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*Bartonella henselae* is associated with heartburn, abdominal pain, skin rash, mesenteric adenitis, gastritis and duodenitis in children and adolescents.

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## Abstract

Ten patients between the ages of 7 and 16 years presented with a history of cat scratches and or tic bites, chronic abdominal pain, esophageal heartburn, a purpuric skin rash, mesenteric adenitis and a positive Immunoglobulin G titer for *Bartonella henselae* (*B. henselae*). Endoscopy assessed the gastrointestinal (GI) mucosa for inflammation and biopsies were examined for *Helicobacter pylori* by microscopy and for *B. henselae* by polymerase chain reaction (PCR). Biopsies were PCR positive for *B. henselae* DNA in all patients. Chronic gastritis and or chronic duodenitis was found in all and associated with the detection of *B. henselae* DNA in the GI tract at the site of the inflammation.

## Introduction

Cat scratch disease is a syndrome that is characterized by regional lymphadenopathy after a cat scratch or bite distal to the involved lymph node. In addition, many patients have atypical presentations other than regional adenopathy such as neurological syndromes, self-limited granulomatous hepatitis, splenitis, osteitis, atypical pneumonitis, endocarditis and a syndrome of prolonged fever of unknown origin in children.<sup>1</sup> Gastrointestinal presentations of cat scratch disease have included hepatosplenic abscess presenting as abdominal pain<sup>2</sup> and posterior pancreatic duodenal lymphadenitis presenting as abdominal pain.<sup>3</sup> We describe a new atypical clinical presentation of cat scratch disease in which pediatric patients presented with heartburn, abdominal pain, mesenteric adenitis and a purpuric skin rash following a cat scratch or tick bite.

## Methods

All patients included had no prior history of GI complaints. They were referred to the Pediatric Gastroenterology and Nutrition service of Jersey Shore Medical Center for evaluation of chronic abdominal pain and heartburn pain which radiated from the sternum up the esophagus and was associated with a purpuric skin rash. Ten consecutive patients satisfying the above clinical criteria who had not been on any steroid medications were evaluated from July 2001 through February 2002 (mean age  $12.5 \pm 3.0$  years, range 7-16). Each case included a history in which two consecutive months of heartburn and esophageal pain were not affected by diet, position or time of day. The pain was not relieved by histamine blocking medications, antacids or proton pump inhibiting medication. There was a documented history of a tick bite or cat scratch one month prior to evaluation and a positive blood IgG titre for *Bartonella henselae* (greater than or equal to 1:64) at the time of endoscopy. A complete blood cell count, liver function tests, esophagoduodenoscopy(EGD) were performed on all patients. As an additional inclusion criteria, prior to endoscopy, CT scan of the abdomen confirmed mesenteric adenitis ( lymph nodes measured 1cm or larger in diameter in all of the patients) without the presence of hepatosplenic granulomas. Stool samples were examined for white blood cells, occult blood, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, ova and parasites and *Clostridium difficile* toxin A and B. GI biopsies assessed the mucosa by microscopy and were evaluated for the presence of *Helicobacter pylori*(*H. pylori*). Biopsy specimens were taken from areas of the GI tract that looked inflamed during EGD. Biopses were reported as acutely inflamed when polymorphonuclear cells were present in the mucosa and chronically inflamed if six or more plasma cells and lymphocytes were

present in the gastric mucosa without polymorphonuclear cells. Chronic duodenitis was diagnosed when greater than six intraepithelial lymphocytes per 100 surface absorptive cells were present in tissue biopsies in conjunction with a distortion in glandular architecture.

Polymerase chain reaction for DNA to *B. henselae* was performed on all biopsies by Medical Diagnostic Laboratories, Mount Laurel, New Jersey as described below.

### **DNA EXTRACTION**

The lymphocytes were dissolved in 470 $\mu$ L of tris-EDTA buffer (10mM tris-hydrochloride [pH8.0] and 1 mM EDTA), 25  $\mu$ L of 10% sodium dodecyl sulfate and 12 $\mu$ L of freshly prepared deoxynuclease-free proteinase K(10mg/ml). The mixture was incubated at 55°C for 2 hours; DNA was extracted by phenolchloroform extraction and ethanol precipitation. The purified DNA was dissolved in pyrogen-free, double-distilled water and quantified using a Genesys-5 spectrophotometer (Spectronics Instruments, Rochester, NY). The purified, quantitated DNA was used as a template for *Bartonella henselae* PCR analysis.

