Comparison of gene expression between pediatric and adult gastric mucosa with *Helicobacter pylori* infection

Running title: Gene expression in *Helicobacter pylori* infection

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Abstract

Background: Although *Helicobacter pylori* infection among adults is a major risk factor for the development of gastric cancer, and initial infection with *H. pylori* may occur before 5 years of age, the direct effects of *H. pylori* infection since childhood on gastric mucosa are unknown. The aim of this study was to evaluate gene expression in the *H. pylori*-infected gastric mucosa of children.

Methods: Gastric mucosal samples were obtained from 24 patients (12 adults and 12 children) who had undergone endoscopic evaluation of chronic abdominal complaints and were examined by the adult and pediatric gastroenterologists at Juntendo University Hospital. Six adult and pediatric patients with and six without *H. pylori* infection were enrolled. Their gastric mucosal samples obtained from the antrum and corpus were used for microarray, real-time polymerase chain reaction, and immunohistochemical analyses to examine the expression of inflammatory carcinogenic molecules.

Results: The expression of inflammatory molecules was up-regulated in the *H. pylori*-infected gastric mucosa from both adults and children. The expression of olfactomedin-4 was only up-regulated in adult patients, while that of pim-2, regenerating islet-derived 3 alpha, lipocalin-2 and C-X-C motif chemokine ligand 13 was equally up-regulated in the infected gastric mucosa of both adults and children.

Conclusions: Because several carcinogenic molecules are up-regulated in *H.pylori* –infected gastric mucosa even in children, early eradication therapy from childhood may be beneficial to decrease the incidence of gastric cancer. Although increased expression of olfactomedin-4 can be important in suppressing gastric cancer in adults, the increase was not detected in children.

Introduction

Helicobacter pylori is a gram-negative, microaerophilic bacterium found in the stomach. It contains a hydrogenase that can be used to obtain energy by oxidizing molecular hydrogen (H_2) produced by intestinal bacteria [1]. It also produces oxidase, catalase, and urease. *H.pylori* is capable of forming biofilms and can convert from a spiral to a possibly viable but nonculturable coccoid form, both of these forms are likely to favor its survival and influence the epidemiology of the bacterium. *H. pylori* infection also causes chronic inflammation of the stomach, which increases the risk of peptic ulcer disease and stomach cancer.

Gastric mucosal inflammation associated with *H. pylori* infection results from damage to the gastric mucosa by ammonia, a byproduct of the reaction between *H. pylori*-produced urease and urea, as well as other *H. pylori* pathogenic factors such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) proteins [2,3]. A large proportion of chronic *H. pylori* infections occur when the patients are under the 6 years, with approximately 10% children in Japan carrying the bacterium [4]. Many children infected with *H. pylori* are asymptomatic and experience no complications; however, research has shown that nodularity in the stomach had a high specificity (98.5 %) and positive predictive value (91.7 %) for the diagnosis of *H. pylori* infection [5]. Chronic infections resulting from an initial infection in adulthood are rare. In Japan, the prevalence of *H. pylori* in non-nodular

gastritis, nodular gastritis, and duodenal and gastric ulcers are 28.8%, 98.5%, 83.0%, and 44.2%, respectively [6].

Although *H. pylori* infection rates have decreased in recent years because of fewer children per family, as well as improvements in hygiene and residential environments, the downward trend in infection rates has plateaued in some developed countries. *H. pylori*-related gastrointestinal disorders include gastric and duodenal ulcers, nodular gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma, and a protein-losing enteropathy, while non-gastrointestinal tract disorders associated with *H. pylori* include chronic idiopathic thrombocytopenic purpura and iron-refractory or recurrent iron deficiency anemia [7]. Multiple studies have demonstrated increased iron stores and improved growth rates following *H. pylori* eradication [8-11].

The International Agency for Research on Cancer declared *H. pylori* as a definite human carcinogen in 1994 [12]. The prophylactic effect of bacterial eradication against the development of gastric cancer is supported by a reported gastric cancer rate of 2.9% in association with *H. pylori* infection, compared with a rate of 0% in an uninfected cohort [13]. Fukase et al. [14] suggested that the prophylactic eradication of *H. pylori* after the endoscopic resection of early gastric cancer is necessary to prevent the development of metachronous gastric carcinoma.

Gastric cancer in the absence of *H. pylori* infection is rare, and as a result, ≤1% of gastric cancer cases are infected with *H. pylori*. *H. pylori* infection usually occurs during infancy, emphasizing the importance of protecting infants from infection. Because parents are often the source of infection, *H. pylori* testing should be performed with bacterial eradication in positive cases in order to eliminate this potential source of infection.

Although *H. pylori* infection among adults is a risk factor for the development of gastric cancer, and the initial *H. pylori* infection may occur before the age of 5 years, the effects of *H. pylori* infection on the gastric mucosa of children is unknown. The aim of this study is to evaluate the immune responses in *H. pylori*-infected gastric mucosa from adults and children using microarray and RT-PCR analyses and to discuss the importance of early eradication of *H. pylori* infection.

Methods

Patients and Samples

All gastric biopsy samples from patients suffering from abdominal symptoms such as nausea, epigastralgia, and vomiting were enrolled in this study. The exclusion criteria were as follows: history of treatment for *H. pylori* infection, malignant disease, inflammatory bowel disease, severe liver disease, heart disease, kidney disease, blood disorders, or other systemic diseases. In addition, patients with histologically moderate or severe nodular gastritis were excluded from the uninfected group. Moderate or severe nodular gastritis was defined as a score of ≥2 on the updated Sydney system [15]. All study protocols were approved by the Institutional Ethics Committee of Juntendo University Hospital, and informed consent for participation was obtained from the patients or the parents of the children prior to enrollment in the study. Upper gastrointestinal endoscopy was performed on all patients, and routine biopsy samples were collected from the antrum and corpus. These samples were used for microarray analysis, real-time PCR, bacterial culture and histological analysis.

Identification of Helicobacter infection and histology

Patients with a positive *H. pylori* culture result and positive results for at least one of the followings were assigned to the *H. pylori* infection group (6 adult and 6 pediatric patients):

serological assay for *H. pylori* serum antibodies, the urea breath test (UBT) or the *H. pylori* stool antigen assay. Adult patients with a negative *H. pylori* culture and UBT result, as well as negative findings on histological microscopic examination, were assigned to the uninfected group (6 adults). Likewise, pediatric patients with negative results for either the serological assay for *H. pylori* serum antibodies or the *H. pylori* stool antigen assay who had negative findings on histological microscopic examination were assigned to the uninfected group (6 pediatric patients).

RNA extraction from biopsy samples

Mucosal biopsy samples were stored in RNAlater solution (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) at -80°C until RNA isolation. In preparation for the microarray and real-time PCR analyses, the samples were disrupted and homogenized in Buffer RLT, and the RNA was extracted using the RNeasy Mini Kit (Qiagen, Germantown, MD, USA). The quantity and purity of the RNA samples were determined using the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, MA, USA) and the Experion RNA StdSens Analysis Kit (Bio- Rad Laboratories, Hercules, CA, USA).

Microarray hybridization and data analysis

To measure the relative gene expression, a microarray assay was performed according to the manufacturer’s instructions for the Ambion WT Expression Kit (Applied Biosystems) and

the Gene- Chip® WT Terminal Labeling and Controls Kit (Affymetrix, Santa Clara, CA, USA). Briefly, total RNA was reverse-transcribed into cDNA using random primers, after which cRNA was synthesized from cDNA by *in vitro* transcription. The resulting cRNA was then used as a template for the second cycle of cDNA synthesis. cDNA was then fragmented, labeled, and hybridized to a GeneChip®Human Gene 1.0 ST Array (Affymetrix) at 45°C for 17 h. This array encompassed approximately 29,000 genes. The chips were washed, stained with streptavidin–phycoerythrin, and scanned using the GeneChip Scanner 30007G (Affymetrix).

The scanned images were converted to CEL files using the Gene-Chip® Command Console® Software (AGCC) (Affymetrix), and data analysis was performed using the GeneSpring GX v11 software (Agilent Technologies, Santa Clara, CA, USA). The raw-intensity data from each chip were normalized using the RMA16 algorithm. Genes that showed a >5-fold difference in signal intensity compared with those in controls, and a significant difference in the levels of expression between patients with *H. pylori* infection and controls (*t*-test, $P < 0.01$) were considered either up-regulated or down-regulated genes.

Real-time polymerase chain reaction

The expression of specific signaling molecules related to inflammation, infection and the presence of lymphoid follicles in the antrum mucosa was examined using RT-PCR. TaqMan probe-based quantitative RT-PCR was performed using cDNA synthesized from total mucosal biopsy RNA preparations (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems) and analyzed using the 7500 Fast Real-Time PCR system (Applied Biosystems) using default protocols. The expression of each gene was normalized for the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the standard curve method. The primers and probes for olfactmedin 4 (OLFM4) , regenerating islet-derived 3 alpha (REG3A) , lipocalin-2 (LCN2), pim-2 oncogene (PIM2), and C-X-C motif chemokine ligand (CXCL) 13 were prepared using TaqMan® Gene Expression Assays (Applied Biosystems).

Immunohistochemical analysis

Immunohistochemical analysis was performed on formalin fixed, paraffin-embedded tissue sections using an automated immunostainer: Benchmark GX and Nexes IHC (Ventana Medical System, Tucson, AZ, USA) and these stainers were used according to the manufacturer’s protocols with minor modifications. Deparaffinized sections were incubated with primary antibodies with following dilutions; OLFM4 (Thermo Fisher Scientific, Rockford,

IL) 1:150, REG3A (Novus Biologicals USA, Littleton, CO) 1:25; LCN2 (Abcam, Cambridge, MA) 1:200, PIM2 (Sigma, St. Louis, MO) 1:100, and CXCL13 (R & D Systems, Inc., Minneapolis, MN) 1:10. Antigen retrieval for REG3A, PIM2, and CXCR3 was performed in 10 mM citrate buffer with a pH of 6.0 at 121°C for 10 min. All heating was performed in an autoclave. After washing, the sections were incubated with a biotin-conjugated 2nd antibody. The sections were then incubated with streptavidin (Dako) for 30 min. The peroxidase activity was detected with 3,3'-diaminobenzidine-tetrahydrochloride containing 0.01% H_2O_2. All sections were counterstained with hematoxylin before examination by light microscopy. Non-specific staining was evaluated in sections without primary antibody staining. For negative controls, normal fraction rabbit immunoglobulin (Dako) or goat immunoglobulin (Dako) was used instead of primary antibodies.

Statistical analysis

Differences in patient age and mRNA expression profiles as measured by RT-PCR were analyzed using the Mann–Whitney *U*-test. For RT-PCR analysis, $P < 0.05$ was considered statistically significant.

Results

From March 2009 to October 2013 at Juntendo University Hospital, 12 adult (age range, 40–58 years; mean age, 44.2 ± 5.1 years) and 12 pediatric (age range, 4.9–15.6 years; mean age, 12.1 ± 2.7 years) patients with (n=6 each) and without (n=6 each) *H. pylori* infection were enrolled in this study (Tables 1 and 2). With regard to concurrent medication use, patients 2 and 4 in the infected adult group were on H_2 blockers, while patients 5 and 6 were on proton pump inhibitors (PPI; proton pump inhibitor). No patients in the uninfected adult group or pediatric group were on medications that could affect the investigation results. With regard to medical history, patients 3 and 5 in the infected adult group had hydronephrosis and hyperlipidemia, respectively, while patient 5 in the uninfected adult group had bronchial asthma. In the uninfected pediatric group, patients 1,2,3,4 and 6 had irritable bowel syndrome.

Gastrointestinal endoscopy revealed chronic and atrophic gastritis with normal to moderate inflammatory cell infiltration in adult patients; in the pediatric patients, the endoscopy confirmed normal to nodular gastritis with mild to severe active gastritis.

Gene expression analysis with or without H.pylori infection

Gene expression was examined in microarray analysis using RNA isolated from the mucosal biopsies; the samples were compared between patients with and without *H. pylori*

infection. In addition, gene expression was compared between the antrum and corpus biopsy samples from infected patients. Among the antrum biopsies with or without infection for the adults, there were 16 up-regulated or down-regulated genes with fold change differences of >5 and a *P* value of < 0.01 (Table 3); for corpus biopsies with or without infection, 8 genes were alternatively expressed (Table 4). However, in the antrum biopsies with or without infection in the children, the number of up-regulated or down-regulated genes with fold change differences of >4 and a *P* value of < 0.01 was 15 (Table 5); for corpus biopsies with or without infection, 12 genes were alternatively expressed (Table 6).

Expression of OLFM4, REG3A, LCN2, PIM2, and CXCL13

Additional quantitative analysis using RT-PCR was performed for five molecules, which were all up-regulated in the *H. pylori*-infected gastric mucosa of children. The following molecules have been putatively associated with inflammation, infection, cancer, and iron absorption: OLFM4, LCN2, REG3A, PIM2, and CXCL 13. The expression of OLFM4 was mostly up-regulated in adults (Figure1-a), while that of PIM2, REG3A, LCN2 and CXCL13 was equally up-regulated in the infected gastric mucosa of both adults and children (Figures 1-b, c, d, and e).

Immunohistochemical analysis of OLFM4, REG3A, LCN2, PIM2, and CXCL13

The staining of OLFM4 was enhanced on infiltrated inflammatory cells in the lamina propria

but not epithelial cells, which was confirmed only in the adult infected gastric mucosa (Figure 2-A). The mild staining of LCN2 was confirmed on the epithelial cells of both adult and pediatric uninfected gastric mucosa. Its staining was enhanced in the infected adults and pediatric gastric mucosa both on epithelial cells and infiltrated inflammatory cells in the lamina propria (Figures 2-C). Epithelial cells of both adult and pediatric uninfected gastric mucosa were also mildly stained with REG3A and PIM2.Their staining were enhanced only in the infiltrated inflammatory cells in the lamina propria but not on epithelial cells both in the adult and pediatric infected gastric mucosa (Figures 2-B and D). CXCL13 staining was confirmed around lymphoid follicules where lymphocytes were migrated both in the adult and pediatric infected gastric mucosa (Figures 3).

Discussion

Previous microarray and RT-PCR studies have examined *H. pylori*-infected gastric mucosa in children [16]; however, the present analysis was the first to compare the gene expression between the *H. pylori*-infected gastric mucosa of adults and children. In this study, the expression of OLFM4 was only up-regulated in adult patients, while that of PIM2, REG3A, LCN2 and CXCL13 was equally up-regulated in the infected gastric mucosa of both adult and pediatric patients.

The OLFM4 gene is linked to gastric cancer. In a study by Yasui *et al.*, OLFM4 expression was observed in 94 of 167 gastric cancer patients (56%). The prognosis is significantly more favorable for patients who are positive for OLFM4 than for those who are negative, indicating its utility as a favorable prognostic factor and serum marker [17]. In the present study, OLFM4 was strongly expressed in the infected adult group but it was not significantly enhanced in the pediatric group. Although OLFM4 was highly expressed in adenocarcinoma [18], our histochemical analysis revealed that OLFM4 was expressed migrated inflammatory cells but not non-malignant epithelial cells in the adult infected gastric mucosa. OLFM4 reportedly acts to suppress the natural immunity against *H. pylori* [19], therefore, non-enhanced expression of OLFM4 in childhood may disregard a biological response and promote tumor-genesis, which can lead to development of gastric cancer in adulthood.

Because OLFM4 is considered to be a fundamental factor of cancer risk and reduced expression of OLFM4 is associated with the development of gastric cancer, increased expression of OLFM4 in adults with *H.pylori* infection can contribute to the suppression of gastric cancer [18]. In contrast, increased expression of OLFM4 may not always be necessary to reduce carcinogenic factors in children with *H.pylori* infection.

The LCN2 protein inhibits pathogen iron absorption and can sequester host iron from *Plasmodium* during malaria infection, this protein can protect the host and reinforce the immune response [20]. In children, LCN2 is thought to interfere with the iron uptake and proliferation of *H. pylori*. The role of LCN2 in preventing iron loss is particularly important because *H. pylori* infection causes iron deficiency anemia. It is thought that the enhanced expression of LCN2 may protect the host against iron deficiency anemia during *H. pylori* infection. Furthermore, LCN2 expression has been confirmed on the surface of the epithelial cells in the mucosa of the gastrointestinal tract, and the lower respiratory tract, in response to inflammation due to bacterial infection [21]. Histochemical analysis revealed that the expression of LCN2 was enhanced in the epithelial cells and infiltrated inflammatory cells in the adult and pediatric infected gastric mucosa. Ikuse, *et al.* reported on the enhanced expression of the LCN2 gene in the *H. pylori*-infected gastric mucosa of pediatric patients [16]. Alpizar-Alpizar *et al.* [22] also reported that LCN2 expression was upregulated in

gastritis mucosa infected with *H. pylori*, whereas it was not upregulated in mucosa with intestinal metaplasia, dysplasia, and gastric cancer. It is possible that LCN2 may protect *H. pylori*-infected gastric mucosa from tumor-genesis and that the enhanced expression of LCN2 may be favorable for pediatric *H. pylori*-infected patients in terms of gastric cancer.

The REG family proteins I-IV are expressed in the epithelial cells of the gastrointestinal mucosa and are over-expressed in inflammatory diseases, such as nodular gastritis and ulcerative colitis [23-26]. REG family proteins may act as growth factors and anti-apoptotic factors in inflammation and carcinogenesis. Fukui *et al*. reported that REG I α gene expression in *H. pylori*-infected gastric mucosa was induced by STAT3 activation elicited by cytokines such as IL-6 and IFN-γ. Meanwhile, PIM2 has also been considered as a novel anti-apoptotic factor in myeloma. The expression of PIM2 is up-regulated in cancerous lesions in several organs including the pancreas, liver, and large intestine. The present study discovered up-regulated expression of REG 3A as well as the PIM2 genes both in adults and children with *H. pylori*-infected gastric mucosa. Histochemical analysis also revealed that REG3A and PIM2 were both mildly expressed in the epithelial cells and infiltrated inflammatory cells in the adult and pediatric infected gastric mucosa. Because these genes may be involved in gastric cancer, the increased expression of REG3A and PIM2 may directly or indirectly increase the risk of tumor-genesis in the *H. pylori*-infected

gastric mucosa of both adult and pediatric patients.

CXCL13 is produced in germinal center dendritic cells and follicular dendritic cells in lymph nodes and the spleen. CXCL13 attracts B cells and Th2 cells in the lymphoid follicle and forms a nodular gastritis in the *H. pylori*-infected gastric mucosa of children. Recent studies have shown that CXCL13 and IL-10 were specific markers of CNS lymphoma [27]. Nakashima *et al.* [28] reported significantly elevated CXCL13 expression levels in *H. pylori*-positive patients compared with those in uninfected controls. The CXCL13 expression levels also correlated with the degree of chronic gastritis and bacterial colonization observed. In the present study, CXCL13 expression was up-regulated in both adults and children (Figure 1-e). Histochemical analysis revealed that CXCL13 staining was confirmed around lymphoid follicules where lymphocytes were migrated in both adults and pediatric infected gastric mucosa (Figures 3).Since number of lymphoid follicules is increased in nodular gastritis, the expression of CXCL13 may also enhanced in the follicule-nodular forming infected gastric mucosa. Because chronic inflammation can be the cause of gastric cancer, increased expressions of CXCL13 may also involve the risk of tumor-genesis in *H. pylori*-infected gastric mucosa.

In conclusion, because there may be several carcinogenic molecules; such as PIM2 and REG3A that have been, up-regulated in *H.pylori* infected mucosa even in childhood, early

eradication therapy for *H. pylori* may be beneficial in decreasing the incidence of gastric cancer. Meanwhile the precise reason of non-enhanced expression of OLFM4 during childhood should be further investigated.

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Table 1. Subject characteristics in adults

Group	Id no.	Age	Sex	^{13}C-UBT	Culture	Pathology	Endoccopy
H.pylori(+)	1	58	Female	Positive	Positive	Severe chronic active gastritis	Reflux esophagitis Angiodysplasia of the stomach
	2	48	Male	Positive	Positive	Moderate chronic active gastritis	Reflux esophagitis Chronic gastritis
	3	40	Male	Positive	Positive	Severe chronic active gastritis	Reflux esophagitis Gastric ulcer scar
	4	37	Male	Positive	Positive	Moderate chronic active gastritis	Reflux esophagitis
	5	43	Male	Positive	Positive	Moderate chronic active gastritis	Atrophic gastritis Hiatus hernia
	6	41	Female	Positive	Positive	Moderate chronic active gastritis	Atrophic gastritis Gastric polyp Duodenal ulcer scar
H.pylori(−)	1	46	Male	Negative	Negative	Moderate chronic active gastritis	Duodenal polyp
	2	45	Male	Negative	Negative	No findings	Reflux esophagitis Erosive gastritis
	3	41	Female	Negative	Negative	Moderate chronic active gastritis	Hiatus hernia Erosive gastritis
	4	46	Female	Negative	Negative	No findings	Atrophic gastritis Gastric polyp
	5	46	Male	Negative	Negative	No findings	Hiatus hernia Gastric polyp
	6	40	Male	Negative	Negative	No findings	Reflux esophagitis Hiatus hernia Atrophic gastritis

Table 2. Subject characteristics in children

Group	Id no.	Age	Sex	IgG	^{13}C-UBT	Stool antigen	Culture	Pathology	Endoccopy
H.pylori(+)	1	14 years 4 months	Female	Positive	Positive	N.D.	Positive	Moderate chronic active gastritis	NG
	2	14 years 2 months	Female	Positive	Positive	Positive	Positive	Severe chronic active gastritis	NG, duodenitis
	3	14 years 1 month	Male	Positive	Positive	Negative	Positive	Moderate chronic active gastritis	NG
	4	12 years 1 month	Female	Positive	Positive	Negative	Positive	Mild chronic active gastritis	NG
	5	14 years 4 months	Female	Positive	Positive	Positive	Positive	Mild chronic active gastritis	NG
	6	10 years 7 months	Male	Positive	Positive	Negative	Positive	Severe chronic active gastritis	NG, GU, DU
H.pylori(−)	1	11 years 6 months	Female	Negative	N.D.	N.D.	Negative	No findings	No lesion
	2	15 years 6 months	Male	N.D.	N.D.	Negative	Negative	No findings	No lesion
	3	11 years 2 months	Female	Negative	N.D.	N.D.	Negative	No findings	No lesion
	4	10 years 1 month	Male	Negative	N.D.	N.D.	Negative	No findings	No lesion
	5	4 years 9 months	Male	Negative	N.D.	Negative	Negative	No findings	No lesion
	6	12 years 1 month	Male	Negative	N.D.	Negative	Negative	No findings	No lesion

N.D., not done; NG, nodular gastritis; GU, gastric ulcer; DU, duodenal ulcer.

