embedded, and stained with hematoxylin–eosin as well as special stains, such as Giemsa or silver, or are used for immunohistochemistry. On examination, special attention is paid to the adenoid crypts, and histologically, *H pylori* presents with a characteristic gull shape. Stains have low sensitivity and low specificity because they highlight all bacteria, whereas immunohistochemistry has higher specificity. Culture of *H pylori* has a reported specificity of 100%, but the bacteria are difficult to culture, which leads to a high number of false-negative results. The rapid urease test (*Campylobacter*-like organism test) is used to identify the urease enzyme, which allows *H pylori* to survive in the acidic gastric environment during colonization, resulting in the production of ammonia and causing cell damage and epithelial inflammation. In children, the rapid urease test has slightly higher specificity and sensitivity than histopathologic examination. The use of fluorescent in situ hybridization or PCR has specificity of 100%, provided the probes/primers used are appropriate (see the next paragraphs). We believe that the findings from this study support the use of PCR techniques to detect *H pylori* in tissue samples.

Previous studies have demonstrated a variable prevalence of *H pylori* detection in hyperplastic adenoids of children. The detection rate in hyperplastic adenoid tissue is variable depending on the methods involved (culture, histologic tests, urease/*Campylobacter*-like organism test, or RT-PCR; Table 1). Using the *Campylobacter*-like organism test, one study found 33% of hyperplastic adenoids (10 of 30) contained *H pylori*, whereas other workers found a 3% (1 of 38) positive detection rate using RT-PCR. A comparison of different methodologies to detect *H pylori* in adenoid tissues by Bitar et al found it to be present in 84% of samples (21 of 25), using the urease test, 16% (4 of 25) by histologic examination, and none using a nested PCR assay. They concluded that the urease test and histologic examination were unreliable because these techniques produced many false-positive results; therefore, nested PCR was deemed to be the most accurate for detecting *H pylori*.

Detection rates of *H pylori* in adenoid tissue using RT-PCR vary from none to 48% (Table 1). This discrepancy is likely related to primer design. The protocols used by Yilmaz et al and Cirak et al involve amplification of bacterial 16S ribosomal RNA to select for bacterial species. However, the Hp1 and Hp2 primers used in the following nested PCR have been reported to amplify contaminating human DNA and produced a false-positive rate of 25% with saliva samples. The higher detection rates of *H pylori* reported in adenoid tissue are likely the result of lower specificity and sensitivity of the primers and perhaps contaminating human DNA. In contrast, we developed a nested RT-PCR protocol with high sensitivity and specificity for the detection of all members of the Helicobacteraceae family rather than just *H pylori*. The key with this assay design is that it incorporated a *Helicobacteraceae* gene-specific primer for the RT reaction, as opposed to primers for bacterial 16S, which selects for all bacteria. This makes our assay specific to Helicobacteraceae family members before PCR amplification and enables the detection of the equivalent of 1 *Helicobacteraceae* bacterium within a specimen. The *Helicobacteraceae*-specific RT was followed by 2 nested rounds of PCR, which selectively amplify the *Helicobacteraceae* cDNA, each round using primers designed with modifications to increase stability/binding within the *Helicobacteraceae* genome. *Helicobacter pylori* was not detected in any of the hyperplastic adenoid specimens using this assay. It has been demonstrated that *H pylori* infection in the stomach results in reduced expression of MUC5AC. Our findings in this study therefore appear to demonstrate that the reduced expression of MUC5AC messenger RNA previously observed in hyperplastic adenoids is not due to *H pylori* colonization. The reduction of MUC5AC expression that we observe in adenoid hyperplasia may simply be the result of the presence of squamous metaplasia in these tissues, as discussed previously.

Diseased adenoids have been shown to be colonized by a variety of bacterial species, including *Haemophilus influenza*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Bitar et al observed bacteria in 68% of specimens by histologic examination, although only 16% were identified as *H pylori*. In contrast, we found surprisingly few bacteria in our hyperplastic adenoid specimens. Two bacteria were observed in 1 of 21 specimens, but morphologically, they did not correlate with *H pylori*. However, identification of bacteria in biopsied tissues is particularly difficult, and histologic examination should not be considered the criterion standard. The PCR-positive hyperplastic adenoid specimen 3 identified *Candidatus W africanus*, but histologic examination showed no bacteria in this tissue. We therefore surmised that hyperplastic adenoids are unlikely to act as a reservoir for bacterial growth on the basis of histologic examination and nested RT-PCR assay results in this study.
Our assay was specific for all members of the Helicobacteraceae family, and we identified 1 sample of 78 specimens that provided a positive PCR product. Our results demonstrated a relatively new member of this family, identified as Candidatus W africanus. This species has been identified in only 4 humans and was associated with squamous cell carcinoma of the esophagus as well as with the digestive tract. Until now, this organism has not been discovered outside the esophagus or stomach of either humans or animals and, of interest, Wolinella species contain some of the genes responsible for coding the pathogenic components of H pylori. This finding supports the notion of gastric refluxate reaching the upper aerodigestive tract and adenoids.

