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## Serologic evidence that *Ascaris* and *Toxoplasma* infections impact inflammatory responses to *Helicobacter pylori* in Colombians

Courtney Ek<sup>1</sup>, Mark T. Whary<sup>1</sup>, Melanie Ihrig<sup>2</sup>, Luis E. Bravo<sup>3</sup>, Pelayo Correa<sup>4</sup>, and James G. Fox<sup>1</sup>

<sup>1</sup>Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

<sup>2</sup>Comparative Medicine Program, The Methodist Hospital Research Institute, Houston, Texas 77030, USA

<sup>3</sup>Department of Pathology, Universidad del Valle School of Medicine, Cali, 25360, Colombia

<sup>4</sup>Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37240, USA

### Abstract

**Background**—*Helicobacter pylori* infected children from coastal Tumaco, Colombia have more parasitism, and adults have lower gastric cancer risk compared to high altitude Pasto/Tuquerres residents. Because helminth and *Toxoplasma gondii* infections alter helicobacter gastritis in rodent models, we determined if seropositivity to *Ascaris lumbricoides* or *T. gondii* was associated with Th2-IgG1 or Th1-IgG2 responses to *H. pylori*.

**Methods**—Sera (240) from the two populations were evaluated for *A. lumbricoides* and *T. gondii* seropositivity and results correlated with IgE and IgG isotype responses to *H. pylori*.

**Results**—Most Tumaco children and adults were seropositive for *A. lumbricoides* (89%, 66%), *T. gondii* (59%, 98%) or both (45%, 66%). In contrast, seropositivity among Pasto/Tuquerres children was much lower (9% *A. lumbricoides*, 11% *T. gondii* and 2% dual positive) but increased in adults (58% *A. lumbricoides*, 82% *T. gondii* and 41% dual positive). *A. lumbricoides* seropositivity correlated with elevated IgE and anti-inflammatory Th2-IgG1 responses to *H. pylori*, while *Toxoplasma gondii* seropositivity was linked to elevated IgE, pro-inflammatory Th1-IgG2, IgG3 and IgG4 responses to *H. pylori*. Individuals with high *T. gondii* titers had reduced Th1-IgG2, IgG3 and IgG4 responses to *Helicobacter pylori*.

**Conclusions**—Results support regional differences for childhood parasitism and indicate *A. lumbricoides* and *T. gondii* infections may impact inflammatory responses to *H. pylori* and partially explain differences in gastric cancer risk in Colombia.

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Corresponding Author: Mark Whary, DVM, PhD, DACLAM, Division of Comparative Medicine, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Building 16-825A, Cambridge, MA 02139, Phone 617-253-9435, Fax: 617-258-5708, mwhary@mit.edu.

**Conflict of Interest Statement:**

The authors do not have any associations that might pose a conflict of interest.

**Previous Presentation of Data:**

Portions of this data were presented at Digestive Disease Week in June 2009 in Chicago, IL (abstract 587616).

## Keywords

*Helicobacter pylori*; *Ascaris lumbricoides*; *Toxoplasma gondii*; immune response; gastric cancer; Colombia

Although gastric adenocarcinoma is initiated with *Helicobacter pylori* (*Hp*) infection (1), the basis for regional differences in the incidence of gastric cancer secondary to helicobacter infection remains unresolved (2). *Hp*-associated disease is most often limited to chronic but subclinical gastritis; other factors likely delay or promote the progression of gastritis to gastric adenocarcinoma (3). This multi-factorial etiology has been attributed to variations in *Hp* strain virulence (4) and origin (5), genetic polymorphisms of the human inflammatory response (6), environmental promoters such as smoking and dietary salt, environmental inhibitors including dietary antioxidants (7), and co-colonization of *Hp*-infected atrophic stomachs with enteric flora (8–9). Rodent models of helicobacter-induced chronic gastritis have provided evidence that concurrent helminth infections reduce gastric atrophy (10–11), a key premalignant lesion. Therefore, parasites may also reduce the risk for *Hp*-associated gastric cancer in humans.

Worldwide, approximately 50% of humans are infected with *Hp* but *Hp* prevalence is reported to be much higher in select populations, typically those of lower socioeconomic status (12–15). Several epidemiologic studies have evaluated gastric cancer risk for Colombian communities with a high prevalence (>90%) of *Hp* infection (16–18). Residents of coastal Tumaco have a lower incidence of *Hp*-associated atrophic gastritis (36.5%) and gastric cancer (6 per 100,000) compared to residents of Pasto/Tuquerres in the Andes Mountains, where the frequency of atrophic gastritis is 90.5% with a gastric cancer incidence of 150 per 100,000 (19–21).