Table 3. Effects of *H.pylori* infection on adult antrum mucosa (fold change >5, P <0.01)

Accession no.	Gene symbol	Gene description	Fold change
NM_138939	REG3A	Regenerating islet-derived 3 alpha	27.58782
NM_006418	OLFM4	Olfactomedin 4	26.76457
NM_001783	CD79A	CD79a molecule, immunoglobulin-associated alpha	12.74575
NM_152866	MS4A1	Membrane-spanning 4-domains, subfamily A, member 1	11.85287
NM_005564	LCN2	Lipocalin 2	9.193067
NC_010162	IGLV7-46	Immunoglobulin lambda variable 7-46 (gene/pseudogene)	9.143877
NM_002343	LTF	Lactotransferrin	7.29566
NM_001085	SERPINA3	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	7.24859
NM_006419	CXCL13	Chemokine (C-X-C motif) ligand 13	6.497946
NM_000064	C3	Complement component 3	6.427261
NM_002426	MMP12	Matrix metallopeptidase 12 (macrophage elastase)	6.143543
NM_007231	SLC6A14	Solute carrier family 6 (amino acid transporter), member 14	6.134971
NM_001191358	SLAMF7	SLAM family member 7	5.452487
NM_006875	PIM2	Pim-2 oncogene	5.325736
NM_004591	CCL20	Chemokine (C-C motif) ligand 20	5.291274
NM_014479	ADAMDEC1	ADAM-like, decysin 1	5.283501

Table 4. Effects of *H.pylori* infection on adult corpus mucosa (fold change >5, P <0.01)

Accession no.	Gene symbol	Gene description	Fold change
NM_005564	LCN2	Lipocalin 2	11.732614
NM_138938	REG3A	Regenerating islet-derived 3 alpha	10.050514
NC_010162	IGLV7-46	Immunoglobulin lambda variable 7-46 (gene/pseudogene)	9.707812
NM_001010905	C6orf58	Chromosome 6 open reading frame 58	8.991477
NM_001783	CD79A	CD79a molecule, immunoglobulin-associated alpha	8.138612
NM_002644	PIGR	Polymeric immunoglobulin receptor	6.0731664
NM_019010	KRT20	Keratin 20	5.749275
NM_003937	KYNU	Kynureninase (L-kynurenine hydrolase)	5.7143874

Table 5. Effects of *H.pylori* infection on child antrum mucosa (fold change >4, $P < 0.01$)

Accession no.	Gene symbol	Gene description	Fold change
NM_152866	MS4A1	Membrane-spanning 4-domains, subfamily A, member 1	16.164877
NM_012598	LCN2	Lipocalin 2	10.438714
NM_001085	SERPINA3	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	9.15701
NM_000064	C3	Complement component 3	8.978772
NM_002988	CCL18	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	8.260766
NM_001783	CD79A	CD79a molecule, immunoglobulin-associated alpha	8.185633
NM_002426	MMP12	Matrix metallopeptidase 12 (macrophage elastase)	6.957664
NM_002343	LTF	Lactotransferrin	5.998344
NM_006419	CXCL13	Chemokine (C-X-C motif) ligand 13	5.6768055
NM_018406	MUC4	Mucin 4, cell surface associated	5.590358
NM_007231	SLC6A14	Solute carrier family 6 (amino acid transporter), member 14	5.4997983
NM_004591	CCL20	Chemokine (C-C motif) ligand 20	5.33443
NM_014479	ADAMDEC1	ADAM-like, decysin 1	5.3015494
NM_002423	MMP7	Matrix metallopeptidase 7 (matrilysin, uterine)	4.5940804
NM_006875	PIM2	Pim-2 oncogene	4.1142826

Table 6. Effects of *H.pylori* infection on child corpus mucosa (fold change >4, P <0.01)

Accession no.	Gene symbol	Gene description	Fold change
NM_012598	LCN2	Lipocalin 2	11.276529
NM_138939	REG3A	Regenerating islet-derived 3 alpha	10.137276
NM_001010905	C6orf58	Chromosome 6 open reading frame 58	6.8478856
NM_002644	PIGR	Polymeric immunoglobulin receptor	6.664708
NM_014080	DUOX2	Dual oxidase 2	6.419147
NM_001783	CD79A	CD79a molecule, immunoglobulin-associated alpha	5.8288584
NC_010162	IGLV7-46	Immunoglobulin lambda variable 7-46 (gene/pseudogene)	5.693495
NM_019010	KRT20	Keratin 20	5.618007
NM_003890	FCGBP	Fc fragment of IgG binding protein	4.914343
NM_152866	MS4A1	Membrane-spanning 4-domains, subfamily A, member 1	4.771285
NM_014211	GABRP	Gamma-aminobutyric acid (GABA) A receptor, pi	4.1858683
NM_022555	HLA-DRB3	Major histocompatibility complex, class II, DR beta 3	4.16585

Figure legends

Figure 1 RT-PCR analysis of gene expression in adult and pediatric gastric mucosa obtained from the antrum and corpus.

The expression of each gene that was normalized to the expression of GAPDH is described with a box-and-whisker plot; the central box covers the interquartile range, with the median indicated by the line within the box. The whiskers extend to the minimum and maximum values.

A) Olfactomedin 4 (OLFM4), B) Regenerating islet-derived 3 alpha (REG3A), C) Lipocalin 2 (LCN2), D) Pim-2 oncogene (PIM2), E) C-X-C motif chemokine ligand 13 (CXCL13)

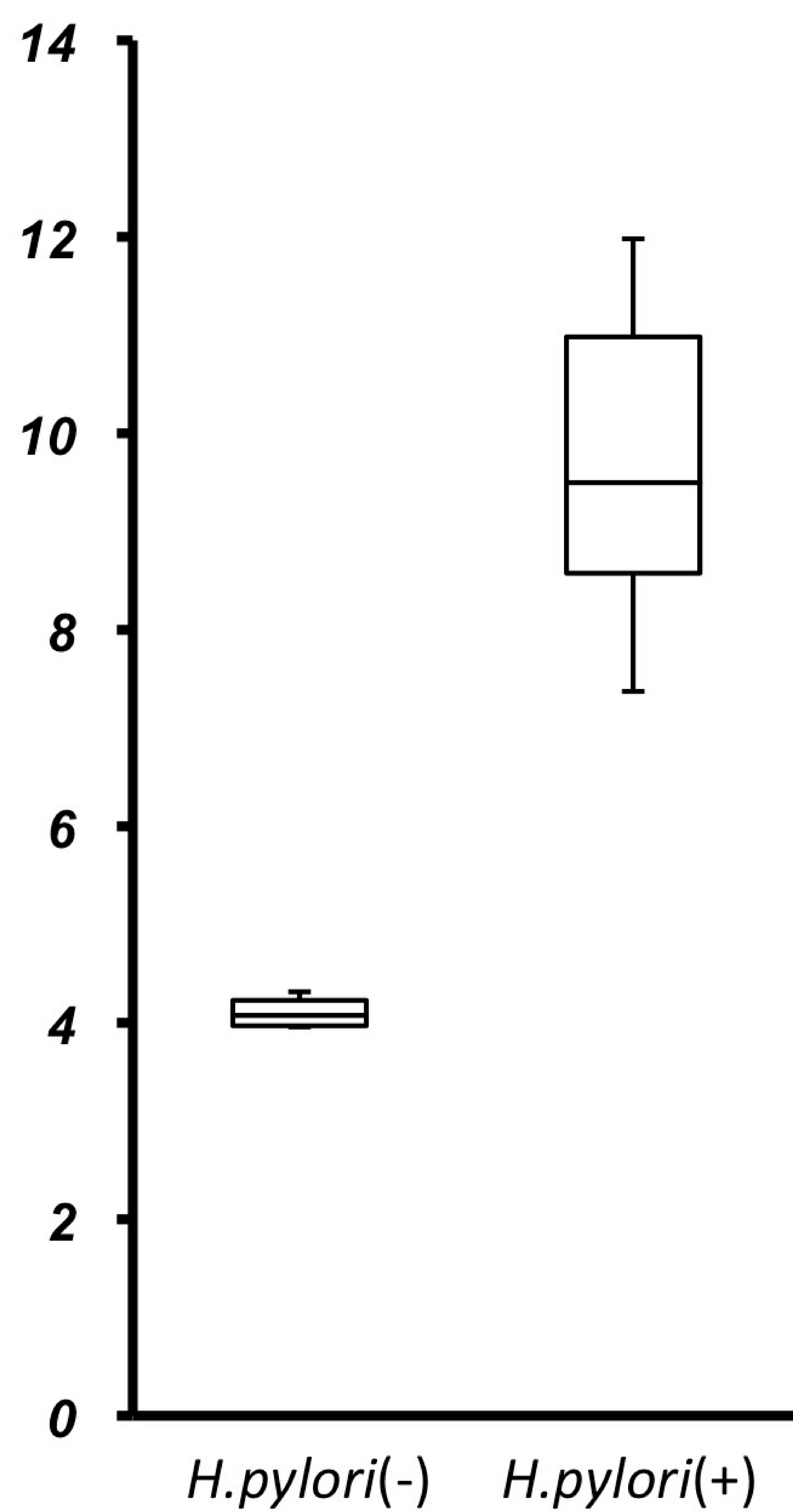
Figure 2 Immunochemical analysis in the adult (A-D; *H. pylori* infected, E-H; not infected) and pediatric (I-L; *H. pylori* infected, M-P; not infected) gastric mucosa. A, E, I, M; OLFM4, B, F, J, N; REG3A, C, G, K, O; LCN2, D, H, L,P; PIM2. Magnification×400.

Figure 3 Immunochemical analysis of CXCL13 in the adult (A) and pediatric (B) *H. pylori*-infected gastric mucosa. Magnification×200.

Adults

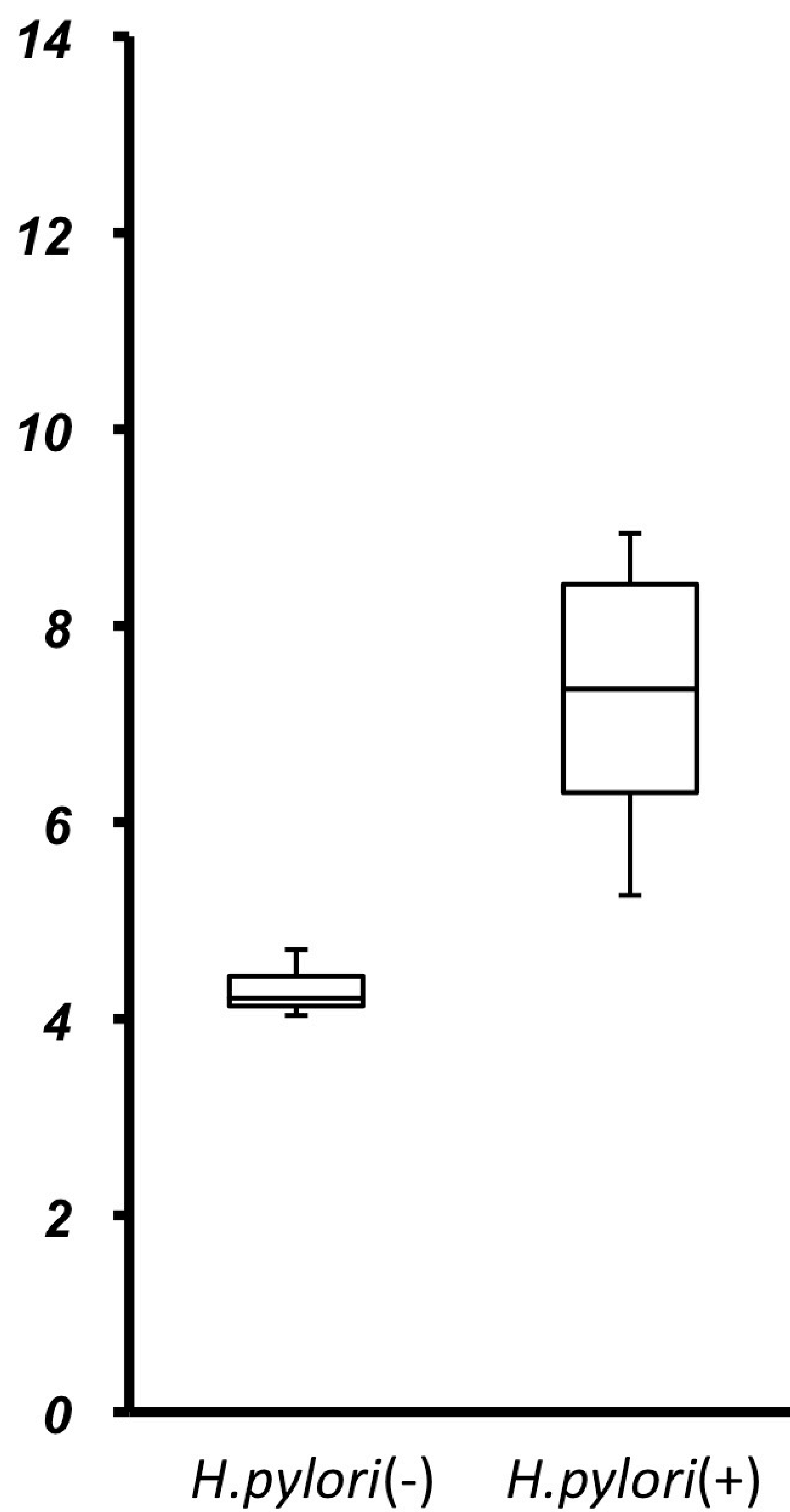
Antrum

P=0.014



Corpus

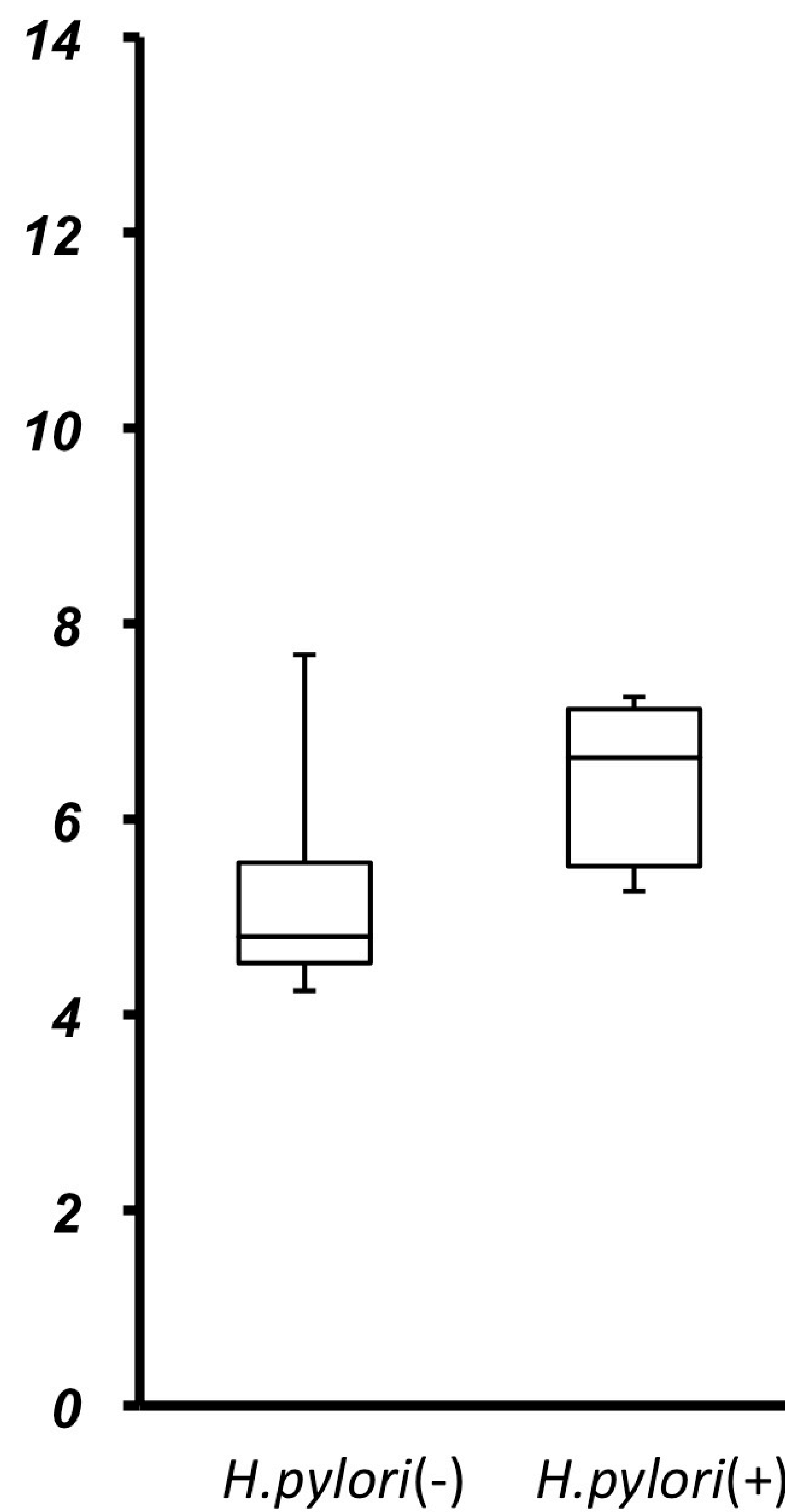
P=0.025



Children

Antrum

P=0.14



Corpus

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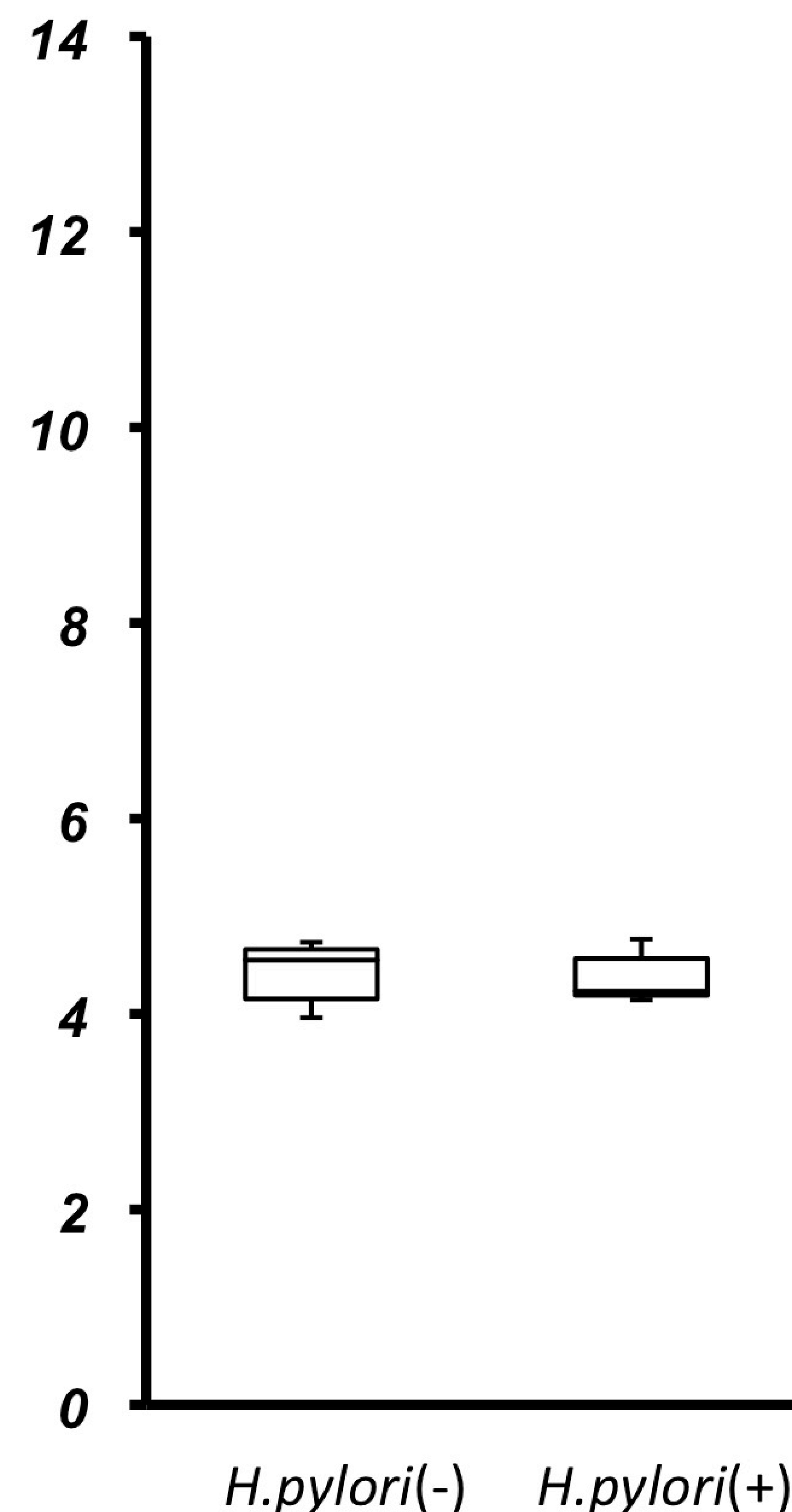

