Assessment of Rectocolonic Morphology and Function in Patients with Chagas Disease in Barcelona (Spain)

Fernando Salvador,* Marianela Mego, Adrián Sánchez-Montalvá, María Morís, Kathleen Ramírez, Ana Accarino, Juan-Ramon Malagelada, Fernando Azpiroz, and Israel Molina

Department of Infectious Diseases, Vall d'Hebron University Hospital, Barcelona, Spain; PROSICS, Barcelona, Spain; Digestive System Research Unit, University Hospital Vall d'Hebron, Barcelona, Spain; Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain; Department of Radiology, Vall d'Hebron University Hospital, Barcelona, Spain

Abstract. The aim of this study was to determine the relationship between colonic symptoms, radiological abnormalities, and anorectal dysfunction in patients with Chagas disease. We performed a cross-sectional study of untreated patients diagnosed with Chagas disease. All patients were evaluated clinically (by a questionnaire for colonic symptoms based on Rome III criteria) and underwent a barium enema and anorectal manometry. A control group of patients with functional constipation and without Chagas disease was included in the study. Overall, 69 patients were included in the study: 42 patients were asymptomatic and 27 patients had abdominal symptoms according to Rome III criteria. Anorectal manometry showed a higher proportion of abnormalities in symptomatic patients than in asymptomatic ones (73% versus 21%, respectively; $P < 0.0001$). Megarectum was detected in a similar proportion in the different subgroups regardless of the presence of symptoms or abnormalities in anorectal functions. Among non-Chagas disease patients with functional constipation, 90% had an abnormal anorectal manometry study. Patients with Chagas disease present a high proportion of constipation with dyssynergic defecation in anorectal manometry but a low prevalence of impaired rectoanal inhibitory reflex, although these abnormalities may be nonspecific for Chagas disease. The presence of megarectum is a nonspecific finding.

INTRODUCTION

Chagas disease is a parasitic infection caused by the protozoan *Trypanosoma cruzi*. An estimated 10 million people are infected worldwide and 10,000 people die annually from the disease.¹ Chagas disease affects mainly Latin America, where the infection is highly endemic; nevertheless, *T. cruzi* infection has been increasingly diagnosed in recent decades in non-endemic countries (Europe, North America, Australia, and Japan) as a result of migrant flows from countries in Latin American countries.^{2,3}

The chronic form of the infection is characterized by the positivity of serologic tests, and the presence of symptoms in about 30–40% of patients who develop cardiac and/or digestive involvement. Prevalence of gastrointestinal involvement in Chagas disease patients varies depending on the tests performed for the evaluation and the geographical area where the study is performed: gastrointestinal involvement prevalence in endemic countries ranges from 14% to 35%, although in non-endemic countries it is 5–14%.^{3–9} Patients with chronic Chagas disease and colonic involvement show slowly progressive constipation and may manifest colonic dilatation or megacolon that can be discovered as a result of radiological examinations. The sigmoid and the rectum are dilated in nearly all cases of megacolon, whereas dilation of more proximal colonic segments is rare. Chagasic megacolon is caused by irreversible destruction of the enteric nervous system during the acute phase of the disease, which results in an absence of the rectoanal inhibitory reflex on anorectal manometry similar to Hirschsprung's disease.¹⁰

This study was designed to evaluate the relationship between the presence of colonic symptoms, radiological abnormalities, and anorectal dysfunction in patients with Chagas disease.

PATIENTS AND METHODS

Study population and data collection. This cross-sectional study was performed at the Infectious Diseases and Gastroenterology Departments of the Vall d'Hebron University Hospital, a tertiary hospital of Barcelona (Spain), from May 2011 to April 2012. Patients who attended the Infectious Diseases Department during the study period and who fulfilled the inclusion criteria were proposed to participate in the study. Selection criteria were patients > 18 years of age and a diagnosis of Chagas disease in the indeterminate or chronic phase. Exclusion criteria were previous treatment of Chagas disease, pregnancy, and not providing signed informed consent. After applying the study protocol, treatment with benznidazole was offered to patients following the World Health Organization (WHO) recommendations.