Nematode infections are prevalent in areas of low socioeconomic status where individuals are commonly co-infected with *Hp* (18, 22). We reported that a higher prevalence of helminthiasis in Tumaco children, particularly *Ascaris lumbricoides* (*Al*), was associated with higher anti-inflammatory Th2-associated IgG1 isotype responses to *Hp* compared to Pasto/Tuquerres residents (18). These findings suggested that other parasites may influence the inflammatory response to *Hp*. *Toxoplasma gondii* (*Tg*), a tissue coccidian acquired by ingesting contaminated water, soil, or undercooked meat, (23) is a major health issue in Colombia (24–25) and worldwide (26). *Tg* stimulates Th1 host defenses (27) and promotes more severe gastritis and premalignant lesions with elevated gastric IFN- $\gamma$  and IL-12 levels in gastritis-resistant BALB/c mice co-infected with *H. felis* (28). In contrast to rodent models of helicobacter gastritis in combination with helminth infections (10–11), co-infection with *Tg* could elevate the risk of *Hp*-associated gastric cancer in humans, although epidemiologic studies have not addressed this hypothesis.

Regional differences in gastric cancer incidence between adults living in Tumaco and Pasto/Tuquerres have persisted since the 1970s, despite nearly ubiquitous *Hp* infection. Given the prevalence of *Hp* infection and differences in parasite burden in Colombian children from these regions (18), we determined whether seropositivity for *Al* and *Tg* were associated with altered pro- or anti-inflammatory IgG isotype responses to *Hp* in a cross-sectional sampling of children and adults.

## Methods

### Study populations

Sera were obtained from volunteer, clinically healthy Colombians attending community health clinics in the regions of Tumaco and Pasto/Tuquerres. From Tumaco, 55 children aged 1–6 years and 41 adults aged 31–84 years were sampled, and serologic responses compared to 105 children aged 1–6 years and 39 adults aged 38–68 years from Pasto/Tuquerres. Individual serum IgE and IgG isotype responses to *Hp* and population-based fecal parasite screening in children were previously reported by our laboratory (18). In this follow-up study, sera that were IgG *Hp* seropositive were further screened for IgG to *Al* and IgM/IgG to *Tg*. Informed consent was obtained from adult participants and from parents of children, and the study was approved by the Massachusetts Institute of Technology Committee on Use of Human Experimental Subjects and the Ethics Committee of Universidad del Valle.

### ELISA for total IgG

Because the previous study focused on childhood exposure to *Hp* and intestinal parasites and sera had been previously thawed and re-frozen, a subset of sera (n=73) from children from both locations was randomly screened to confirm that adequate total IgG levels remained using the Total Human IgG Assay (AlerCHEK, Inc., Portland, ME, USA). According to manufacturer instructions, sera were diluted 1:100,000 and compared to a standard curve with a dynamic range of 0.156 – 1 µg/ml. IgG levels were within the reference range for children (29) and did not differ by geographic location (data not shown).

### ELISA for IgG response to *A. lumbricoides*

Sera were assayed for qualitative IgG antibody levels to *Al* antigen using the *Ascaris lumbricoides* IgG ELISA kit (IBL International GMBH, Hamburg, Germany) with ≥ 95% sensitivity and specificity for *Al*. According to manufacturer instructions, diluted sera and kit-supplied positive, negative, and cut-off controls were incubated in 96 well plates pre-coated with *Al* antigens. Antigen-antibody complexes were detected by horseradish peroxidase-labeled Protein A conjugate reacting with tetramethylbenzidine substrate. Samples were considered negative if the absorbance was lower than 10% below the cut-off value, positive if the absorbance was higher than 10% over the cut-off value or equivocal if the absorbance value was 10% above or below the cut-off value.

### ELISA for IgM and IgG response to *T. gondii*

Qualitative IgM and quantitative IgG levels to *Tg* antigen were determined using the Platelia Toxo IgM and IgG ELISA kits (Bio-Rad Laboratories) following manufacturer instructions. Standard control sera provided in the kit were calibrated by the manufacturer against the WHO standard (TOXM 185) (30) and cut-off values established by comparison of 200 sera to an indirect immunofluorescence and direct agglutination test. Diluted samples and controls were incubated in 96 well plates coated with antibody to either human IgM or IgG. *Tg* antigen derived from tachyzoites and horseradish peroxidase-conjugated monoclonal antibody to *Tg* were sequentially incubated in each well followed by peroxidase substrate and chromogen. Samples were categorized as negative for IgM to *Tg* if the optical reading was < 80% of the cutoff value, equivocal when ≥ 80% but < 100% of the cutoff value, and positive when ≥ 100% of the cutoff value. For quantitative IgG responses to *Tg* based on a standard curve, sera were categorized as seronegative if values were less than 6 IU/ml, equivocal for values ≥ 6 but < 9 IU/ml, and seropositive for values ≥ 9 but < 240 IU/ml; ≥ 240 IU/ml were categorized as high titer values.