Adults

Children

Antrum

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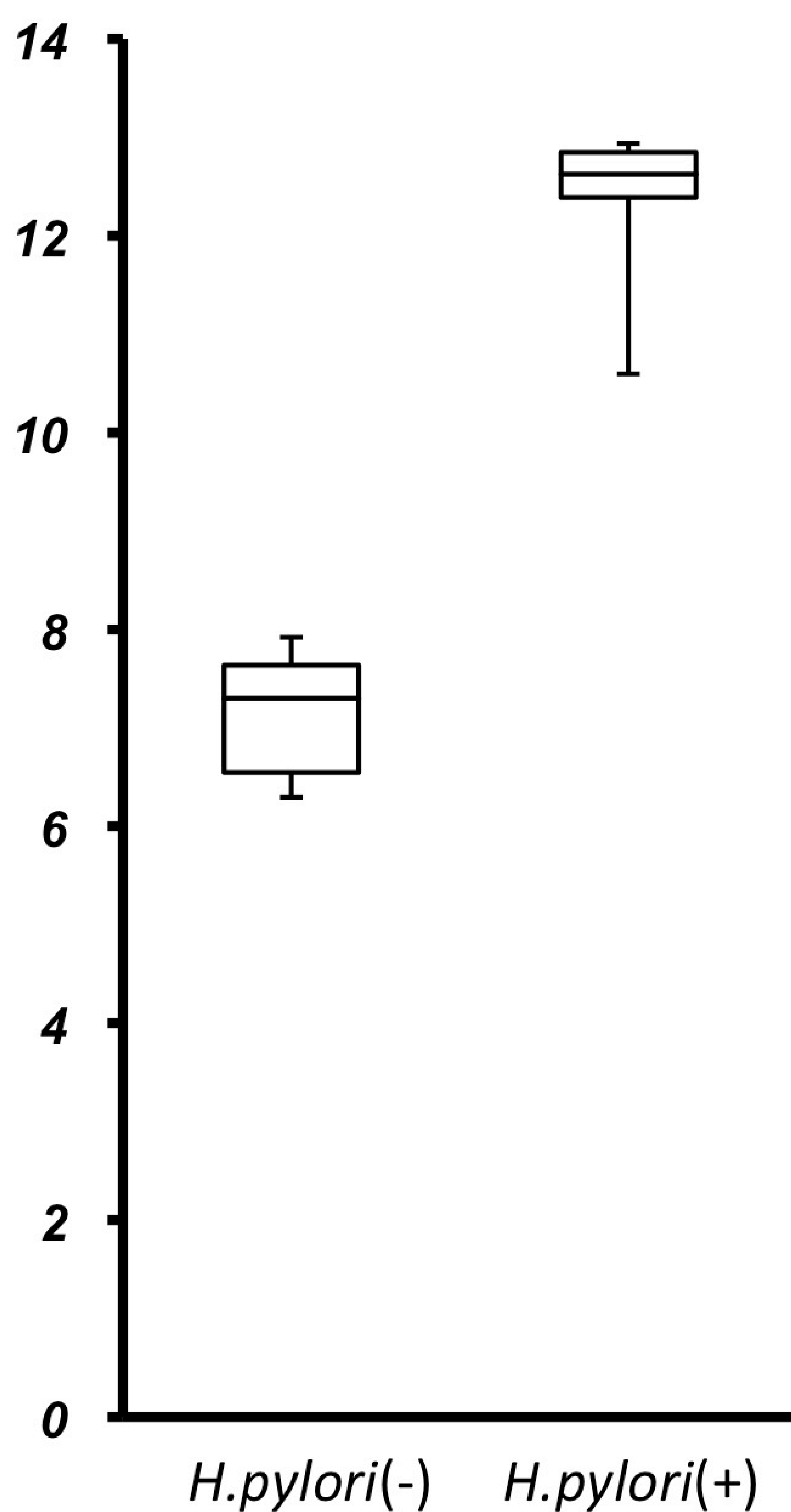

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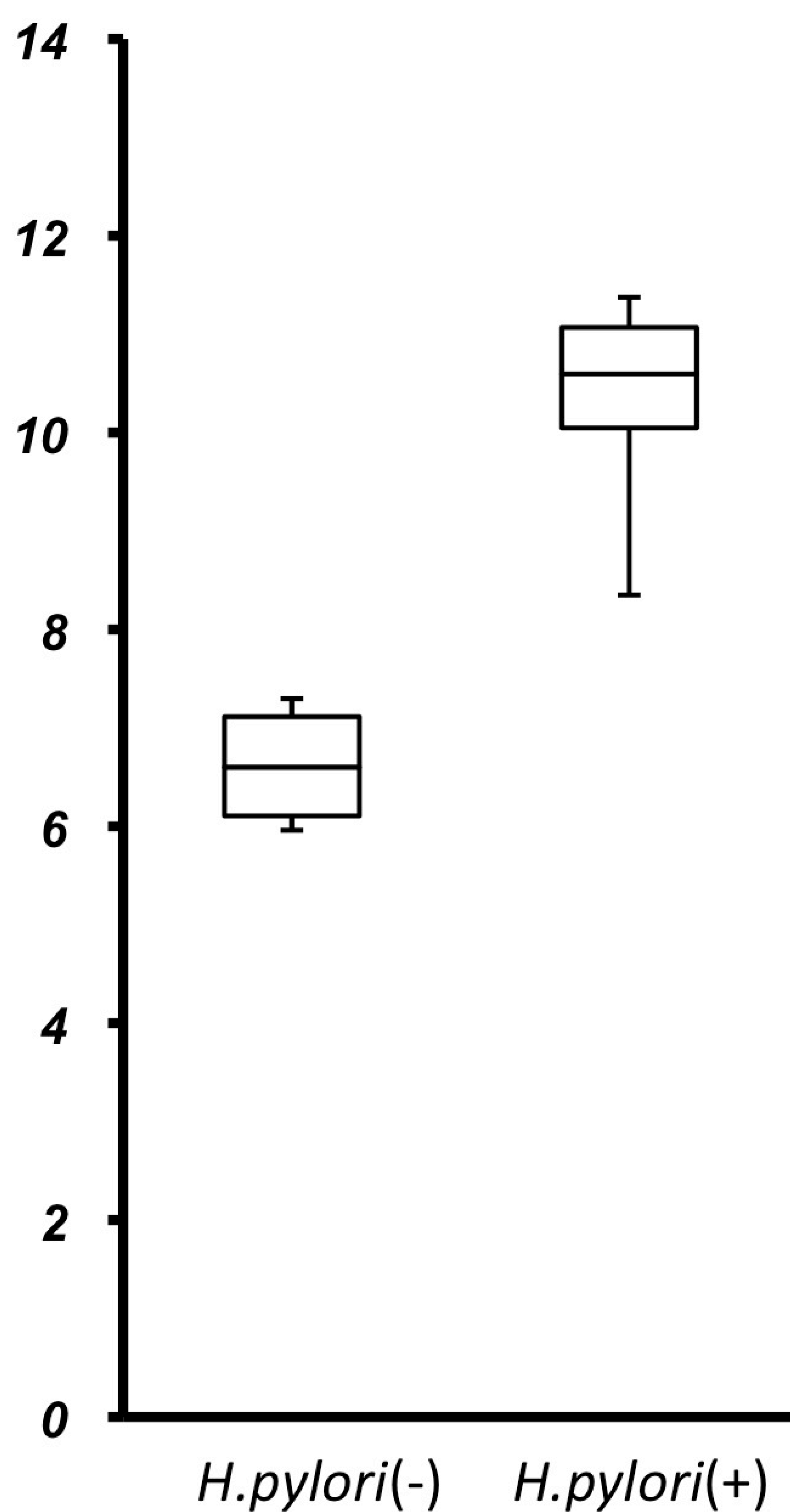

Antrum

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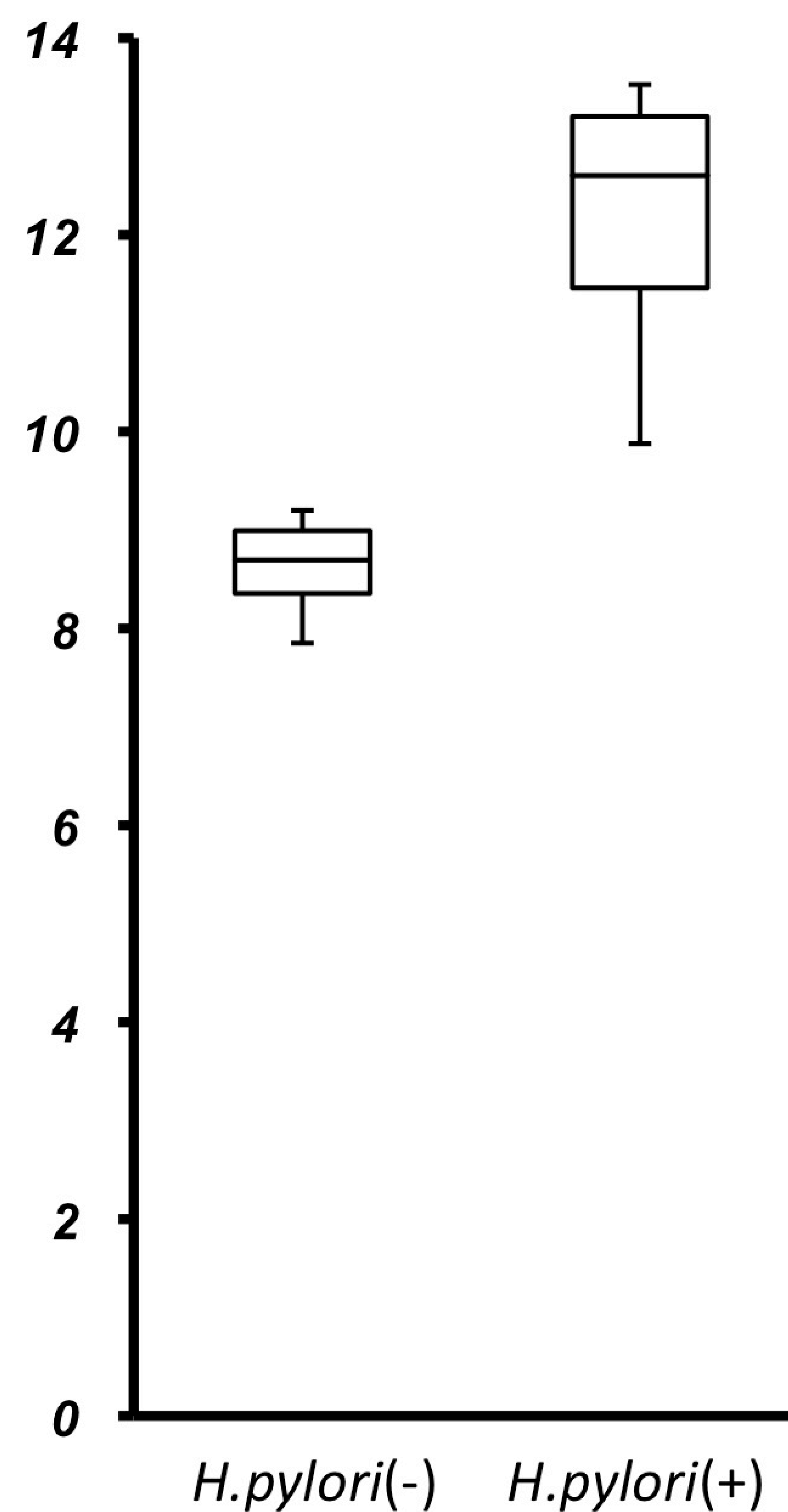

Corpus

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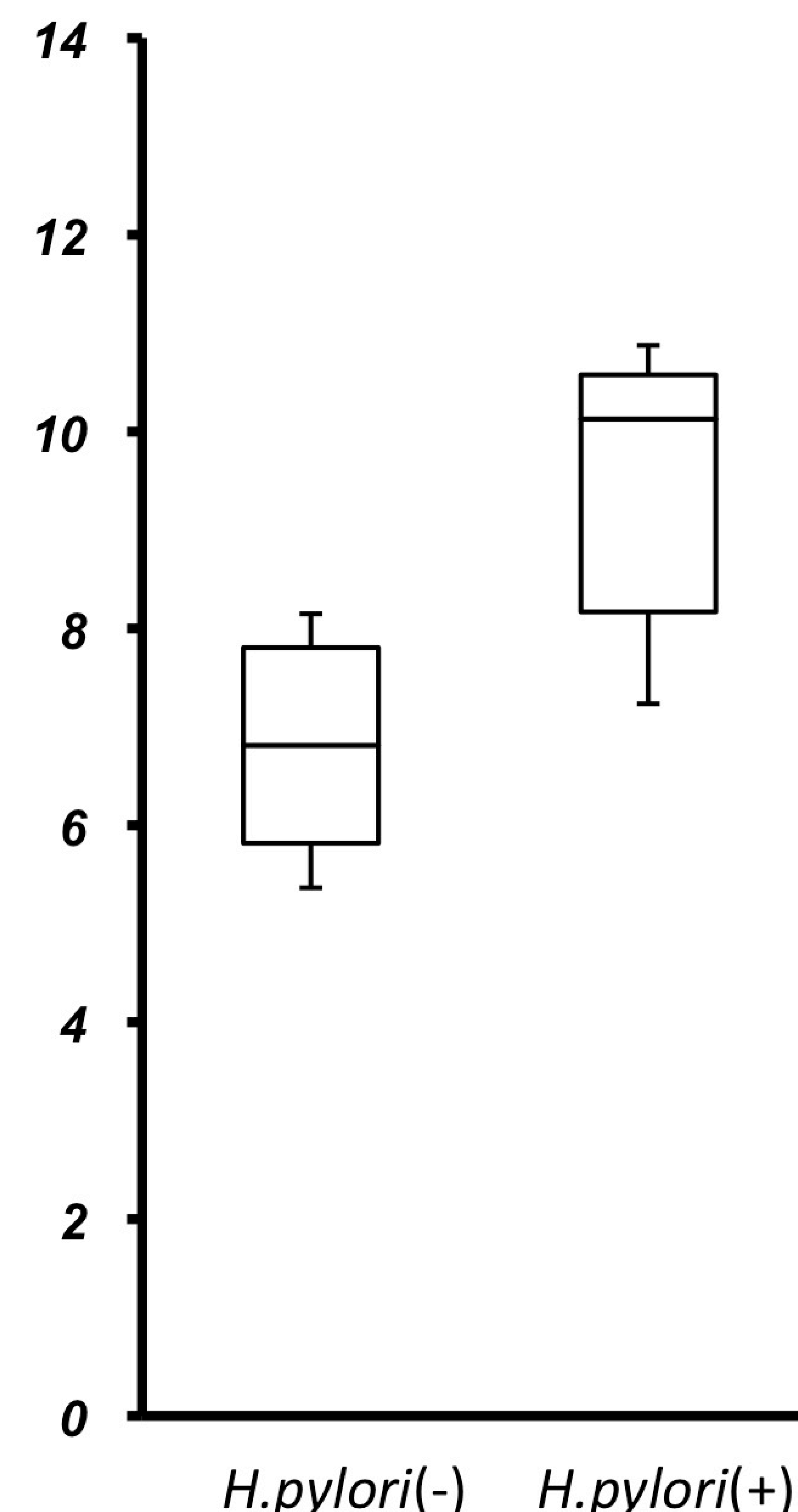

Adults

Antrum

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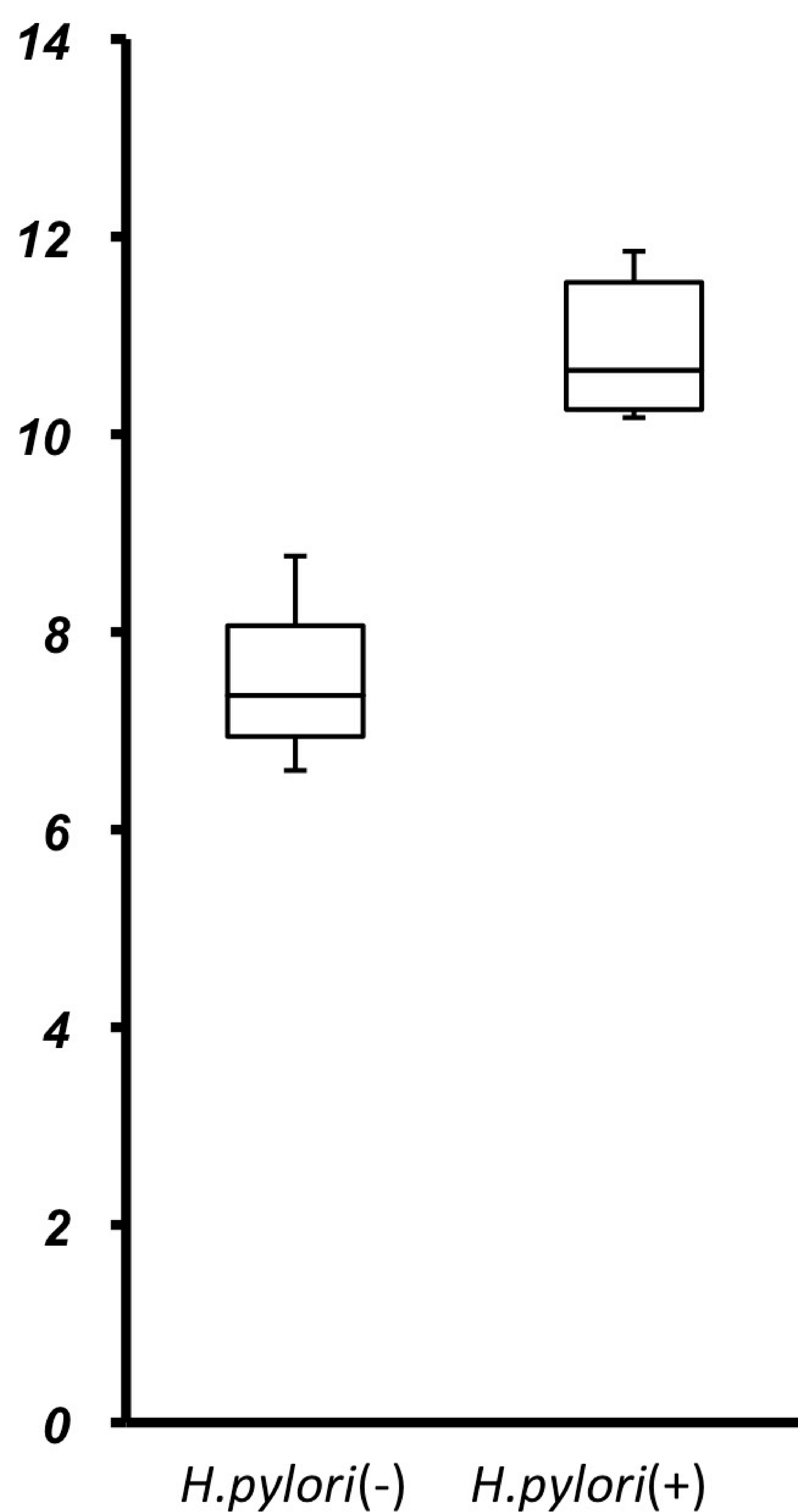