### **PRIMERS**

The PCR primers for the identification of *B. henselae* have been described.<sup>4</sup> The primers were synthesized by Research Genetics (Huntsville, Ala) and purified by high-performance liquid chromatography. Their sequences are given in table 1 and previously reported by Eskow, Rao and Mordechai.<sup>4</sup>

### **POLYMERASE CHAIN REACTION**

The PCR mixtures (50 $\mu$ L) contained extracted DNA (5 $\mu$ L, 0.2 $\mu$ g/ $\mu$ L), P24E and P12B primers (50nM), 10mM tris-hydrochloride (pH,8.3), 50mM potassium chloride, 3mM magnesium chloride, 0.001% (wt/vol) gelatin, the nucleotides dATP, dCTP, dGTP, and

dTTP (each at a concentration of 200mmol/L) and 2.5U of Taq DNA polymerase (Perkin-Elmer, Foster City, CA). The PCR was carried out in 0.2 ml tubes. The thermocycler was a Perkin-Elmer Gene AMP PCR system 2400. The PCR program ran for 3 minutes at 94°C, followed by 40, one-minute cycles at 94°C, 1 minute at 56°C and 1.5 minutes at 72°C. The program finished with an additional 10-minute extension step at 72°C. A 30- $\mu$ L sample of the the final reaction product was run on a 1% agarose gel containing 0.5 $\mu$ g of ethidium bromide per milliliter and the gel was photographed under UV light. For optimization of the PCR conditions for clinical specimens, normal blood was artificially spiked with in vitro-cultivated *B henselae*. A controlled number of *B henselae* (American Type Culture Collection 49882, ATCC, Rockville, MD), ranging from 10<sup>1</sup> to 10<sup>5</sup> pathogens was added to 5ml of whole blood. These spiked samples were treated as described above.

### **HISTONE PCR**

Aliquots (5 $\mu$ L) of the newly extracted DNA were mixed in a 50 $\mu$ L PCR reaction mixture containing 10X PCR buffer (Perkin Elmer), 3 mM magnesium chloride, 200mM dNTP, 2.5 $\mu$ L of Taq DNA polymerase (5U/ $\mu$ L) and 1 $\mu$ L (8pmol) OF 5'-and 3' histone amplifier primer set. The histone primers are complementary to the DNA of a constitutively expressed human histone gene H3.3 as decribed.<sup>15</sup> The amplification process was subjected to 30 cycles of PCR (each cycle at 94°C for 45 seconds, 60°C for 45 seconds and 72°C for 90 seconds) in a 2400 Perkin-Elmer DNA thermocycler. The histone primers served as internal controls for the sample's DNA integrity, presence of inhibitors and intersample equivalency of total amount of DNA analyzed.

**PRECAUTIONS AGAINST CONTAMINATION**

The extraction of DNA and PCR were performed under sterile conditions and in separate rooms. All positive samples were confirmed by reextraction from the original sample, followed by amplification in triplicate. *Bartonella henselae* DNA- positive status was defined as samples that were positive initially and in at least one of the replicates after reextraction. Pyrogen free water was used in the isolation of DNA from the biopsy specimens. The Eppendorff microcentrifuge tubes and the PCR tubes were sterilized in an autoclave and UV irradiated. New Finn pipettes were used solely with the filter tips for PCR. Disposable plastic trays were used to prepare PCRs in a UV irradiated PCR biohood. The laboratory performing the PCR analysis was blinded to the diagnosis of all specimens they received. Blood and CSF samples (n=10 of CSF and n=5 of blood) from individuals with no evidence of cat scratch disease were used in the PCR assays as negative controls.

## Results

The skin rash was a purpuric, serpinginous, nodular, papular rash that did not blanch upon applying pressure to the affected area. In patient 3, the rash presented in the right armpit as three distinct 10cm long vertical lines. In patient 4, it presented in the left groin as three 5cm long serpinginous lines that ran horizontally. The rash did not appear on any other body parts. In patients 7 and 8, the rash appeared only on the lower back in a christmas tree pattern that faded away from the spine and spanned a vertical distance of 15cm(Phot01). In patient 9, the rash was found on his left breast only (Photo 2). There were lines emanating from the areola in a centrifugal pattern. In patient 10, the rash was on the posterior aspect of the right knee only and no other lesions were visualized on the body. (Photo 3).

*B. henselae* DNA was detected in either the stomach (8 of 10) or the duodenum (8 of 10) of all of the patients. The IgG titer for *B. henselae* was positive in all patients and the IgM titer was negative in all of them. All of the patients with a positive PCR DNA for *B. henselae* had an associated biopsy proven gastritis and/or duodenitis at the site of the infection(Table 2). All biopsies were negative by microscopy for *H. pylori*. There was no evidence of acute inflammation, granulomas or ulcers on EGD. White blood cells, ova and parasites, occult blood in the stool, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter* and *Clostridium difficile* toxins A and B were not detected in any of the stool samples. CT of the abdomen did not reveal any gallstones, biliary tract disease, pancreatitis or thickening of the distal ileum.