## Statistical analysis

Statistical analysis was performed using STATA (version 11.0; StataCorp LC, College Station, TX, USA) and Prism (GraphPad Inc., La Jolla, CA, USA). To achieve normal distribution as confirmed by probability plots, quantitative IgG levels to *Tg* and total IgE levels were log transformed, and optical density readouts for the IgG isotype responses to *Hp* were divided by the subject's total IgG response to *Hp*. Sera with equivocal *Al* or *Tg* results were assigned a missing value in STATA. Chi square analysis, including Fisher's exact test, of serology results generated odds ratios and 95% confidence intervals for evaluating relationships between age, sex, and geographic location and *Al* and *Tg* serologic status. *Al/Tg* seropositivity was further analyzed by ANOVA. Data were stratified by *Al* and *Tg* serology status to analyze differences in *Hp*-specific IgG isotype responses using the two-tailed Student's t-test. Significance was set at  $p < 0.05$ .

## Results

Previous evaluation indicated that 95% of 251 Tumaco and Pasto/Tuquerres residents were seropositive to *Hp* (18). Although a variety of intestinal parasites were detected in feces from children from both regions, helminth infections were statistically more prevalent in Tumaco children, presumably due to the tropical coastal environment compared to the drier, colder Andes. This current study was restricted to *Hp* seropositive sera to further evaluate if serologic responses to *Al* and *Tg* were associated with select IgG isotype responses to *Hp*. Of 240 *Hp* seropositive sera used to establish the impact of location, sex, and age on exposure risk to *Al* and *Tg* (Table 1), 24 sera yielded equivocal results for IgG to *Al* or IgM/IgG to *Tg*; therefore sample size varied by assay as indicated in figure legends.

### Seropositivity to *A. lumbricoides* and *T. gondii* was more common in coastal Tumaco compared to high altitude Pasto/Tuquerres

Serology data indicate a significant environmental risk for human exposure to *Al* and *Tg* infections, particularly in Tumaco (Table 1). Tumaco residents were nearly 13 times more likely to be seropositive for *Al* ( $p < 0.001$ ) and 8 times more likely to be seropositive for *Tg* ( $p < 0.001$ ) compared to Pasto/Tuquerres residents. Males and females were at similar risk, but adults were nearly 3 times more likely than children to be *Al* seropositive ( $p < 0.001$ ) and 28 times more likely to be *Tg* seropositive ( $p < 0.001$ ). Concurrent seropositivity for *Al* and *Tg* was 5 times more likely than for either parasite alone ( $p < 0.001$ ).

A high percentage of Tumaco children (89%) were seropositive for *Al* and 59% were seropositive for *Tg*; 45% were dual seropositive and no Tumaco children were seronegative for both parasites (Table 2). Nine children in Tumaco (17%) had both IgM and IgG titers to *Tg*, and 21 children had mature *Tg* IgG titers without IgM. High titers to *Tg* ( $>240$  IU/ml) were measured in 37% of sera from Tumaco children (Figure 1). In contrast, only 9% of Pasto/Tuquerres children were *Al* seropositive, 11% were *Tg* seropositive with the majority (9 of 11) of these having high *Tg* titers. Just 2% were seropositive for both parasites.

### Positive serology for *A. lumbricoides* and *T. gondii* increased with age, and prevalence converged between adult populations

Most adults (66%) in Tumaco had antibodies to both parasites (Table 2). One third of adults (34%) were seropositive for *Tg* only with an overall *Tg* seroprevalence of 98%, all IgM negative and IgG positive with 39% categorized as high titer to *Tg*. Only one of 41 Tumaco adults was seronegative for both *Al* and *Tg*. In Pasto/Tuquerres, low seroprevalence for *Al* and *Tg* in children contrasted with much higher prevalence in adults. Serology data from the adults indicated 58% were seropositive for *Al* and 82% for *Tg* with 12% of *Tg* seropositive individuals having high IgG titers. Titers to both parasites were noted in 41% of the adults

from Pasto/Tuquerres. Thus, seroprevalence in adult residents of Tumaco and Pasto/Tuquerres were much more similar than regional differences between children. Indeed, *Al* seroprevalence was so common in adults that regional differences were not statistically significant but *Tg* seroprevalence was significantly lower in Pasto/Tuquerres adults ( $p<0.05$ ).

### **IgG responses to *A. lumbricoides* and *T. gondii* were associated with elevated serum IgE levels**

We previously reported that serum IgE levels were elevated in Tumaco residents compared to Pasto/Tuquerres residents and attributed the difference to higher helminthiasis in Tumaco children compared to Pasto/Tuquerres children (18), acknowledging that parasite test results were not available for adults. Current data enabled sorting IgE levels based on serologic evidence for parasite exposure. Seropositivity for *Al* or *Tg* was similarly associated with elevated IgE ( $p<0.01$  and  $p=0.01$ , respectively; Figure 2), supporting that IgE levels developed principally in response to parasitism.