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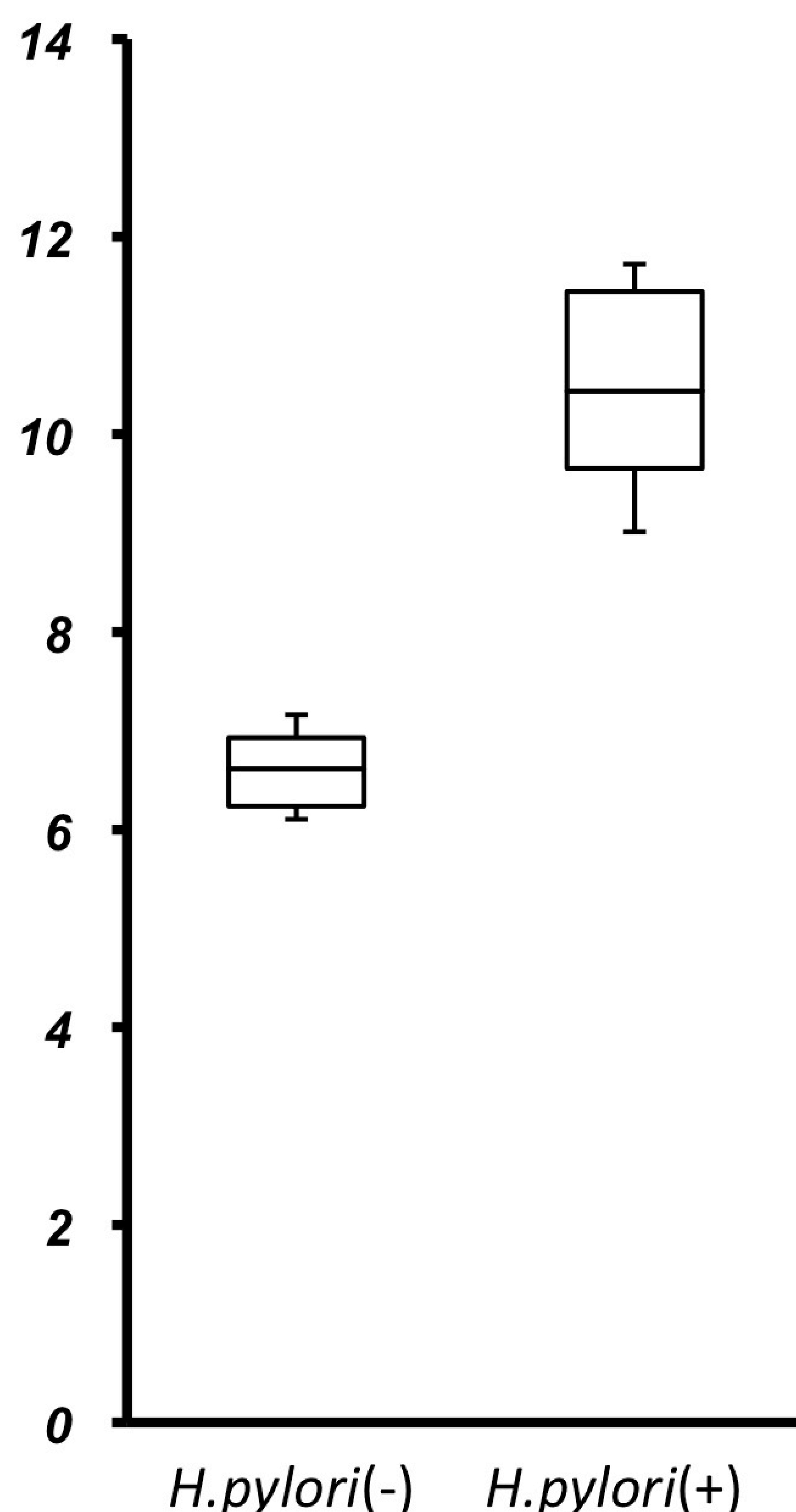

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Antrum

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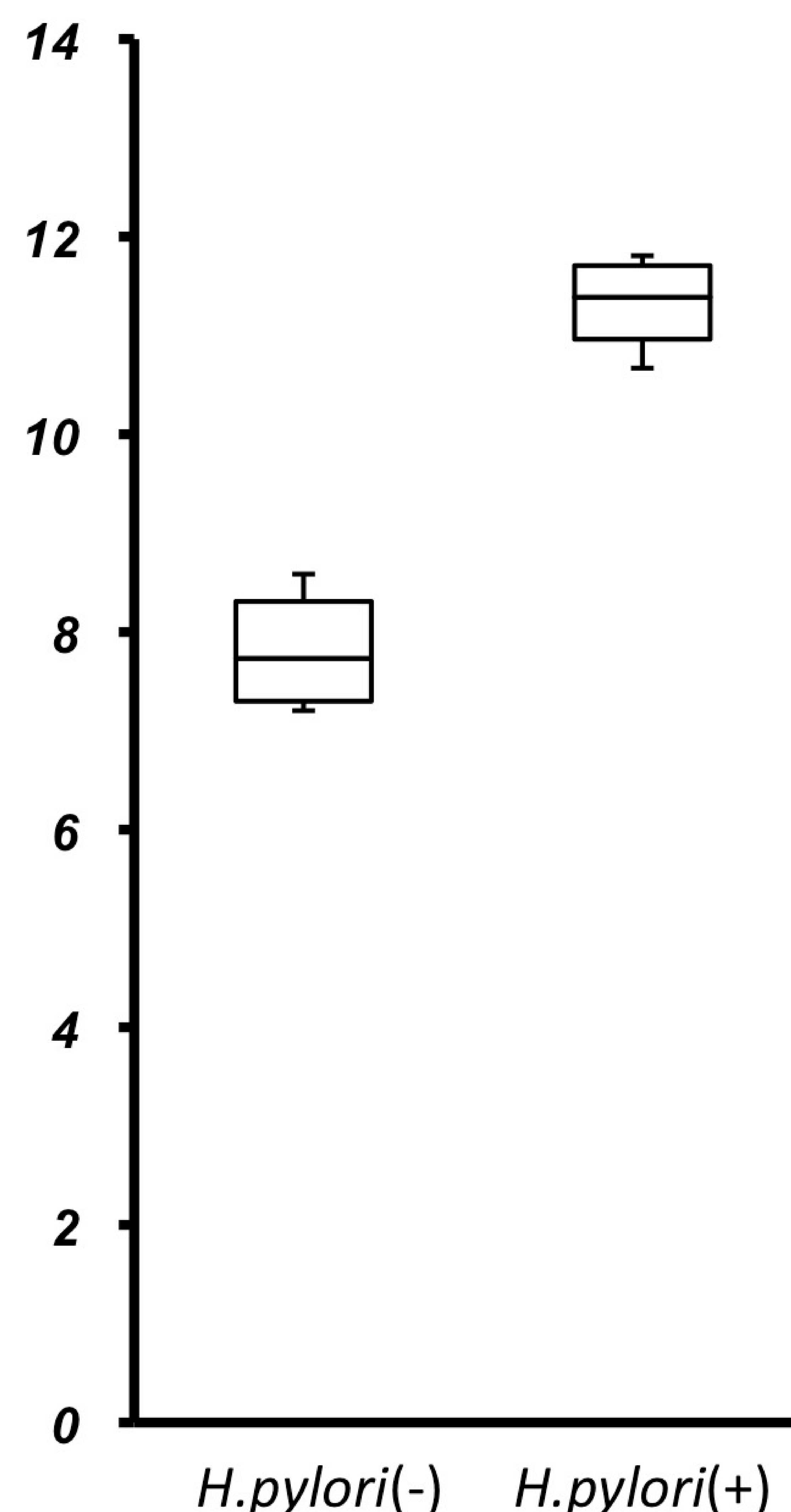

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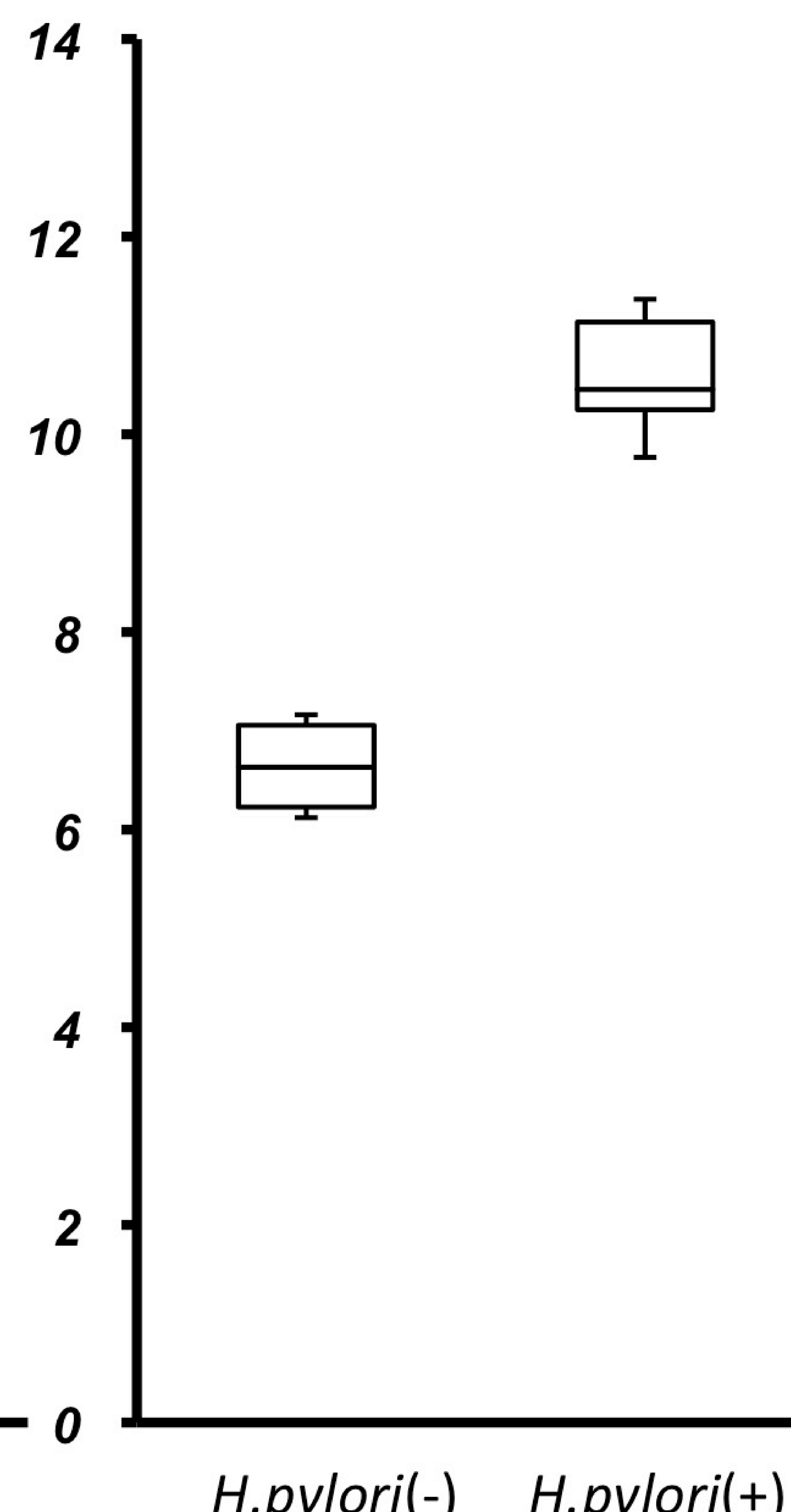

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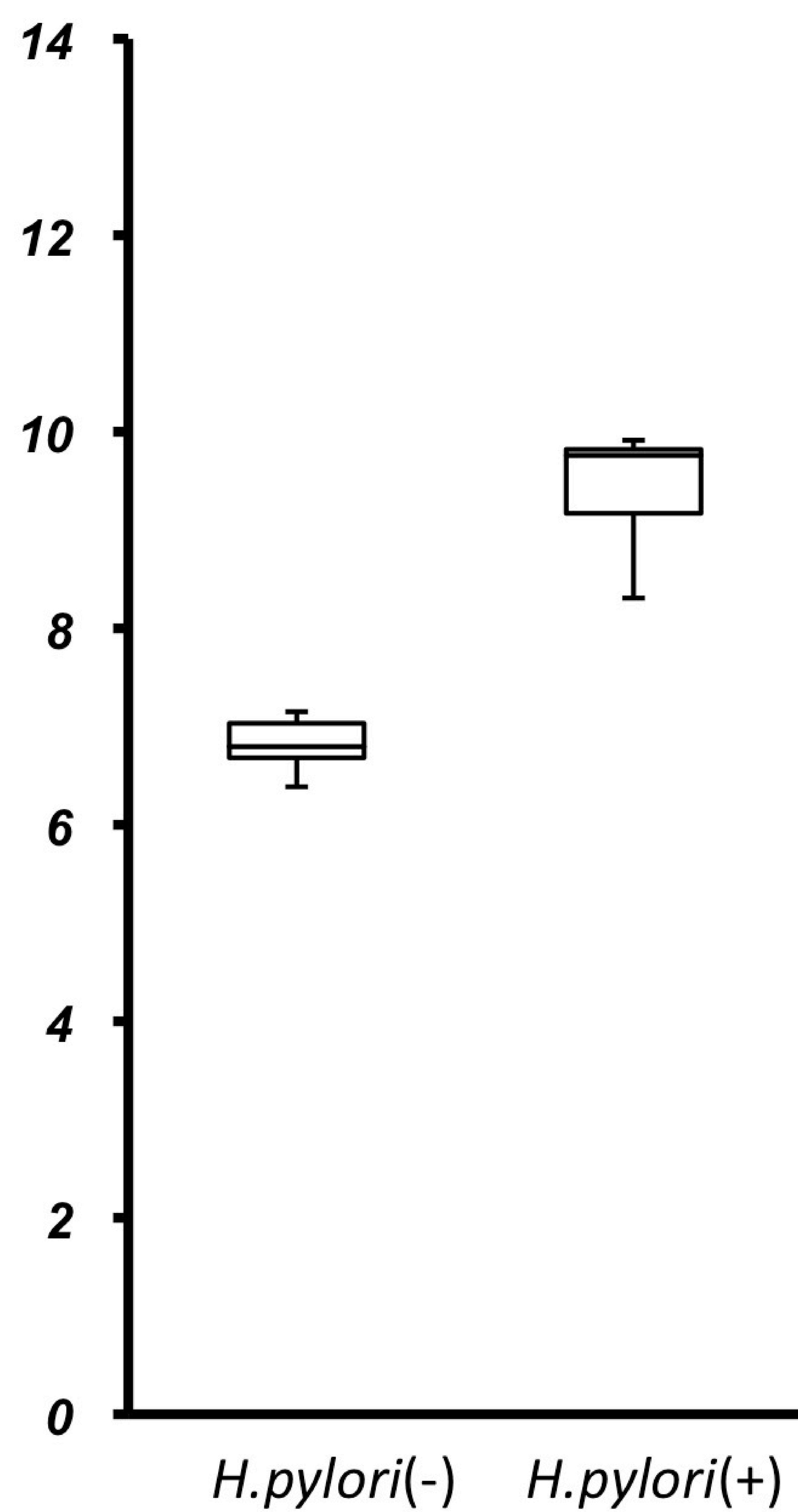
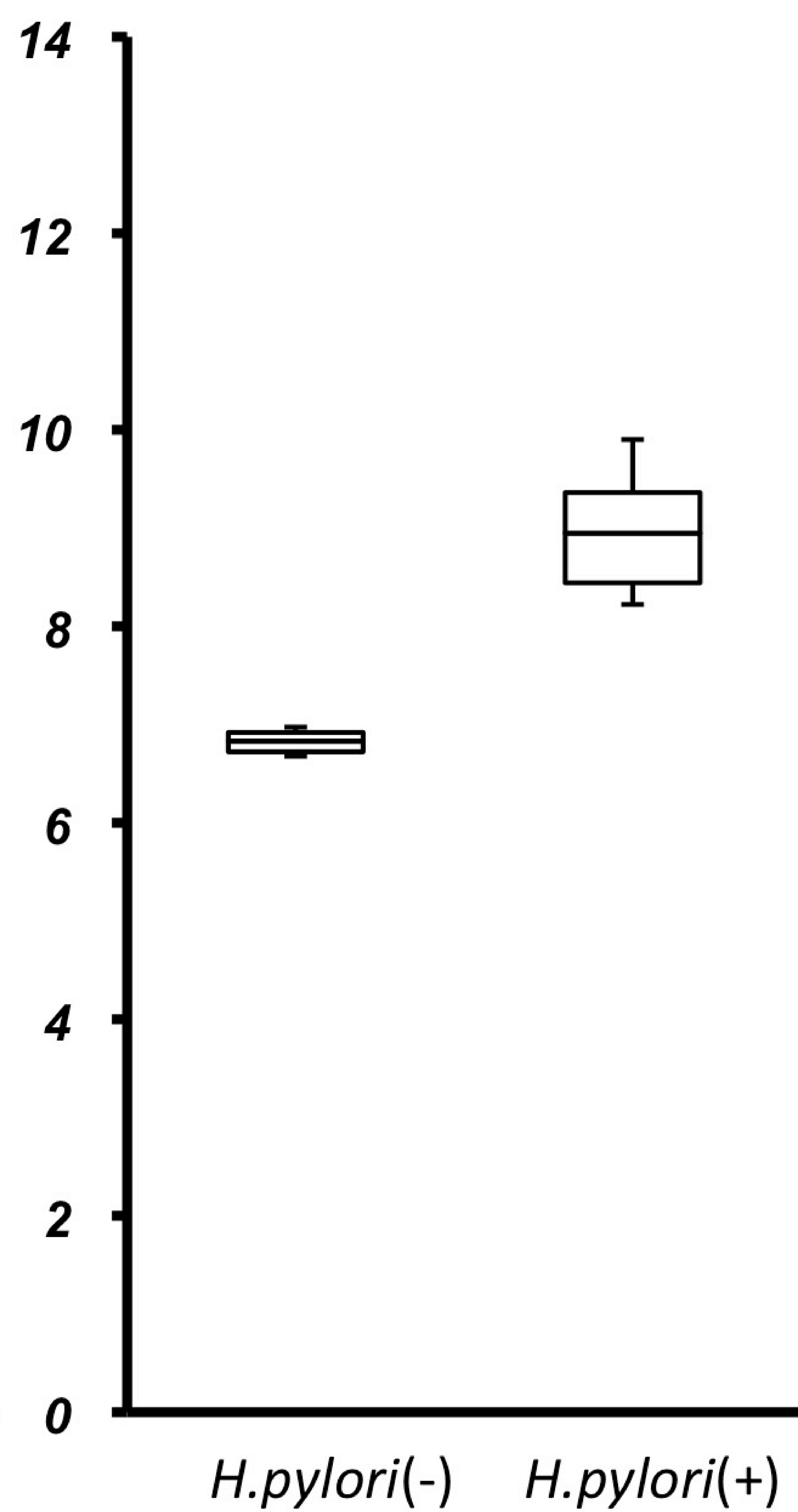
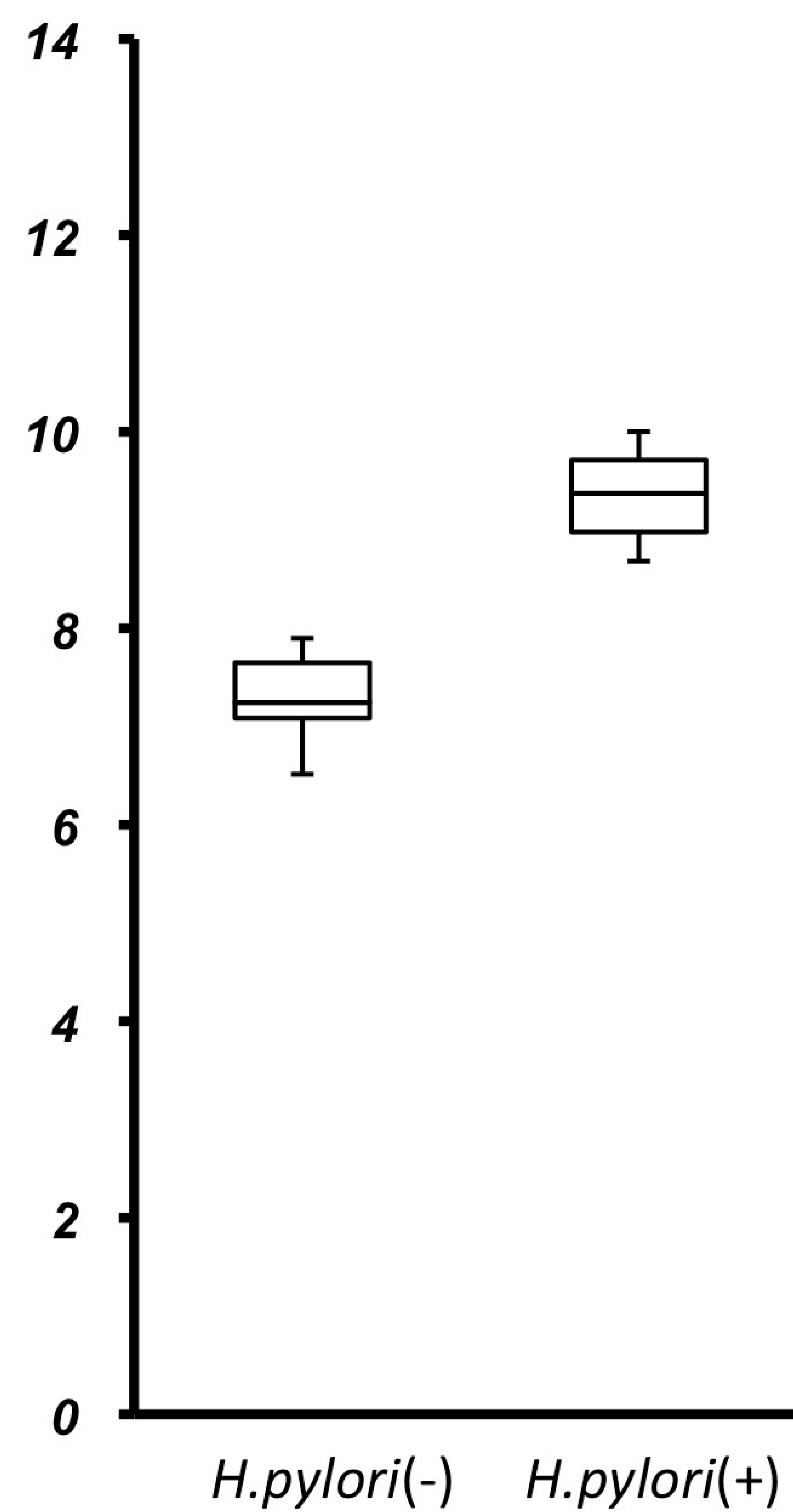
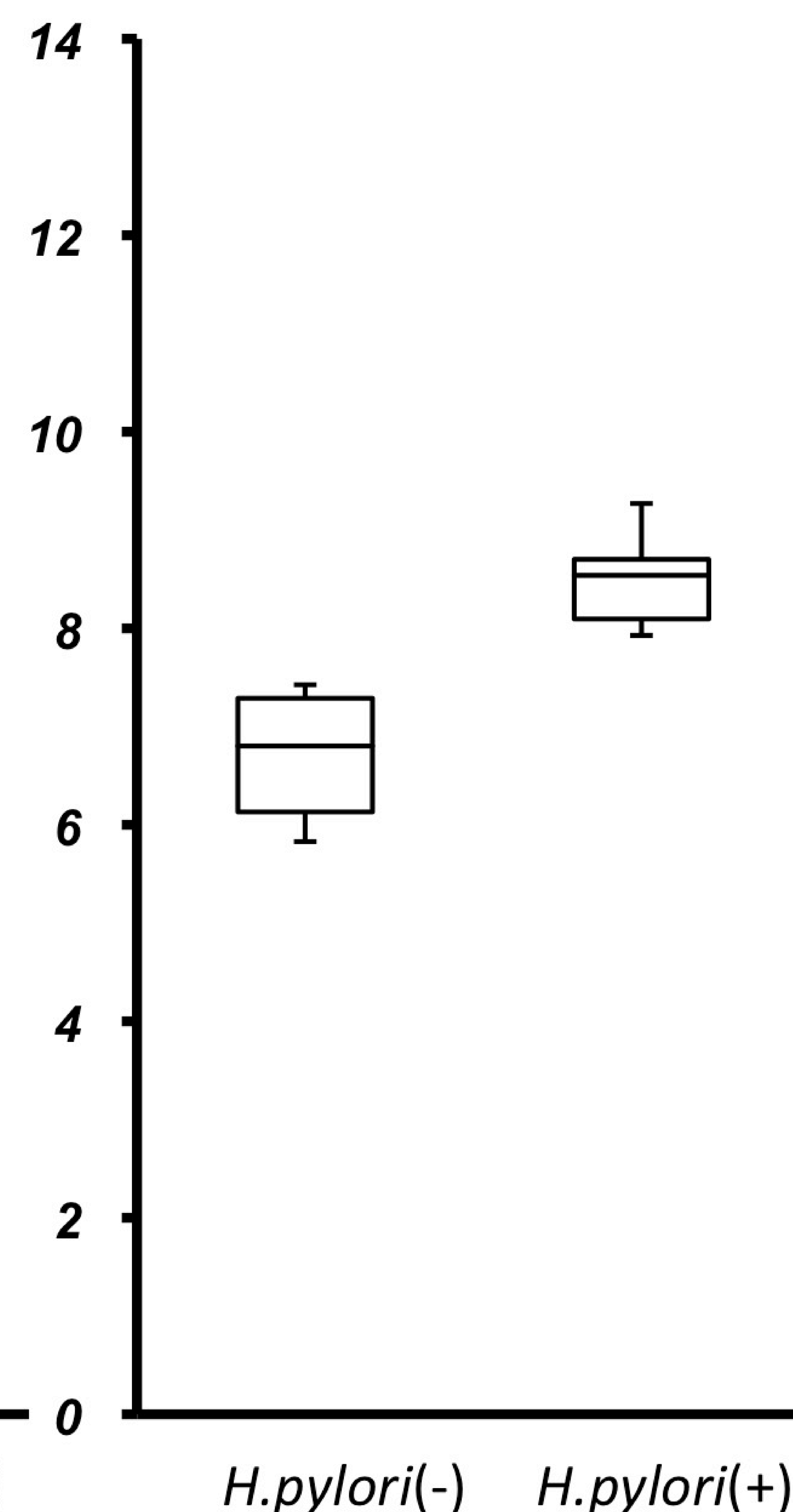
Children