Serologic diagnosis of Chagas disease was performed using in parallel two enzyme-linked immunosorbent assays (ELISAs), one with recombinant antigen (Bioelisa Chagas, Biokit, Lliçà d'Amunt, Spain) and the other with a crude antigen (Ortho *T. cruzi* ELISA, Johnson and Johnson, High Wycombe, UK), according to WHO's diagnostic criteria. We considered a positive or negative result if both tests were concordant. All sera showing discordant results were tested by an in-house Western blot method using a lysate from *T. cruzi* epimastigotes, and diagnosis was based on the positivity of this test.¹¹

Assessment of colonic involvement. Clinical symptoms were evaluated by a structured questionnaire based on Rome III criteria.¹² This questionnaire included evaluation of the following symptoms: abdominal pain, abdominal distention, early satiety, postprandial fullness, number and consistency of the stools, characteristics of defecations (straining, sensation of

* Address correspondence to Fernando Salvador, Infectious Diseases Department, Hospital Universitari Vall d'Hebron, PO Vall d'Hebron 119-129, 08035 Barcelona, Spain. E-mail: fmsalvad@vhebron.net

TABLE 1
Rome III diagnoses

Functional bowel disorders
Irritable bowel syndrome
Functional bloating
Functional constipation
Functional diarrhea
Unspecified functional bowel disorders
Functional abdominal pain syndrome
Functional anorectal disorders
Functional fecal incontinence
Functional anorectal pain
Functional anorectal disorders

incomplete defecation, sensation of anorectal obstruction (blockage, manual maneuvers), and relationship of the symptoms with physiological events (eating, defecation). The Rome III diagnoses are summarized in Table 1.

Barium enema and anorectal manometry were performed in all patients. The barium enema was performed according to the method described by Ximenes.¹³ In brief, patients underwent the radiological study without prior preparation (i.e., without the administration of laxatives or enemas before the examination). Contrast solution was obtained in advance by diluting 300 mL of standard barium contrast into 1,200 mL of water. Patients were placed in the right lateral position and the 1,500 mL of test solution instilled in the rectum. Patients were asked to remain still in this position for the next 5 min, during which time three radiological projections were obtained: dorsal decubitus, ventral decubitus, and lateral. Megarectum and/or megasigmoid diagnosis was established when the luminal diameter exceeded 6.5 cm.

Anorectal manometry was performed using a low-compliance manometric perfusion system (0.1 mL/min perfusion rate) and a 4 radial-lumen polyvinyl catheter with a distensible balloon in the tip of the probe (2.4-mm outside diameter, Standard Anorectal-4 channels, Medical Measurement Systems, Dover, NH). With the patient in the left lateral position and the legs flexed 90°, the manometry catheter was introduced into the rectum. The following parameters were registered during the test: 1) resting anal canal pressure, 2) anal canal squeeze pressure (peak pressure and duration), 3) the rectoanal inhibitory reflex, 4) anal canal pressure in response to a cough, 5) defecatory maneuver, and 6) rectal sensitivity in response to balloon distension. The rectoanal inhibitory reflex was triggered by inflation of the rectal balloon with air, as follows: phasic rectal distensions of 10 s duration were performed at 1 min intervals, and with volumes of 20, 40, and 60 mL of air while measuring the reflex relaxation of the internal anal sphincter (anal pressure drop). Volumes of 100 and 150 mL of air were tested in case the rectoanal inhibitory reflex was absent with lower vol-

umes. The normal rectoanal inhibitory reflex was defined as a sphincter pressure drop superior to 20 mmHg below the resting pressure. The dynamics of defecation were studied by means of the same catheter described for the evaluation of neural reflexes, with the intra-rectal balloon inflated with 25 mL air, and the manometric ports located in the anal canal. Patients were asked to attempt defecation, and both abdominal compression (intra-rectal pressure increment) and anal relaxation (anal pressure drop) during straining were measured. Normally, the anal canal completely relaxes, and all manometric ports exteriorize recording atmospheric pressure. Defecatory maneuver was considered normal when abdominal compression increased ≥ 20 mmHg, and pressure in all manometric ports decreased by at least 20% of the resting pressure. Rectal distension was produced by an intra-rectal balloon with 20 mL stepwise increments every 15 s, while measuring subjective sensations. Rectal sensitivity was defined as the volume that induced the first sensation and urge to evacuate.