### **IgG to *A. lumbricoides* was associated with enhanced Th2-IgG1 and IgG to *T. gondii* with higher Th1-IgG2 responses to *H. pylori***

Serum levels of *Hp*-specific IgG1, IgG2, IgG3, and IgG4 from *Hp*-positive children and adults were stratified by seronegative or seropositive status for *Al* and *Tg* and compared by the two-tailed Student's t-test. IgG titers to *Al* were associated with enhanced anti-inflammatory Th2-associated IgG1 responses to *Hp* in both *Tg* seronegative and seropositive individuals ( $p<0.01$  and  $p<0.05$ , respectively) (Figure 3A) with no significant association with IgG2, IgG3, or IgG4 responses to *Hp* (Figure 3B–D). IgG to *Tg* did not impact Th2-IgG1 responses to *Hp* but was associated with enhanced Th1-associated IgG2 responses to *Hp* ( $p<0.0001$ ), as well as elevated IgG3 ( $p<0.0001$ ) and IgG4 responses ( $p<0.01$ ), but only in *Al* seronegative sera. Notably, Th2-IgG1 responses to *Hp* were unaffected by high IgG titers to *Tg* (data not shown). For unexplained reasons, the Th1-IgG2, as well as IgG3 and IgG4 responses to *Hp* were all reduced in high titer *Tg* sera ( $p<0.0001$ ,  $p<0.001$ ,  $p<0.003$ , respectively, data not shown).

## **Discussion**

This study is the first to establish regional differences in *A. lumbricoides* (*Al*) and *T. gondii* (*Tg*) seroprevalence in Colombians residing in Tumaco and Pasto/Tuquerres and is supported by results from our previous fecal screening for parasitic ova in children living in these two areas (18). Although differences in childhood seroprevalence to *Al* and *Tg* were dramatic, increasing age was a significant risk factor for *Al* (odds ratio 2.6) and even more dramatically for *Tg* exposure (odds ratio 27.7). *Al* seroprevalence was so common in adults that regional differences were not statistically significant but *Tg* seroprevalence was significantly lower in Pasto/Tuquerres adults. Importantly, the matched data for *Hp*-, *Al*-, and *Tg*-induced antibody responses within these Colombian populations validate that *Al* and *Tg* infections were common and may have biased inflammatory response to *Hp* infection. Despite high seroprevalence of *Al* and *Tg* in adults from both areas, it was possible to detect a significant association between *Al* and *Tg* seropositivity and higher Th2-IgG1 and Th1-IgG2 responses to *Hp*, respectively. Consistent with the hygiene hypothesis (31), these results suggest that pediatric infections with *Hp*, *Al*, and *Tg* may be representative of a spectrum of illnesses that condition a balanced host immune response to exogenous antigens. Infections early in life may be protective by inducing regulatory networks capable of preventing or delaying onset of sequelae such as gastric cancer, that develop after decades of *Hp*-associated gastritis.

Previously, only population-based fecal parasitology data was available for Tumaco and Pasto/Tuquerres children without epidemiologic evidence for either fecal shedding or serologic evidence of parasites in adults (18). Compared to Pasto/Tuquerres, Tumaco children had higher fecal shedding of *Al*, *Trichuris trichiura* (whipworms), and *Giardia* amongst a spectrum of other parasites including hookworms, strongyles, pinworms, tapeworms, and 8 different protozoa (18). Because parasite ova shedding can be intermittent and Colombians with gastrointestinal symptoms living in underdeveloped areas are often treated with anthelmintics (32), seroprevalence rates indicate higher helminth exposure than we reported by fecal testing and likely represent a more accurate estimate of environmental exposure. The children and adults evaluated in this study were attending rural health clinics and may have received anthelmintics. Although helminth eradication has been shown to not restore expression of select innate immunity genes to control levels (33), anthelmintic therapy is unlikely to have impacted seroprevalence of *Al* or *Tg*. The serology data clearly indicate that Tumaco children were at much greater risk for *Al* and *Tg* exposure compared to Pasto/Tuquerres children, likely due to the colder and drier environment in Pasto/Tuquerres. Tropical conditions in Tumaco are comparable to other coastal areas of Colombia with similar problematic parasitic infections (34). Tumaco residents also likely have significant contact with contaminated water sources as shown for *Tg* infections in Brazil (35).

The shift from comparatively low seroprevalence of *Al* and *Tg* in Pasto/Tuquerres children to higher prevalence in adults may be due to low but chronic exposure to fewer infective oocysts in the environment of the Andes compared to the coast. A low but chronic exposure to oocysts may also explain the lower prevalence of high *Tg* titers in Pasto/Tuquerres adults compared to Tumaco. Compared to children, increased seroprevalence in Pasto/Tuquerres adults may potentially reflect greater exposure of adults to *Tg*-contaminated meat. Importantly, lower exposure of Pasto/Tuquerres children to *Al* and *Tg* may be key to the higher gastric cancer risk in adults compared to Tumaco where childhood parasitism was endemic. *Al* and *Tg* infections in children are maintained by parasite-specific immune responses shown to have significant effects on dendritic cells (36) and may alter responses to unrelated antigens such as vaccines (37–38). Indeed, the hygiene hypothesis attributes the increasing trend of allergic, autoimmune, and other inflammatory diseases in industrialized countries to decreased antigenic stimulation during childhood, resulting in more polarized immune responses when individuals are eventually exposed to a specific microorganism (31).