Adults

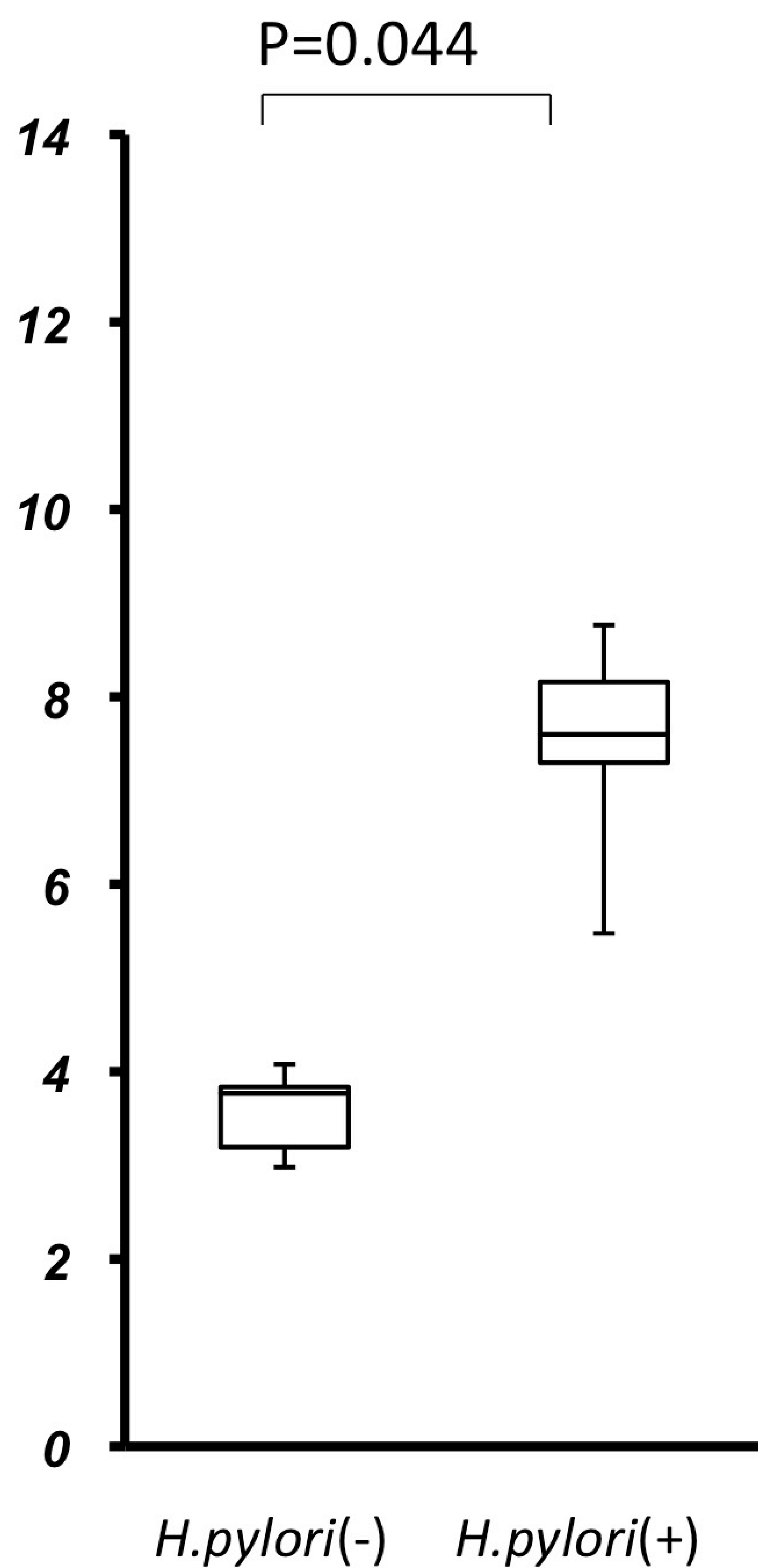
Children

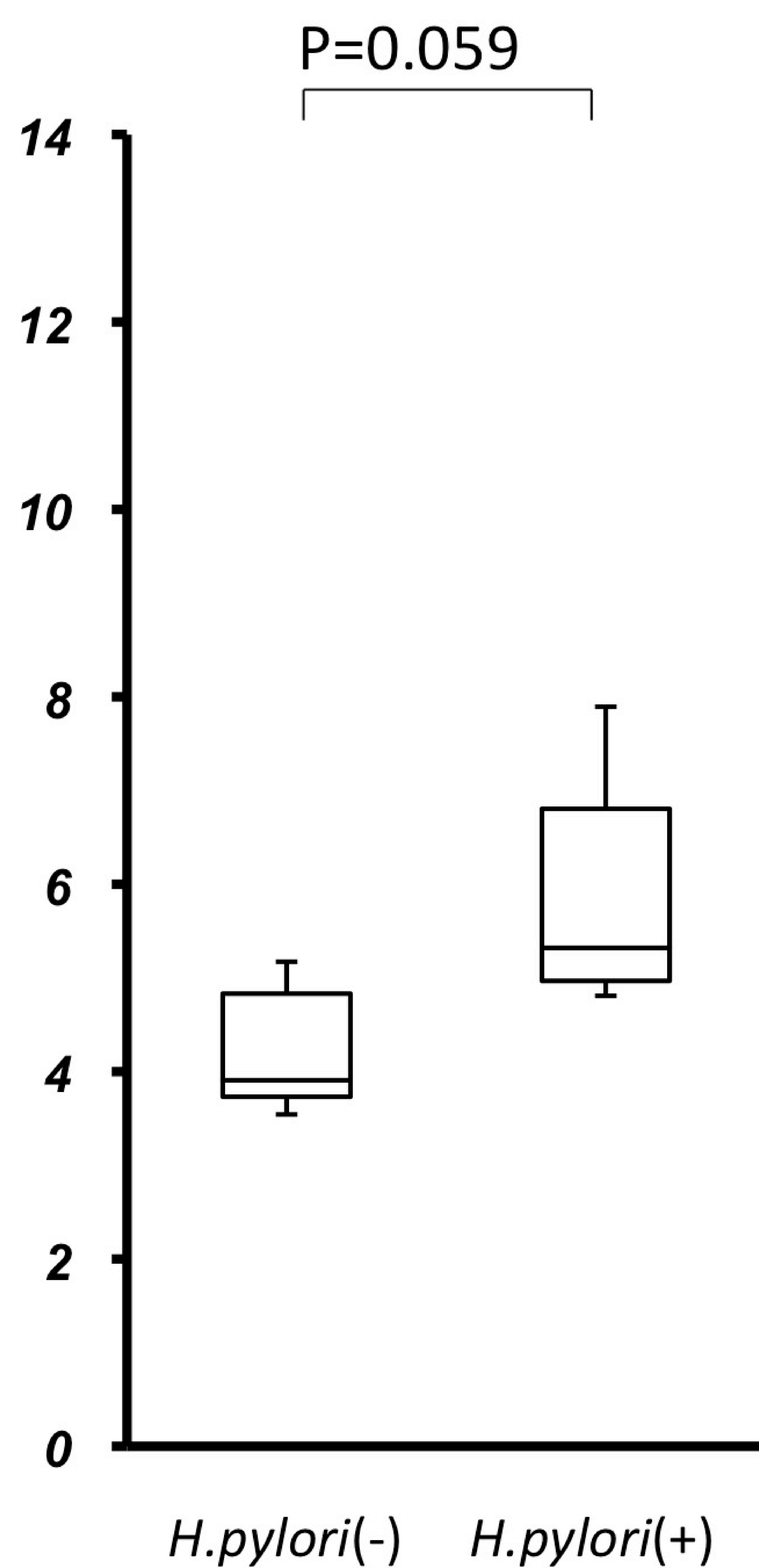
Antrum

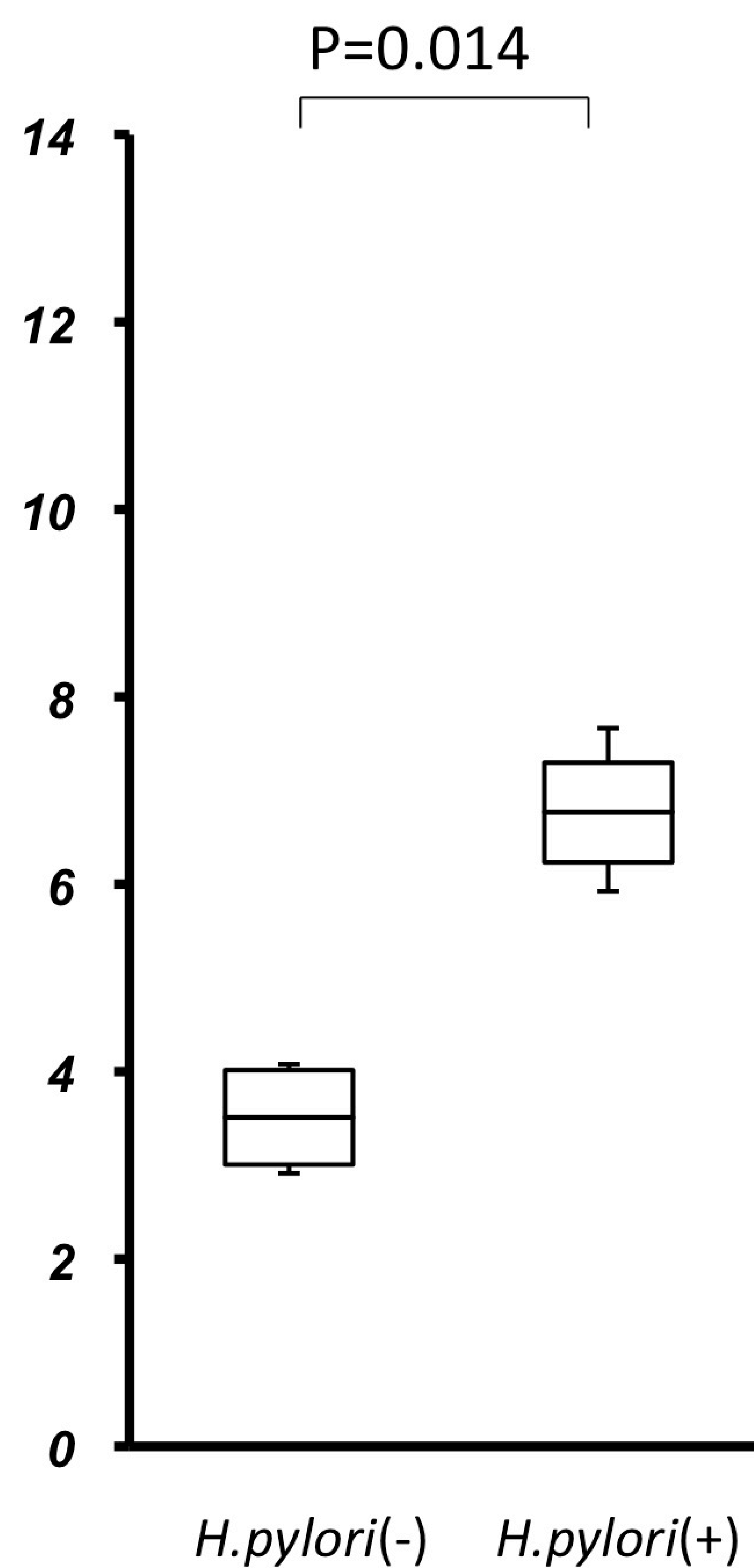
Corpus

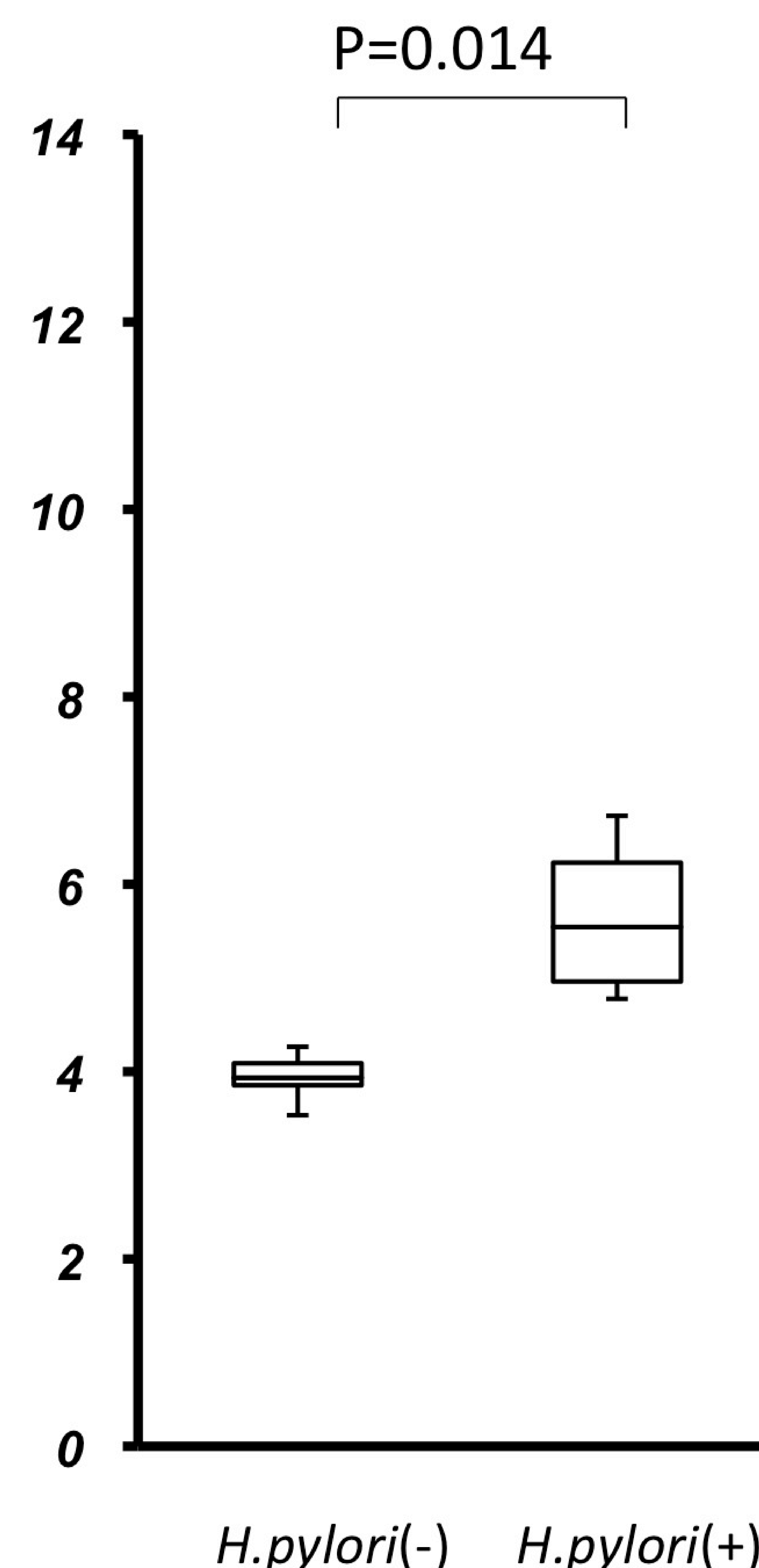
Antrum

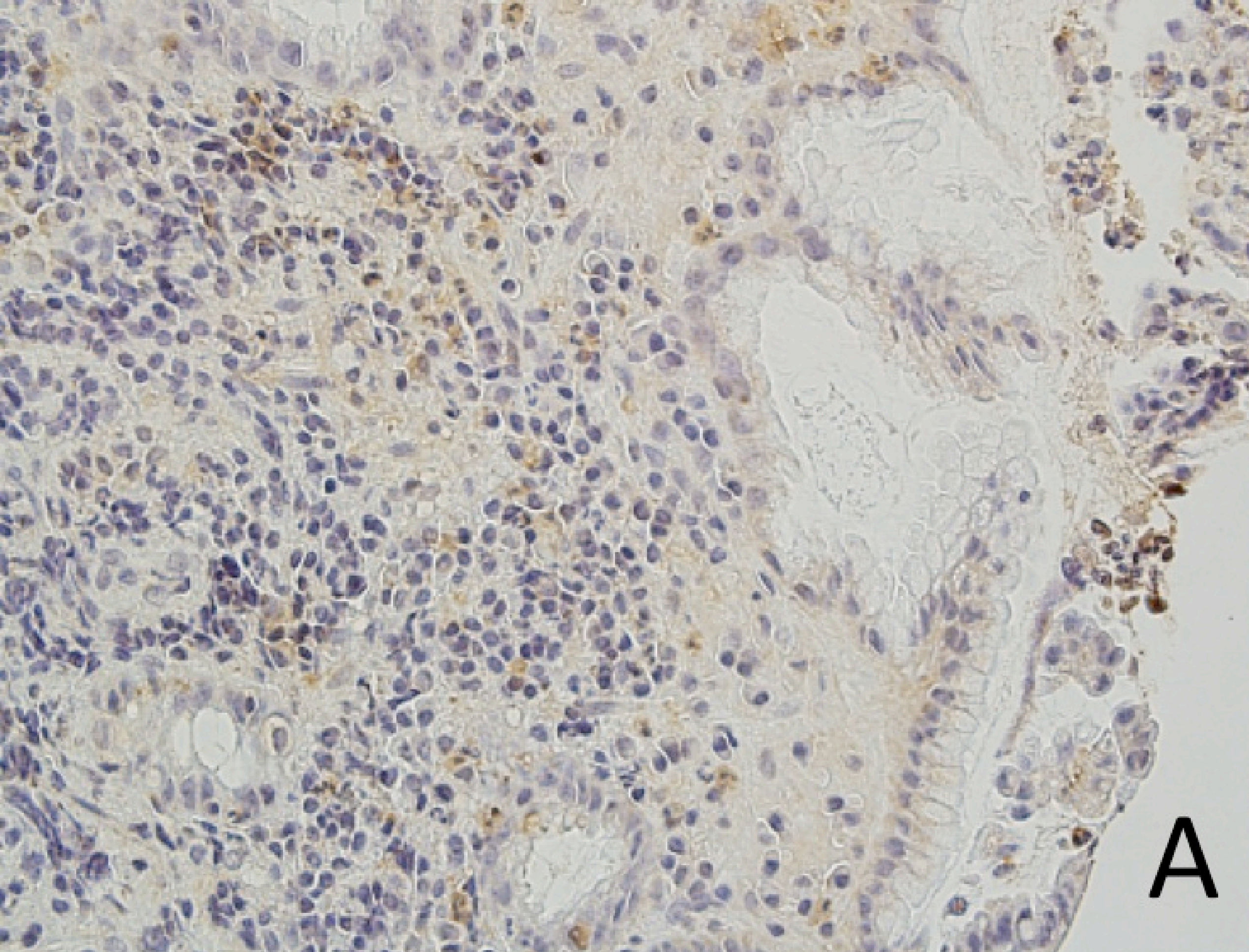
Corpus







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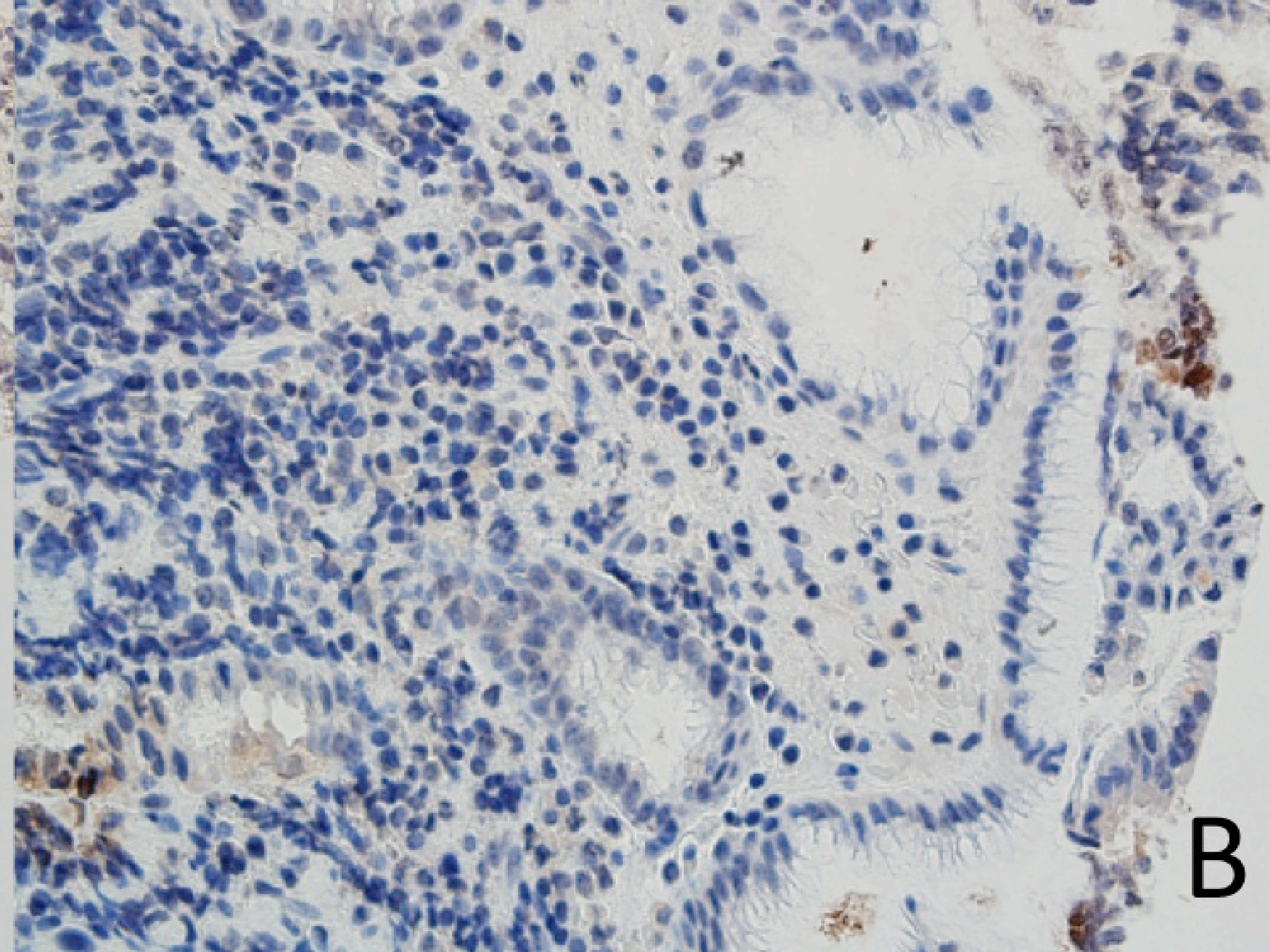
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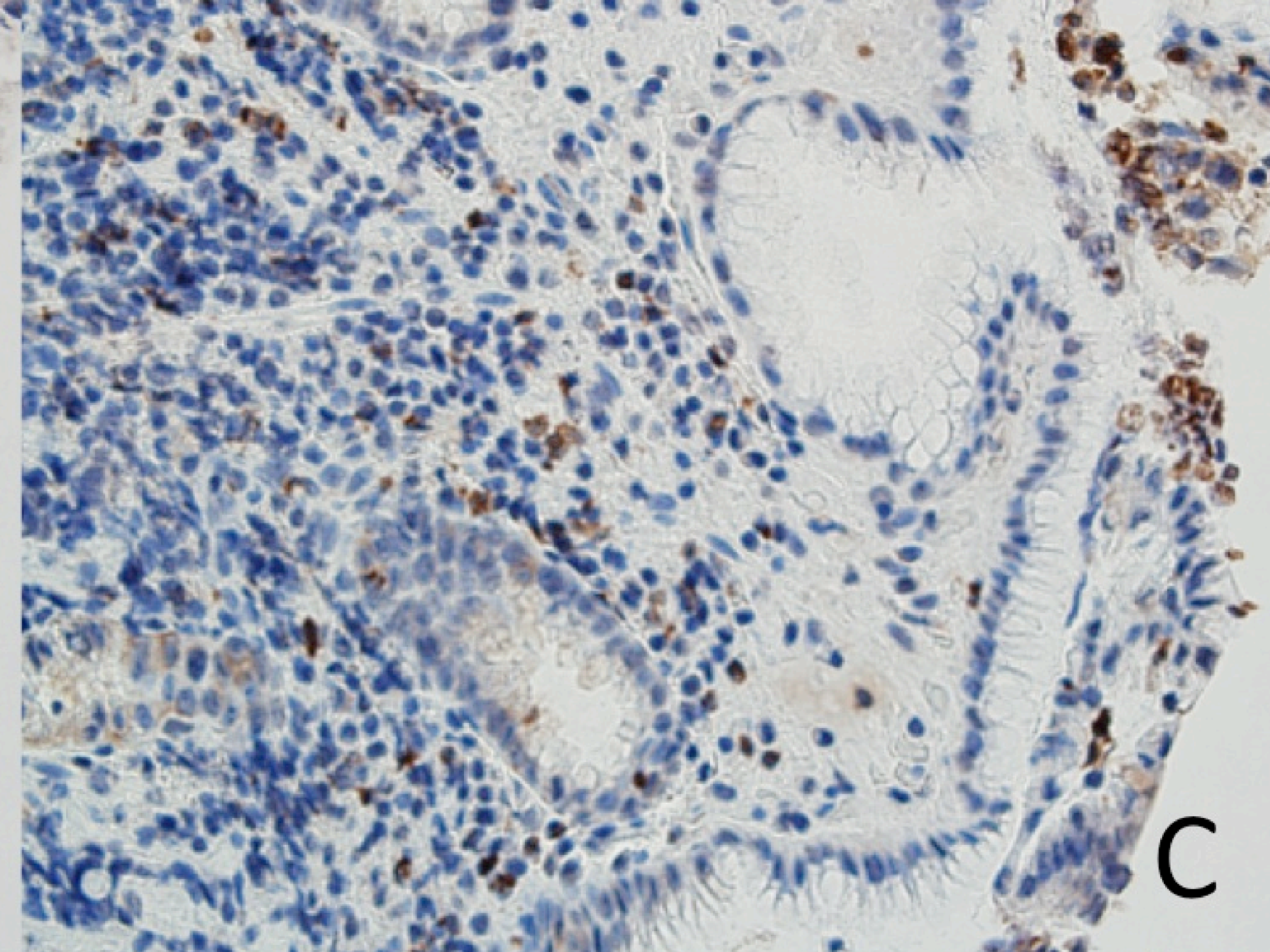