The findings of H pylori in the upper aerodigestive tract (adenoids, tonsils, eustachian tubes, and effusion fluid of otitis media) in the literature, by various methods, depend on the prevalence in the community of H pylori gastric infections. Helicobacter pylori is transmitted by the fecal-oral route, usually in childhood, with the prevalence of infection inversely related to the quality of household and public sanitation. The infection rate in developing countries is approximately 80%, whereas in the United States it is about 30%. In Australia, H pylori has a prevalence ranging from 4% in children aged 1 to 4 years to 23% in individuals aged 50 to 59 years. Of interest, many of the reports of H pylori in the adenoid and tonsils are from the Middle East (Turkey and Lebanon). In these countries, the prevalence of H pylori infections in the community may be considerably higher; this may explain the associated higher detection rates reported.

We used the RSI to identify children who were likely to have extraesophageal reflux; however, we did not perform tests to determine how many of these had a gastric H pylori infection. The criterion standard for diagnosis of extraesophageal reflux is ambulatory 24-hour dual-probe (esophageal and pharyngeal) pH monitoring. However, there is evidence suggesting that the sensitivity of hypopharyngeal pH probe recordings is as low as 40%, depending on placement of the probes, leading to inaccurate results/diagnosis and low clinical use of this technique. Multichannel intraluminal impedance, combined with pH monitoring, offers a number of advantages over pH monitoring alone because it can detect the direction of bolus movement, refluxate constitution, and, more important, the detection of nonacidic reflux. However, this technique is expensive, invasive, may not be well tolerated by pediatric patients, and has limited availability. Furthermore, there is no universally accepted criterion for the diagnosis of LPR in children, which is compounded by a lack of normative data regarding the frequency of “physiologic” regurgitation events in children. The RSI has been developed as a noninvasive alternative to identify children likely to have LPR. The RSI is a 9-item, self-administered outcomes instrument that documents the severity of LPR in adults and correlates well with the Reflux Finding Score, which is obtained using laryngeal endoscopy. In a study of children with dysphonia and chronic, refractory cough, which are generally accepted symptoms of extraesophageal reflux, the RSI correlated with the Pediatric Voice Outcome Survey, thereby supporting the clinical usefulness of the RSI in a pediatric population.

A definitive investigation regarding the significance of reflux on the etiologic characteristics of adenoid hyperplasia requires a prospective study acquiring objective reflux data on children with adenoid hyperplasia and on those with positive test results who are being treated with medical therapy (antacids, proton pump inhibitors, or alginate rafting solutions, such as Gaviscon [Reckitt Benckiser, West Ryde, Australia]) to ascertain whether the adenoid hyperplasia could be successfully treated without surgical intervention. However, given the difficulties mentioned in acquiring objective reflux data, in our study we used the RSI as a parent-proxy instrument for detecting the likelihood of LPR in children. We found that 41% of the children with hyperplastic adenoids experienced symptoms likely to be consistent with LPR. In comparison, 43% of children with hyperplastic adenoids had no evidence of LPR symptoms. However, some questions could not be adequately answered by the parent because of the limited understanding of the child regarding terms such as heartburn, which may result in an underrepresentation of LPR in these children. Further studies investigating the benefits of medical treatment of reflux in children with adenoid hyperplasia may be useful.

In conclusion, our highly sensitive and specific PCR assay results cast doubt over claims by other authors of detecting high colonization rates of H pylori in adenoid tissue. We believe that our findings show that adenoid tissue does not serve as a reservoir for species of the Helicobacteraceae family. This suggests that colonization of the tissue by these bacteria is not a factor contributing to adenoid hyperplasia. However, the detection of Candidatus W africanus in a hyperplastic adenoid sample indicates that gastric contents can reach the adenoid, so occasional reflux episodes may form part of the pathophysiologic characteristics of this disease.

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Author Contributions: Drs Hussey, Woods, Ooi, and Carney had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Hussey, Woods, and Carney. Acquisition of data: Hussey, Woods, Harris, Carney. Analysis and interpretation of data: Hussey, Woods, Harris, Thomas, Ooi, and Carney. Drafting of the manuscript: Hussey, Woods, and Harris. Critical revision of the manuscript for important intellectual content: Hussey, Woods, Thomas, Ooi, and Carney. Obtained funding: Woods, Harris, and Carney. Administrative, technical, and material support: Hussey, Woods, Harris, Ooi, and Carney. Study supervision: Hussey and Carney.

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