*Al* seropositive individuals had enhanced IgE and higher anti-inflammatory Th2-IgG1 responses to *Hp*, consistent with prior reports that children in undeveloped areas frequently become infected with *Hp* and parasites within the first few years of life and respond with a Th2 immune response (13, 18, 39). Robust IgE and other Th2-predominant immune responses to helminth infection are promoted by Th2-associated cytokines, including IL-4 and IL-5, and commonly persist in children despite anthelmintic treatment (40). *Hp*-induced gastritis in adults living in low socioeconomic conditions is also commonly characterized as Th2-predominant (13, 18, 39). In contrast, pro-inflammatory Th17/Th1 responses are more typical of helicobacter gastritis in developed areas of the world (41–43). Unfortunately, suitable samples for evaluating cytokine responses to *Hp* in these populations were not collected.

*Tg* was evaluated because epidemiologic evidence supports its high prevalence in Colombian human and animal populations (24–25). The predominance of IgG titers to *Tg* in the absence of IgM, particularly in adults from both areas, indicates primary exposure was not recent and likely reflects a mature immune response to ongoing re-exposure from the environment. Human and animal studies have demonstrated that Th1-skewed immune

responses driven by *Tg* can counter Th2 responses induced by fluke and nematode infections, respectively (44–45). This study links *Tg* seroprevalence to higher Th1-IgG2 responses to *Hp*. *Tg* seroprevalence, especially high titers in children, also impacted IgG3 and IgG4 titers to *Hp*. The significance of IgG3 and IgG4 responses to *Hp* are poorly understood but IgG3 has been associated with peptic ulcer disease and antral inflammation in *Hp* infected children (46). IgG4 production is promoted by Th2 anti-inflammatory cytokines such as IL-10 and is believed to be most relevant to non-microbial, allergy conditions (47) and helminth infections in humans (48). Elevated IgE was associated with seropositivity to both parasites. Although IgE reduces *Al* numbers in the gut, IgG4 can block IgE directed against *Al*, decreasing resistance to infection particularly in children (49).

Rodent models (10–11) and epidemiologic studies (18, 50) support the hypothesis that concurrent helminth infection delays progression of *Hp*-induced premalignant lesions to gastric cancer. Our study demonstrates that Colombians living in Tumaco, and less so in Pasto/Tuquerres, are likely continually exposed to *Hp*, *Al*, *Tg*, and a broader spectrum of enteric pathogens including parasitic infections as previously detected by fecal testing (18). *T. trichiura* is a candidate for future study because it was prevalent in Tumaco children (18), and *Al* and *T. trichiura* infections in poor Brazilian children were associated with enhanced IL-5 and IL-10 production by peripheral blood mononuclear cells along with hyporesponsiveness to mitogen stimulation in culture (51). Our data are also consistent with elevated serum levels of IL-5, IL-10, and TNF $\alpha$  with an elevated IL-10/IL-12 ratio in asymptomatic African refugee children concurrently infected with *Hp*, malaria and helminths (52).

This study was limited to a cross-sectional analysis by serology, but future work will focus on a longitudinal analysis pairing parasite infection status, anthelmintic therapy, and cytokine responses with gastric pathology in *Hp*-infected adults from these same regions. Recent gastric biopsy data indicated eosinophil density was significantly higher in men from Tumaco compared to Pasto/Tuquerres, and eosinophil numbers increased in parallel with lesion progression to atrophic gastritis, while mast cell density increased in parallel with lesion progression in both populations (53). Eosinophils were decreased in Tumaco men with intestinal metaplasia and dysplasia compared to Pasto/Tuquerres men with similar lesions. This disparate association may be an indicator of anti- or pro-inflammatory response to *Hp*, since eosinophils not only increase in *Hp* gastritis but are a key responding cell type in parasitic diseases.

These results support regional differences in Colombia for childhood parasitism as one of the risk factors for gastric cancer in adults against a background of other potentially important epidemiological factors. These include differences in diet with fish as the predominant food source along with more antioxidants in fresh fruits and vegetables in Tumaco and higher grain and salt intake in the Pasto/Tuquerres region; the significance of some of these factors along with smoking have remained controversial (54). There has been no analysis of genetic polymorphisms related to gastric cancer as done elsewhere (55) and ethnicity between regions is quite different. The Tumaco population is largely mixed in African and European ancestry and Pasto/Tuquerres ‘Mestizos’ are of Amerindian descent with European admixture (7, 11, 16). Notably, more is known about the genetics of the *Hp* strains infecting residents of Tumaco and Pasto/Tuquerres (5) than the indigenous genetic polymorphisms that likely also contribute to the disparity in gastric cancer risk. Consistent with human migration to these areas, multi-locus sequencing of 64 *cagA* and *vacA* s1m1 positive *Hp* strains were of European origin in high risk Pasto/Tuquerres and in low risk Tumaco, most residents (66%) were infected with African *Hp* strains, with the remainder infected with European strains. Thus, as with exposure to *Al* and *Tg*, environmental exposure to specific *Hp* strains likely contributes to gastric cancer risk. Our study provides

further insight into the complex relationship between parasites, *Hp*, and the inflammatory response and therefore may partially explain regional differences in gastric cancer risk for Colombian populations with similar high infection rates with *Hp*.