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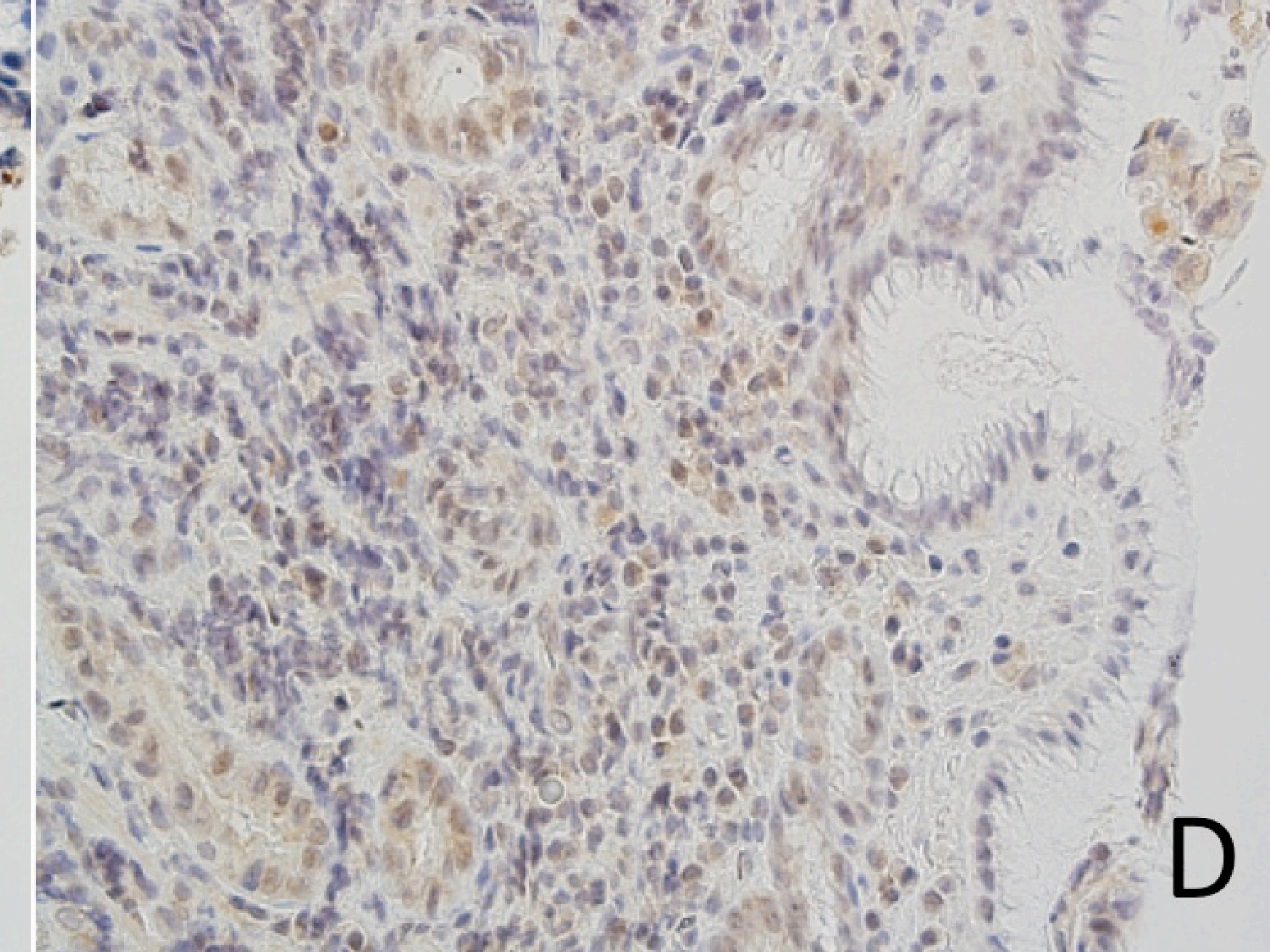
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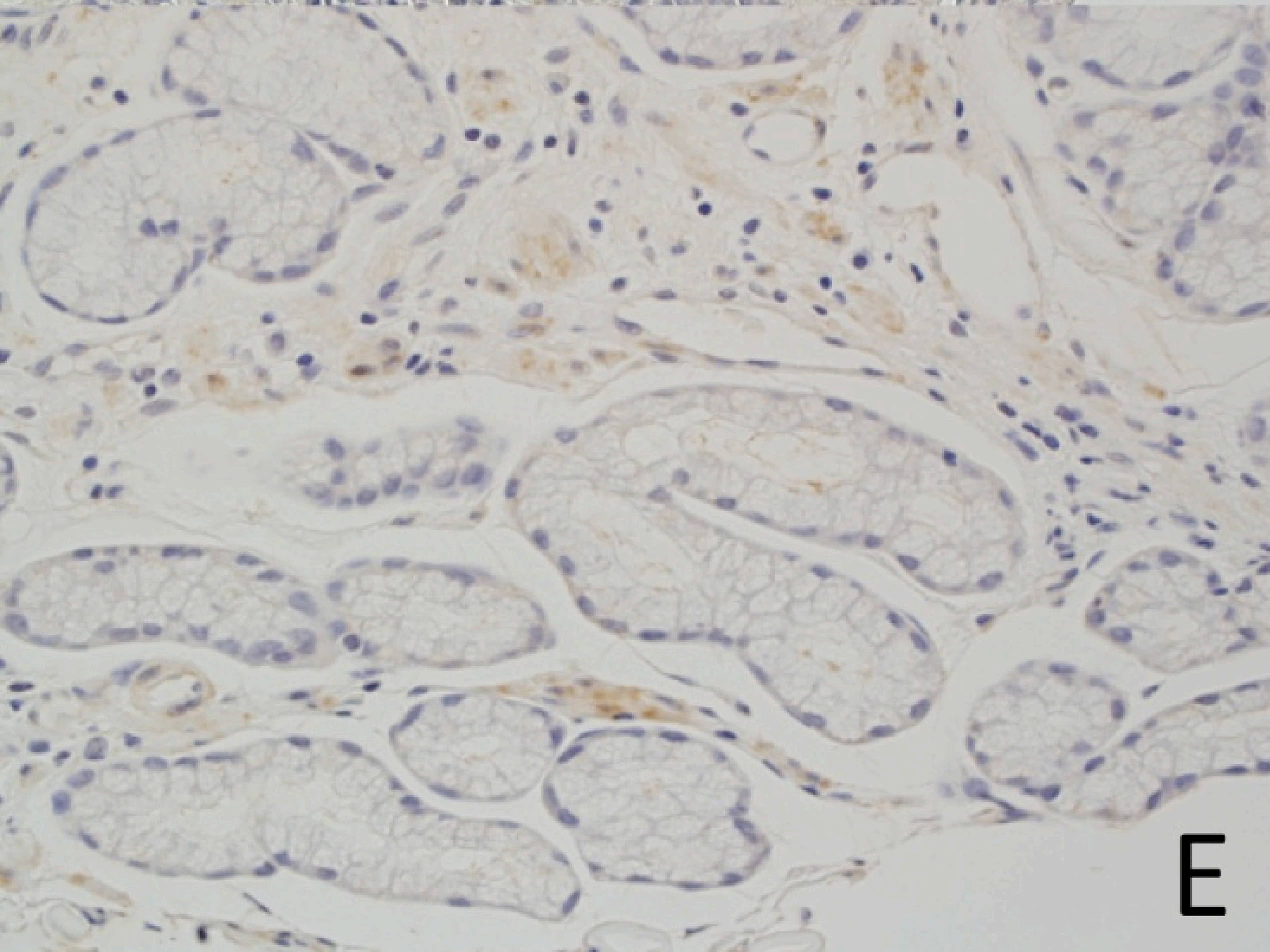
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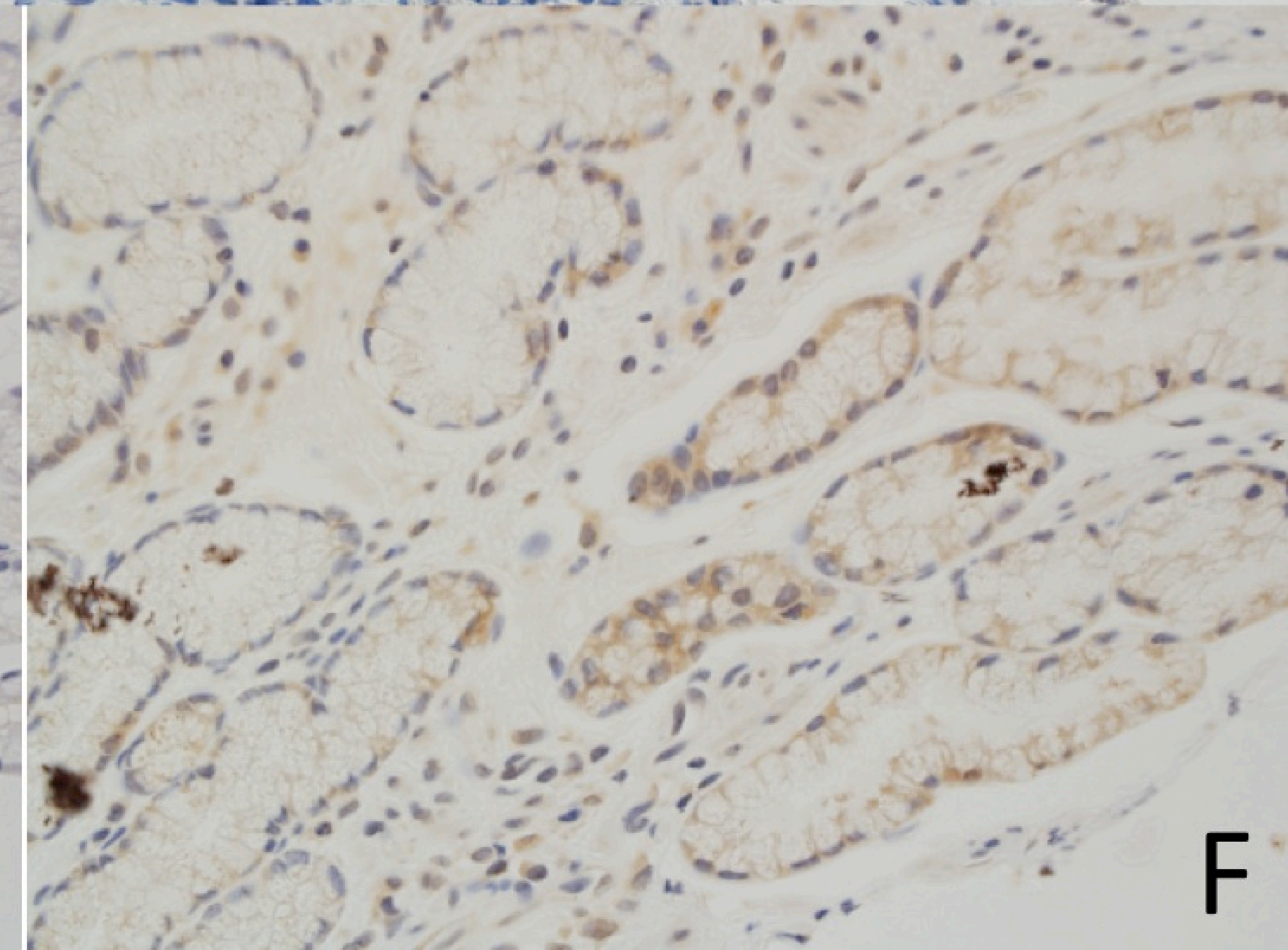
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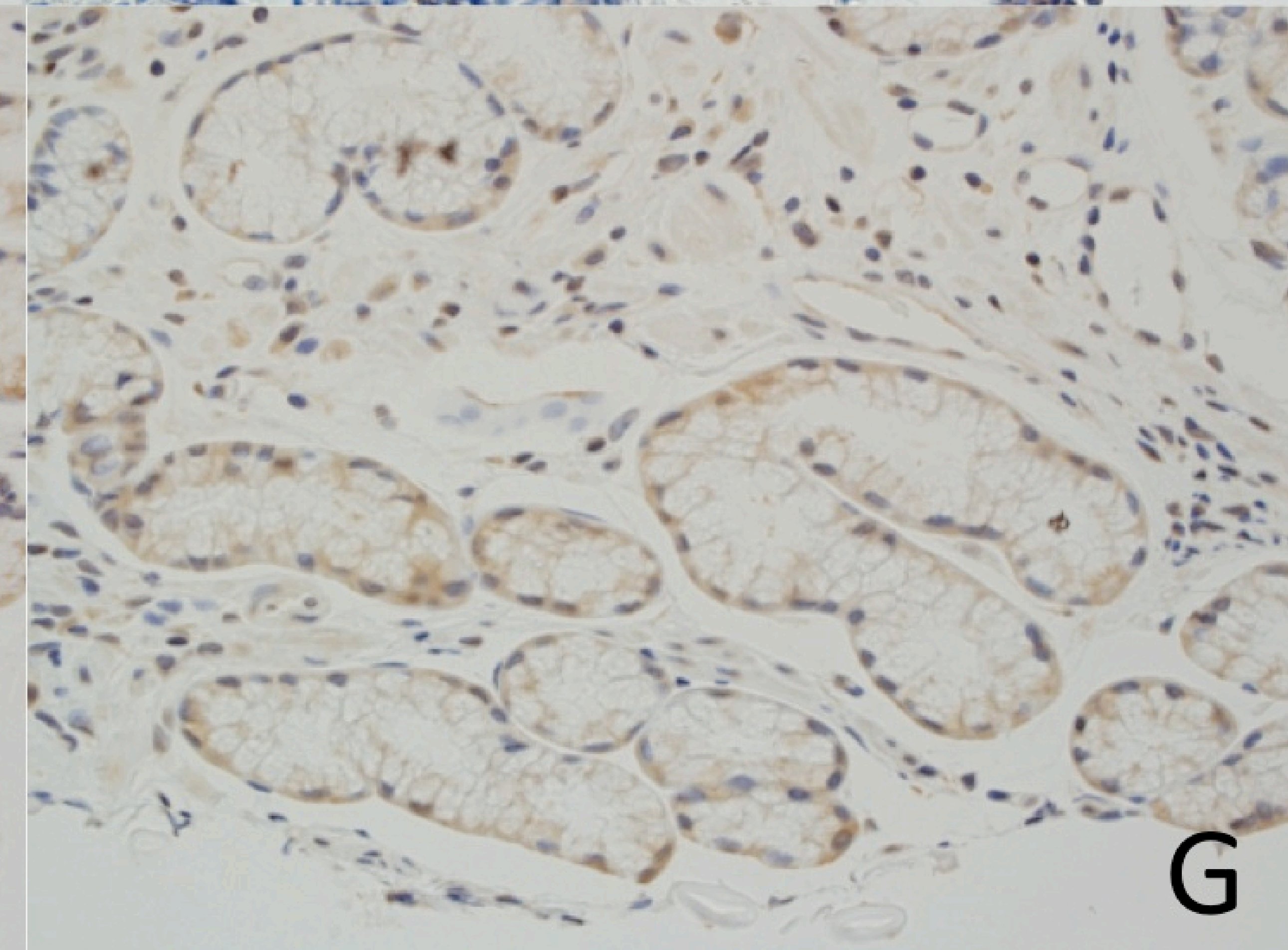
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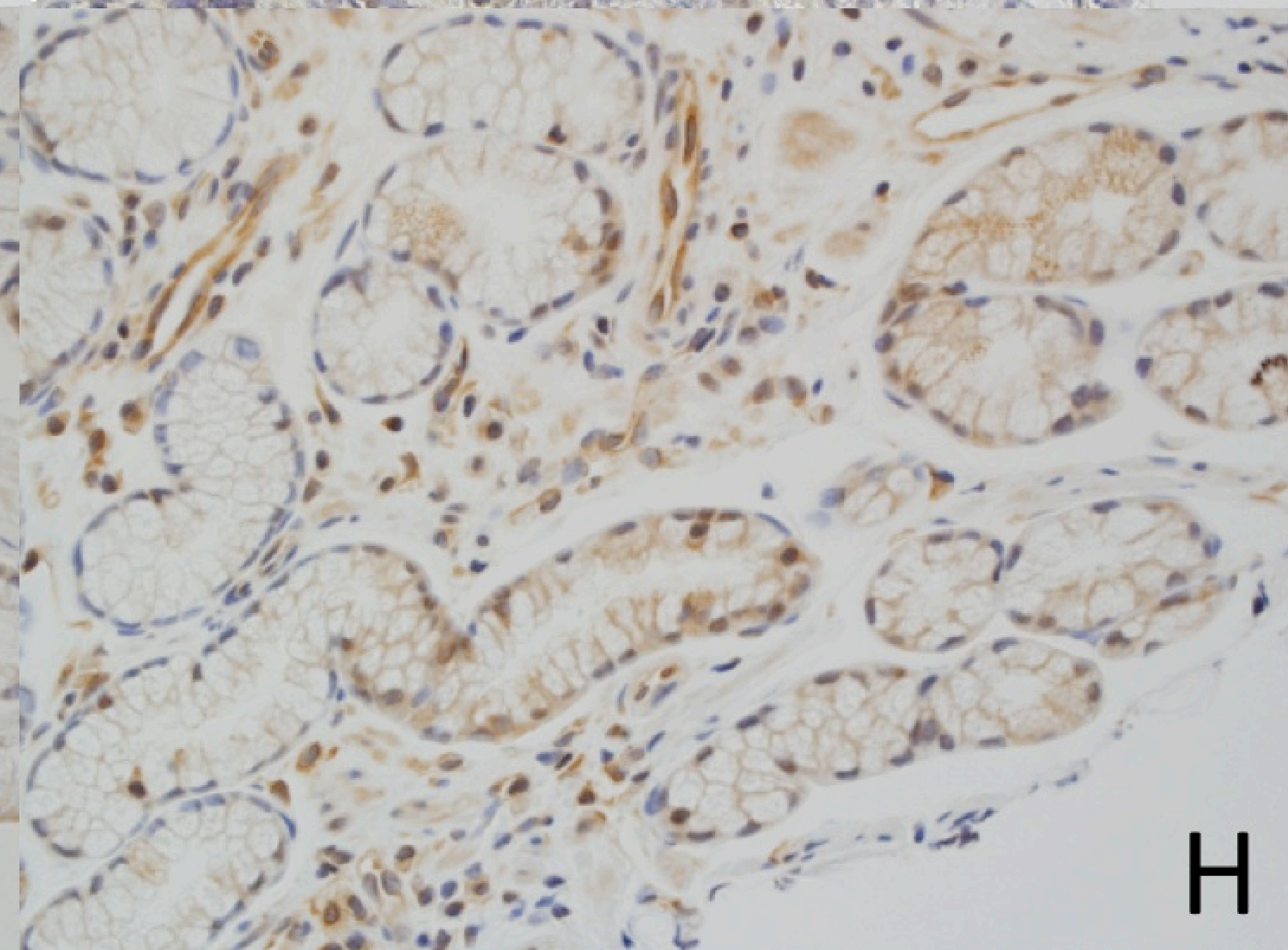
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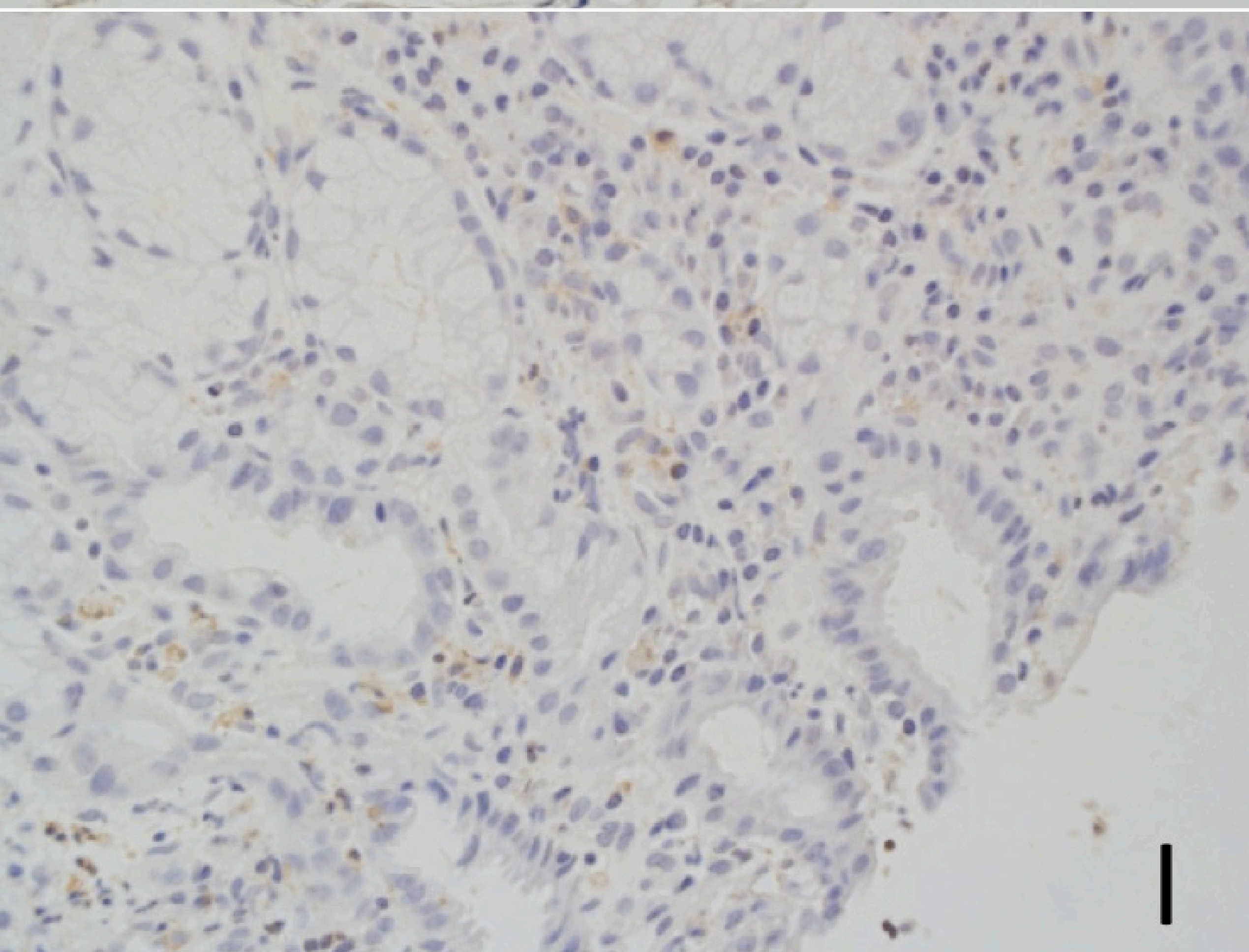
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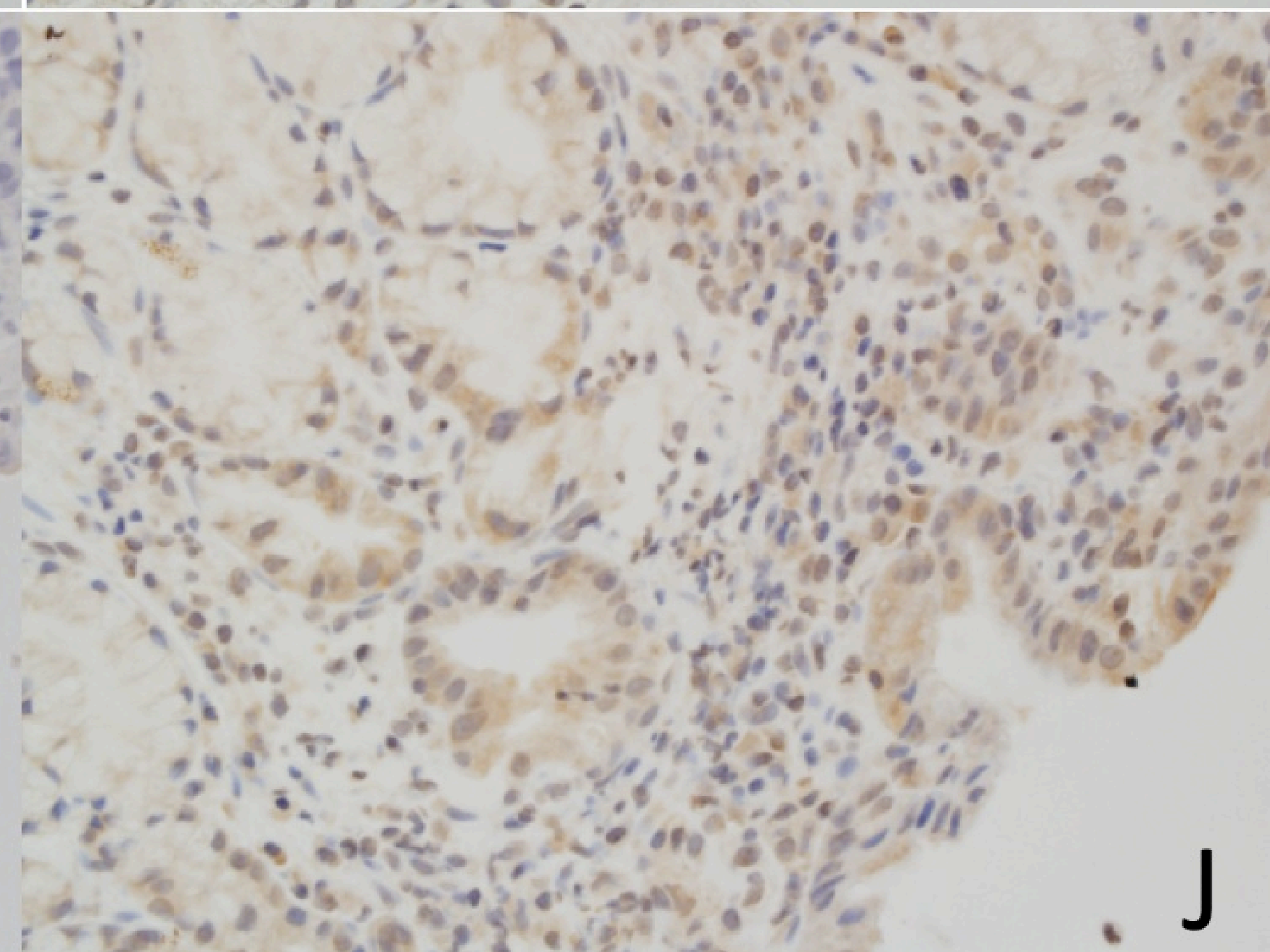
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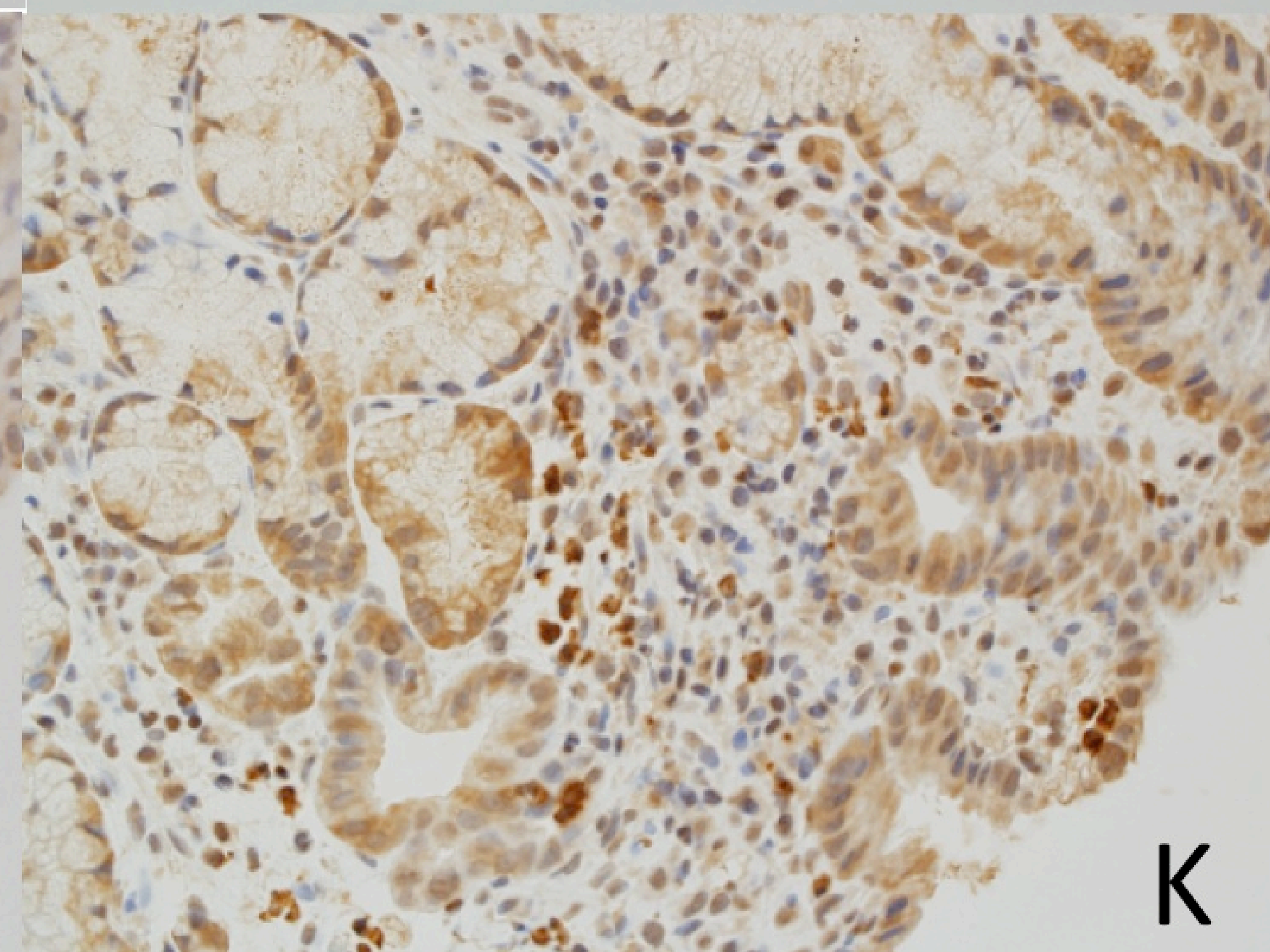
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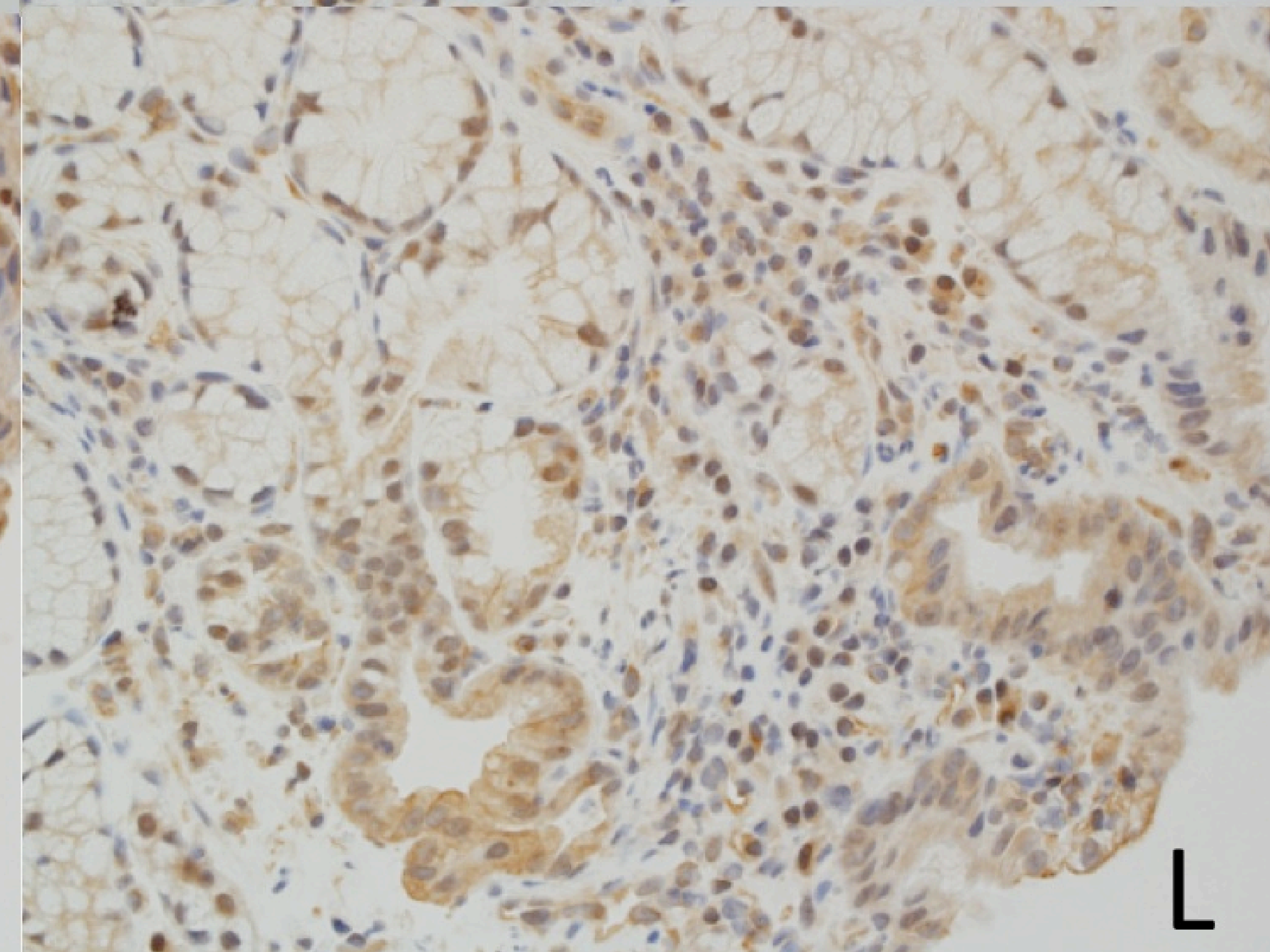
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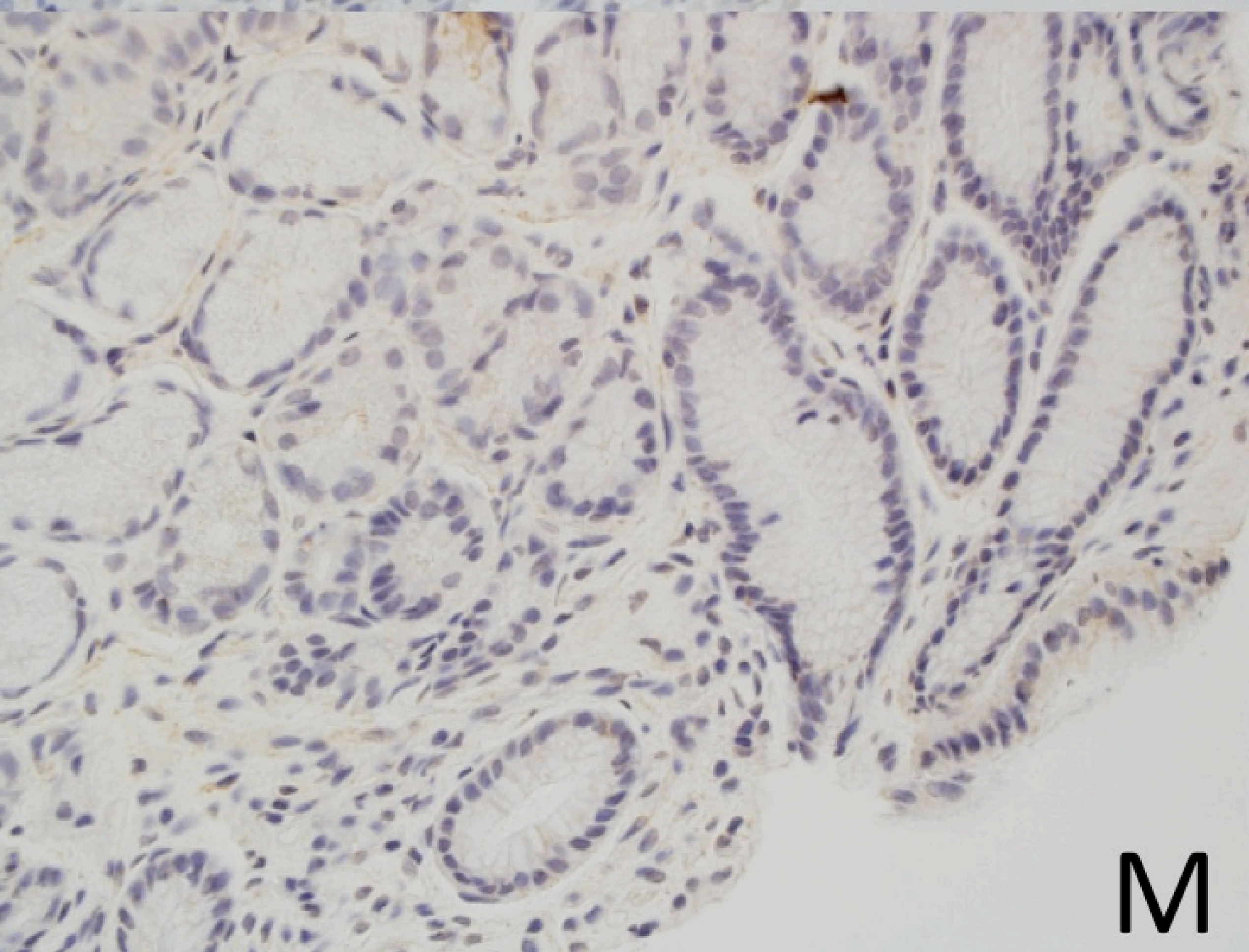