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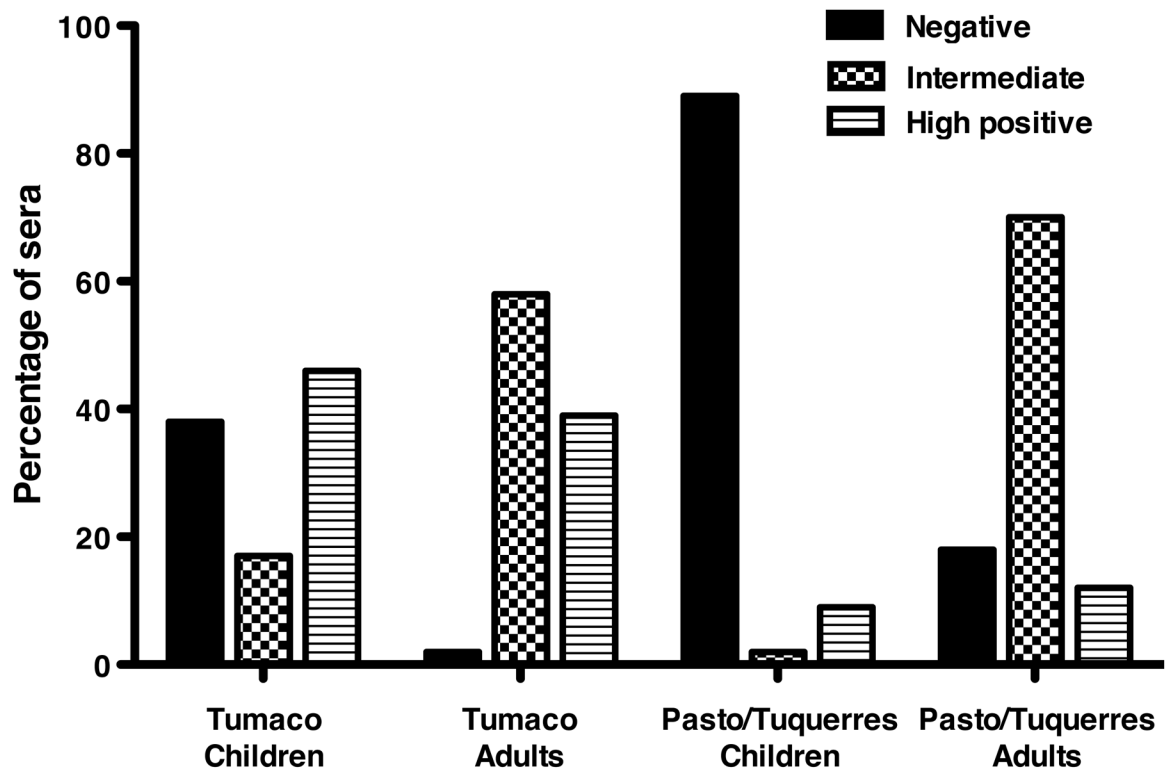
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