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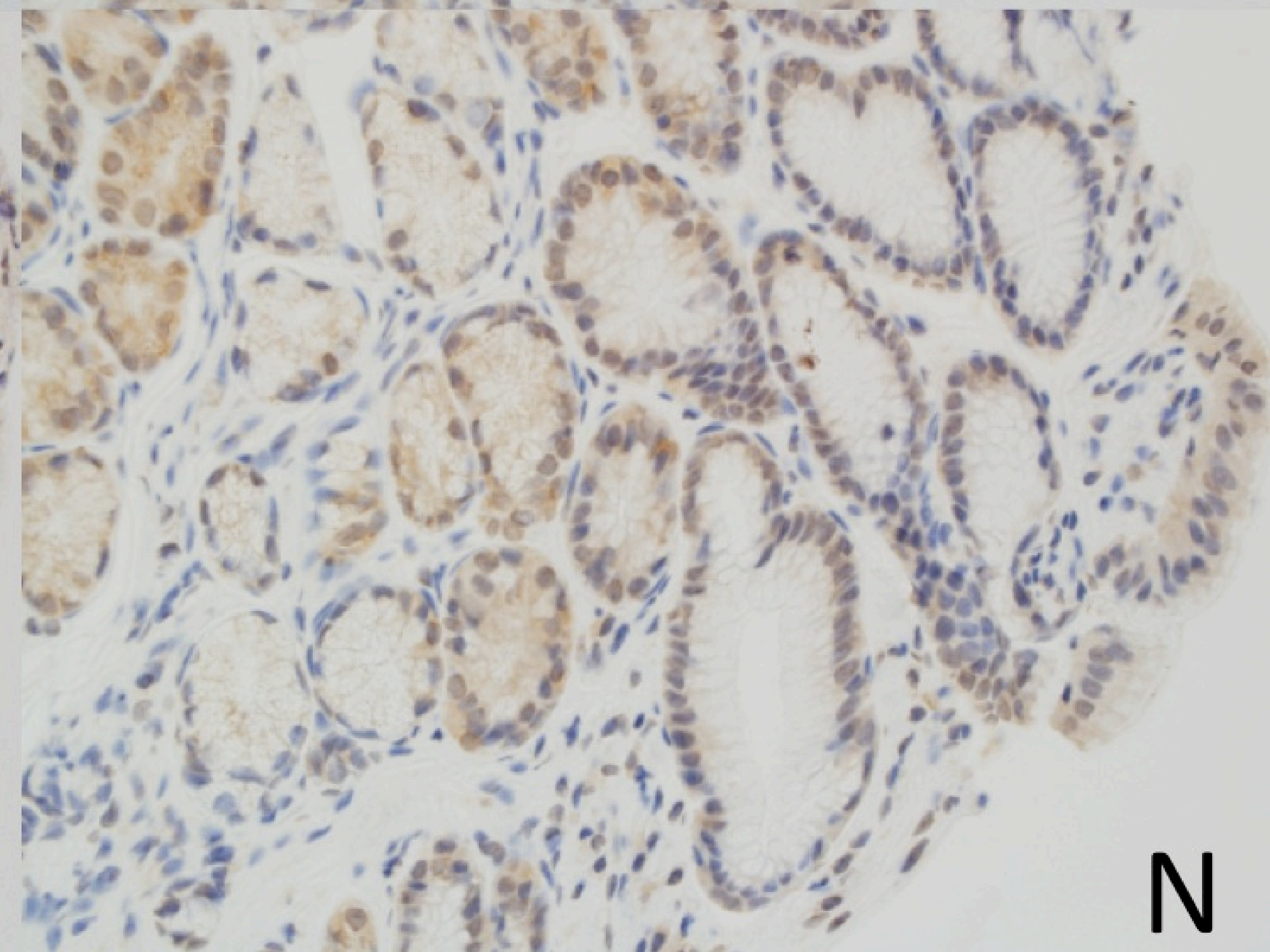
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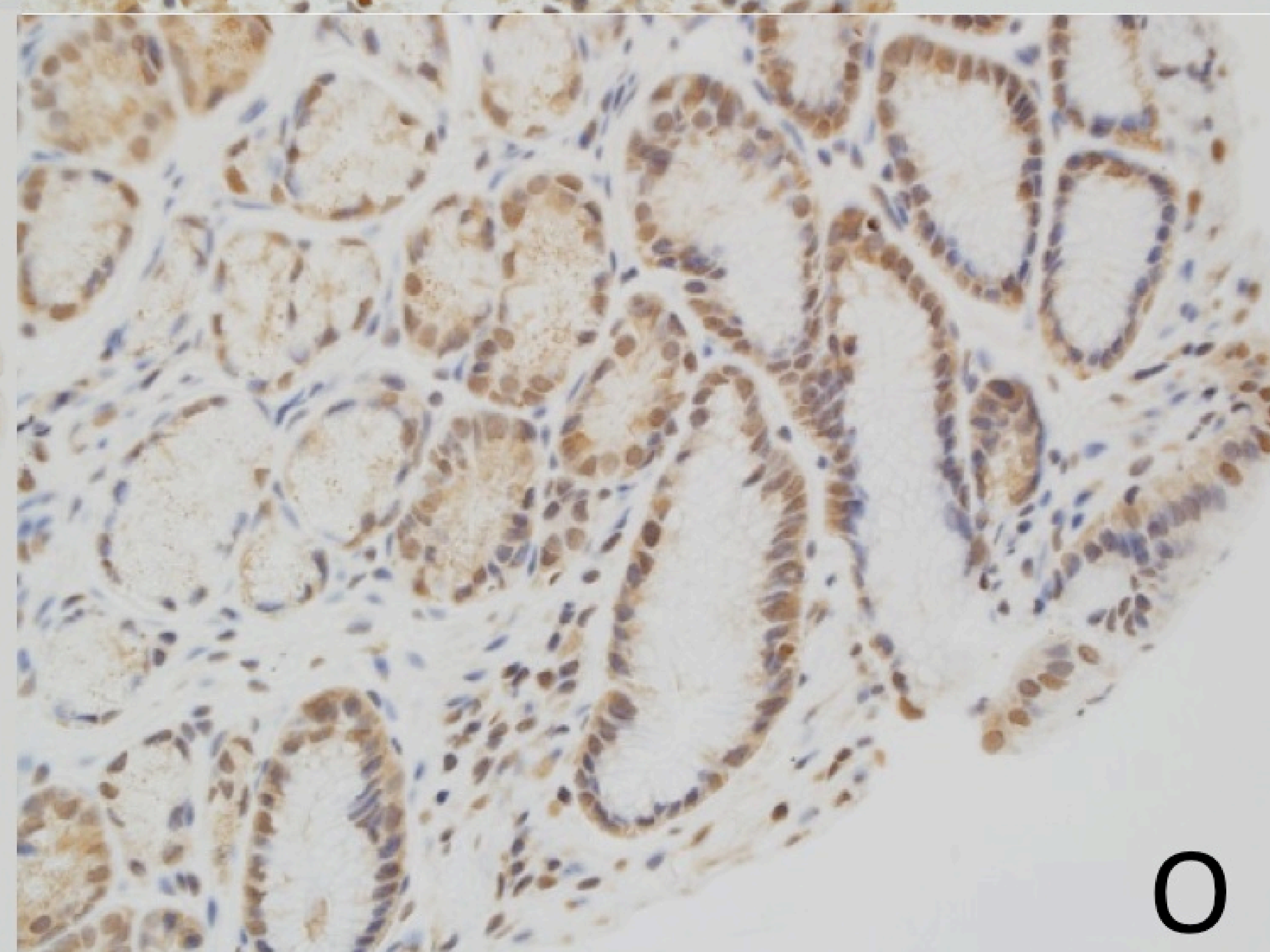
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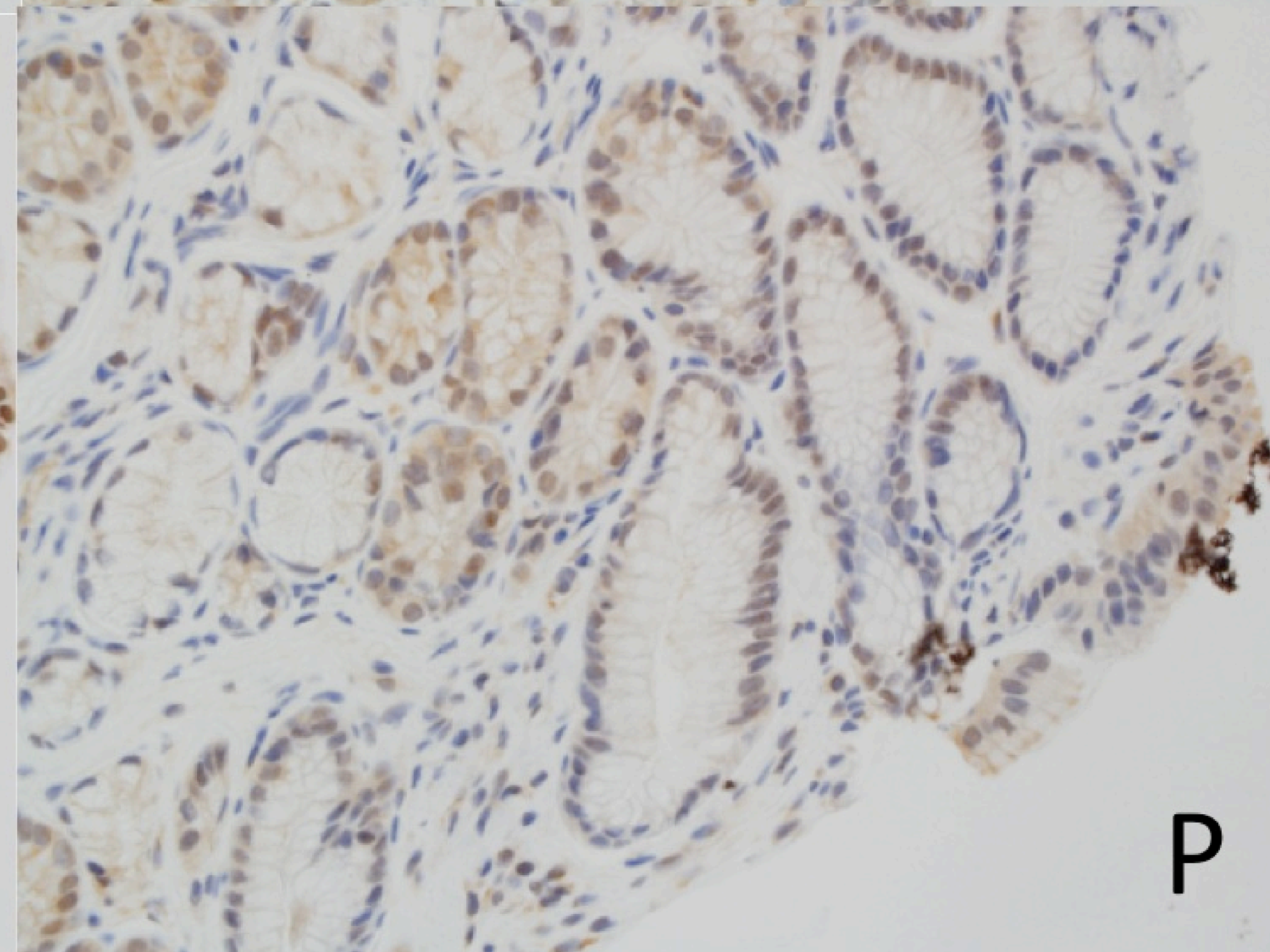
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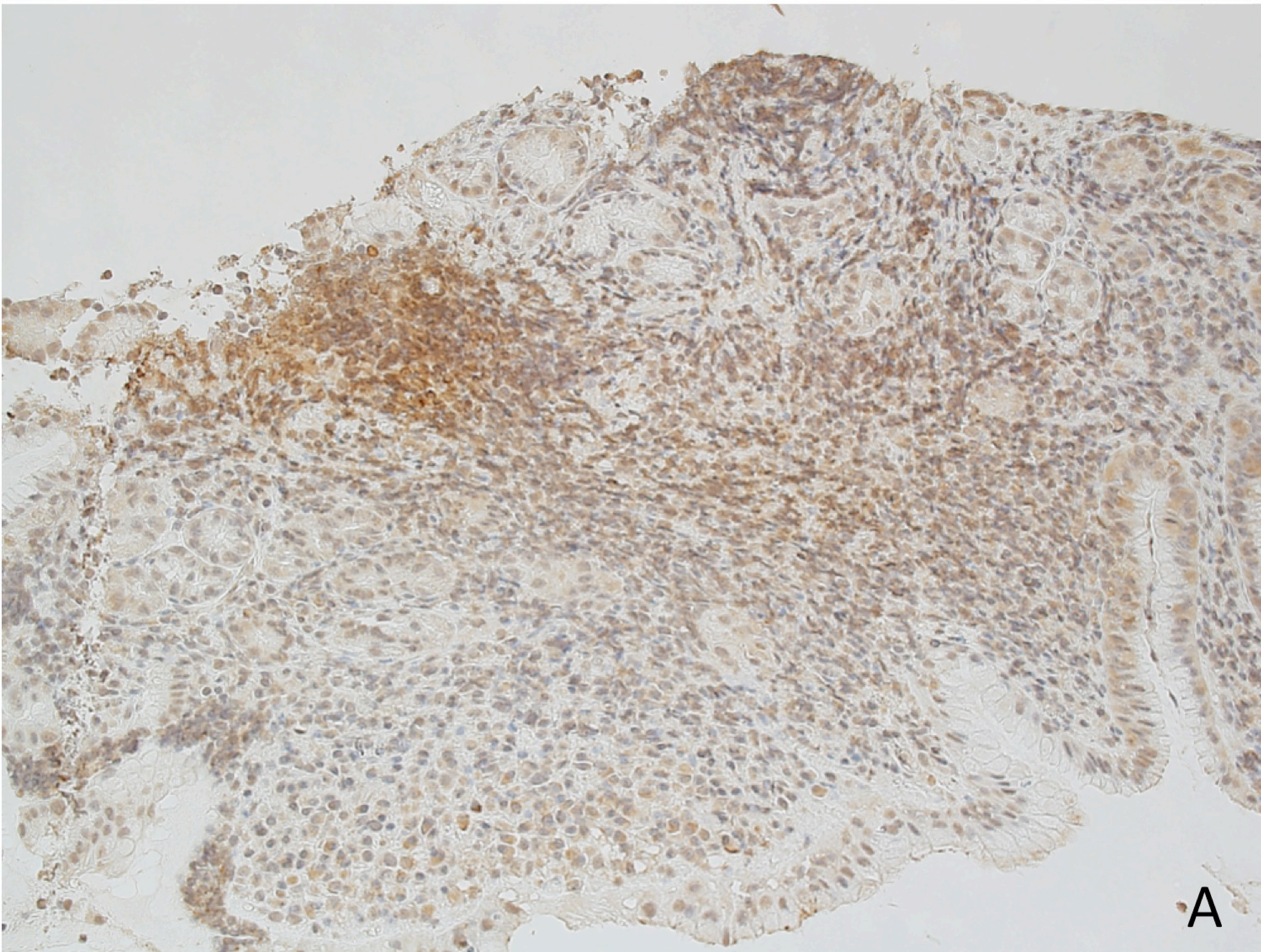
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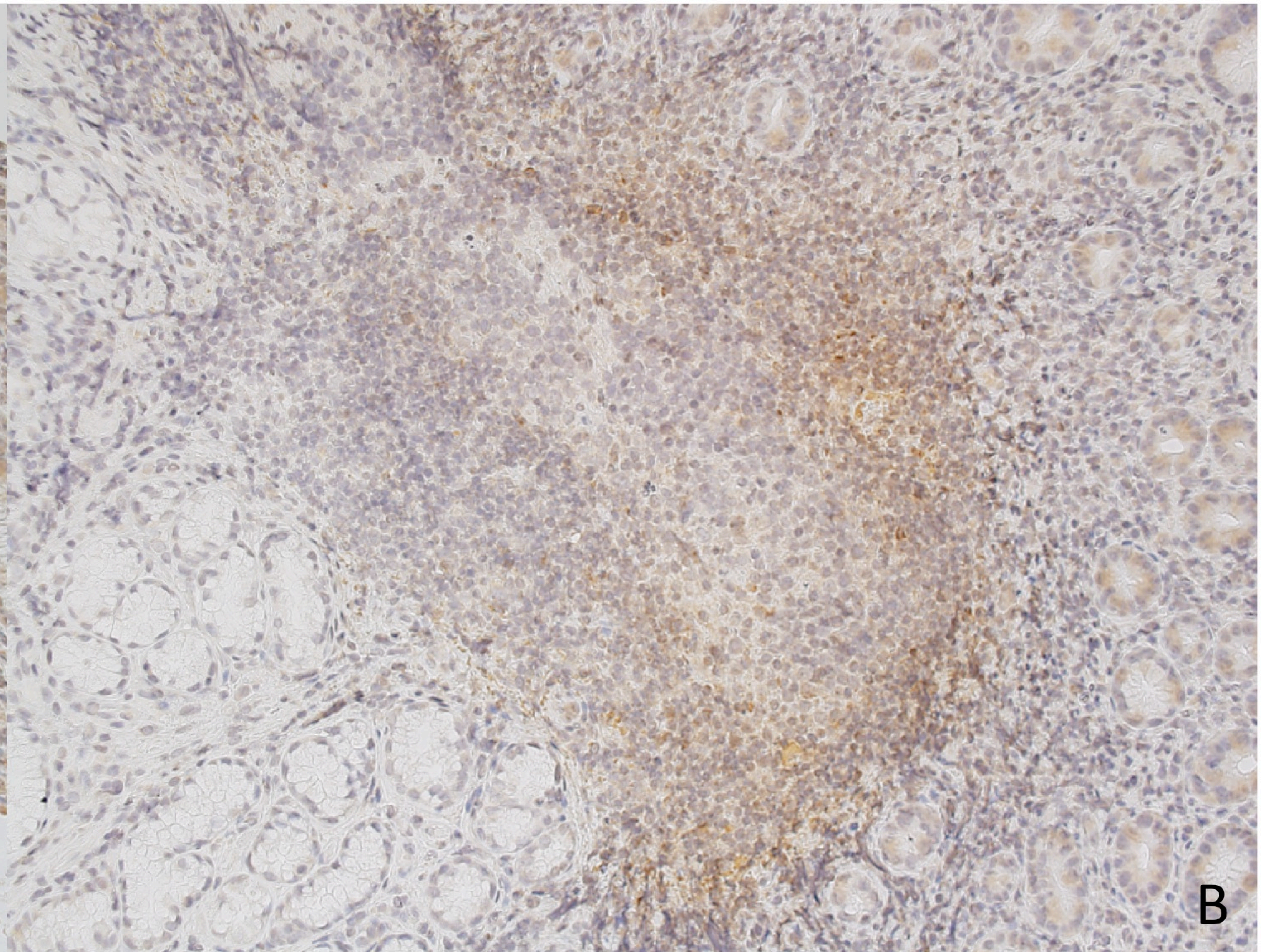
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