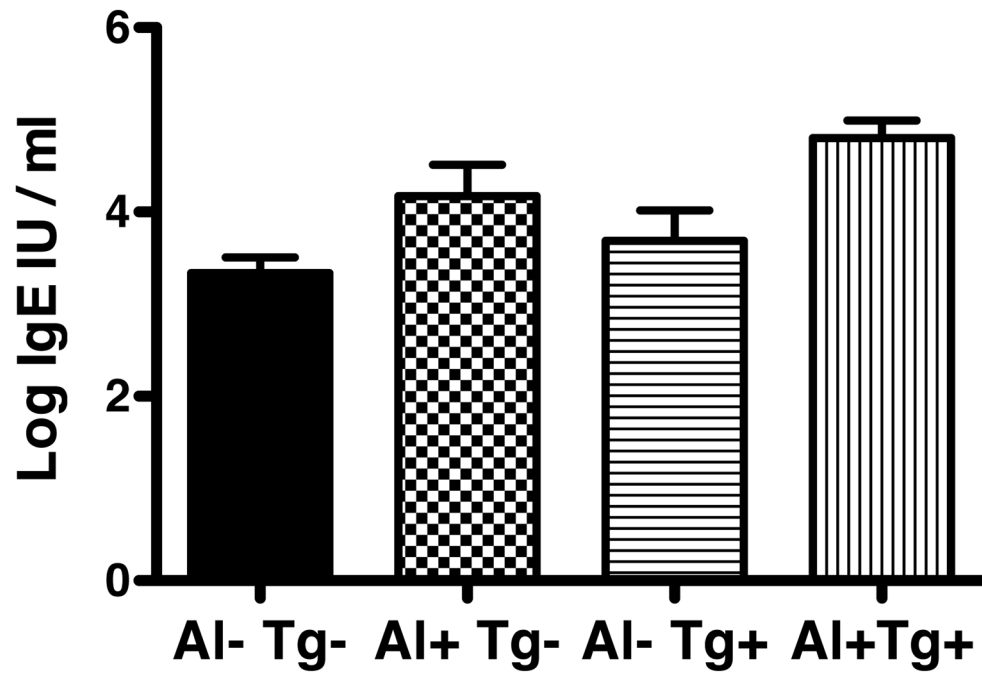
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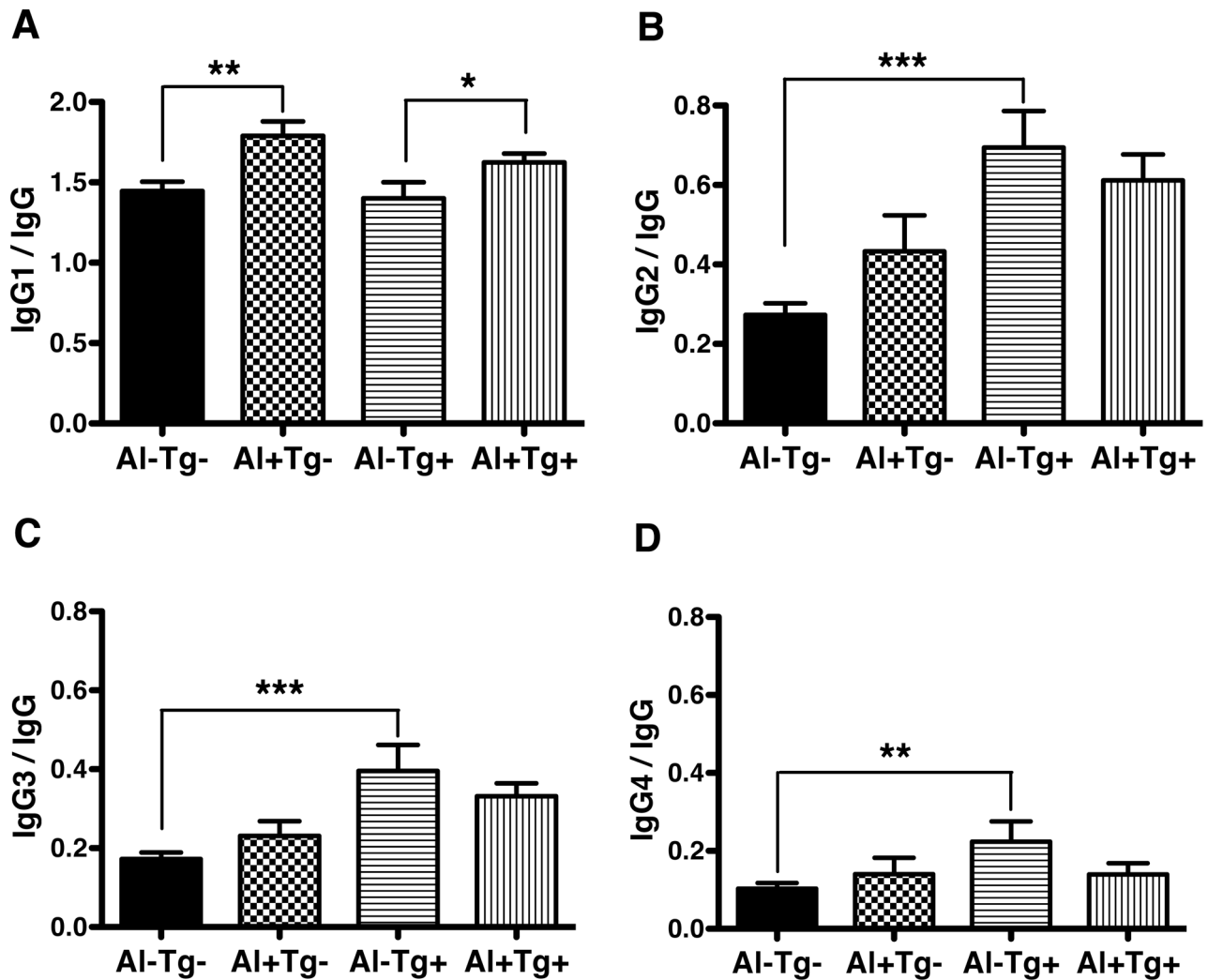


**Figure 1.**

Percentage of sera with negative (<6 IU/ml), intermediate (> 9 but < 240 IU/ml) or high *Tg* titers (>240 IU/ml). In children and adults from Tumaco, 37% and 39% of sera, respectively, were categorized as high titer. Although only 11% of Pasto/Tuquerres children were *Tg* seropositive, most had high titers. High titers to *Tg* were observed in only 12% of Pasto/Tuquerres adults. Absolute sample numbers were as follows; Tumaco children negative n=21, intermediate n=8, high titer n=22; Tumaco adults negative n=1, intermediate n=24, high titer n=16; Pasto/Tuquerres children negative n=92, intermediate n=2, high titer n=9; Pasto/Tuquerres adults negative n=6, intermediate n=23, high titer n=4.



**Figure 2.** Serum IgE levels in Colombians that were seronegative (-) or positive (+) for *Al* and *Tg* were compared by ANOVA. Seropositivity for *Al* or *Tg* ( $p < 0.001$ ,  $p = 0.01$ ) were significantly associated with elevated IgE. Sample numbers were as follows *Al-Tg-*  $n = 80$ ; *Al+Tg-*  $n = 36$ ; *Al-Tg+*  $n = 30$ , *Al+Tg+*  $n = 70$ . Bars represent standard error of the mean.



**Figure 3.**

Mean ratios of serum *Hp*-specific IgG1 (A), IgG2 (B), IgG3 (C) and IgG4 (D) from *Hp*-positive children and adults from Tumaco and Pasto/Tuquerres were stratified by seronegative (–) or positive (+) status for *Al* and *Tg* and compared by the two-tailed Student's t-test. IgG titers to *Al* were associated with enhanced anti-inflammatory Th2-associated IgG1 responses to *Hp* in both *Tg* seronegative (\*\*  $p < 0.01$ ) and *Tg* seropositive individuals (\*  $p < 0.05$ ). *Tg* seropositivity was linked to higher pro-inflammatory Th1-IgG2 (\*\*\*)  $p < 0.0001$ ), as well as IgG3 (\*\*\*)  $p < 0.001$ ) and IgG4 responses to *Hp* (\*\*  $p < 0.01$ ) in *Al* negative sera. Sample numbers were as follows *Al*–*Tg*–  $n = 80$ ; *Al*+*Tg*–  $n = 36$ ; *Al*–*Tg*+  $n = 30$ , *Al*+*Tg*+  $n = 70$ . Bars represent standard error of the mean.

Table 1

Impact of location, sex and age on exposure to *A. lumbricoides* (Al) and *T. gondii* (Tg) in *H. pylori*-seropositive children and adults from Tumaco and Pasto/Tuquerres

		<i>A. lumbricoides</i>	Odds ratio <sup>b</sup>	95% CI <sup>c</sup>	p value
Location	Tumaco	96 (76; 79%) <sup>d</sup>	12.6	6.4–25.2	<0.001
	Pasto/Tuquerres	134 (31; 23%)	-		
Sex	Male	100 (45; 45%)	0.9	0.5–1.6	0.7
	Female	130 (62; 48%)	-		
Age	Adult (31–84 yrs.)	79 (49; 62%)	2.6	1.4–4.8	<0.001
	Child (1–6 yrs.)	151 (45; 30%)	-		
Concurrent IgM/IgG to Tg	Yes	100 (80; 80%)	5.2	2.8–9.7	<0.001
	No	116 (36; 31%)	-		
<i>T. gondii</i>					
Location	Tumaco	90 (68; 76%)	8.0	4.2–15.4	<0.001
	Pasto/Tuquerres	136 (38; 26%)	-		
Sex	Male	95 (41; 43%)	0.8	0.4–1.4	0.3
	Female	131 (65; 50%)	-		
Age	Adult (31–84 yrs.)	74 (67; 92%)	27.7	11.3–76.4	<0.001
	Child (1–6 yrs.)	152 (39; 27%)	-		
Concurrent IgG to Al	Yes	100 (80; 80%)	5.2	2.8–9.7	<0.001
	No	116 (36; 31%)	-		

<sup>a</sup> number of sera evaluated, number and percent positive

<sup>b</sup> odds ratio in comparison to baseline (-) condition

<sup>c</sup> 95% CI, 95% confidence interval

Table 2

*A. lumbricoides* and *T. gondii* serology status of *H. pylori*-positive children and adults from Tumaco and Pasto/Tuquerres

	<i>Ascaris lumbricoides</i> (AI)				<i>Toxoplasma gondii</i> (Tg)				AI & Tg			
	IgG		IgM		IgG		IgG		IgG			
	Neg	Pos	% Pos	Neg	Pos	% Pos	Neg	Pos	% Pos	DbI Pos	% DbI Pos	
Tumaco	6	49	89%	44	9	17%	21	30	59%	0	25	45%
	14	27	66%	40	0	0%	1	40	98%	1	27	66%
Pasto Tuquerres	87	9	9%	105	0	0%	92	11	11%	77	2	2%
	16	22	58%	36	1	3%	6	27	82%	2	16	41%

Neg = negative serology; Pos = positive serology; DbI = double; % = percent. All data represent absolute numbers and percentage of samples that yielded non-equivocal serology status for AI and Tg.

\* No equivocal results for IgG to AI, 2 sera equivocal for IgM, 6 equivocal for IgG to Tg;

\*\* No equivocal results for IgG to AI, 1 serum equivocal for IgM, none equivocal for IgG to Tg;

\*\*\* 9 equivocal results for IgG to AI, none equivocal for IgM, but 2 sera equivocal for IgG to Tg;

\*\*\*\* 1 equivocal result for IgG to AI, 2 sera equivocal for IgM, 6 for IgG to Tg. Final sample size was 216 sera with unequivocal results.