A control group of 30 patients without Chagas disease referred to our Hospital Motility Unit for evaluation of functional constipation, in whom anorectal manometry study was performed, was included for comparison. The study protocol was approved by the Ethical Review Board of the Vall d'Hebron University Hospital and written informed consent was obtained from all patients. All data were anonymized and the confidentiality of the information was respected at all times in accordance with the ethical standards of the Declaration of Helsinki.

Statistical analysis. Categorical data are presented as absolute numbers and proportions, and continuous variables are expressed as medians and ranges. The χ^2 test or Fisher exact test, when appropriate, was used to compare the distribution of categorical variables, and the Mann-Whitney *U* test for continuous variables. Results were considered statistically significant if the two-tailed *P* value was < 0.05 . The SPSS software for Windows (Version 19.0; SPSS Inc., Chicago, IL) was used for statistical analyses.

RESULTS

Overall, 69 patients were included in the study. Forty-eight (69.5%) were women and the median age was 39 (24–75) years. Sixty-eight (98.5%) patients came from Bolivia and one (1.5%) came from Ecuador. After evaluation of symptoms using the structured questionnaire, 27 (39.1%) patients were classified as symptomatic patients. Of these, based on Rome III criteria, 17 patients had chronic constipation, 9 patients had irritable bowel syndrome, and 1 patient had chronic functional abdominal pain. Table 2 summarizes the main radiologic and manometric features observed.

Colonic morphology and anorectal motility. Among the 42 patients who were asymptomatic the barium enema revealed

TABLE 2
Main radiological and manometric features observed in Chagas disease patients who attended Vall d'Hebron University Hospital

	Overall (N = 69)	Symptomatic patients (N = 27)	Asymptomatic patients (N = 42)	P value
Presence of megarectum (barium enema)	18 (26.1%)	10 (37%)	8 (19%)	0.097
Abnormal anorectal manometry	28 (40.6%)	19 (70.4%)	9 (21.4%)	< 0.001
Defecation impairment	24 (34.9%)	17 (63%)	7 (16.6%)	< 0.001
Sphincter insufficiency	2 (2.9%)	1 (3.7%)	1 (2.4%)	1
Impaired anorectal inhibitory reflex	1 (1.4%)	0 (0%)	1 (2.4%)	–
Hypertonic anal sphincter	1 (1.4%)	1 (3.7%)	0 (0%)	–

no abnormalities in 34 patients, but a megarectum was identified in 8 patients. Of the 27 symptomatic patients, the barium enema study revealed no abnormalities in 17, but 10 patients were seen to have a megarectum. In these same 42 asymptomatic patients, anorectal manometry showed normal function in 33 patients and abnormal function in 9 patients (defecation impairment in 7, sphincteric insufficiency in 1, and impaired anorectal inhibitory reflex in 1). Among the 27 symptomatic patients, anorectal manometry results were normal in 8 patients and abnormal in 19 patients (defecation impairment in 17, sphincteric insufficiency in 1, and hypertonic anal sphincter in 1).

Relationship between abnormal anorectal motility and barium enema images. Among the 42 asymptomatic patients, 7 of the 9 patients with abnormal anorectal manometry results had a normal barium enema, whereas 27 out of the 33 patients with normal anorectal manometry results also had normal barium enema results. Thus, the majority of asymptomatic patients had normal colon morphology (34 of 42 patients). Regarding the minority of patients who had megarectum, there was no difference in the proportion of those who, in addition to megarectum, had an abnormal manometry result (2 of 9 patients with abnormal manometry findings compared with 6 of 33 patients who had a normal manometry result). Hence, in asymptomatic patients with Chagas disease there seems to be no relationship between megarectum and motor abnormalities demonstrable by anorectal manometry.

Among the 27 symptomatic patients, 19 patients had an abnormal anorectal manometry result, and 8 of those patients were found to have megarectum as well. In 8 of the 27 symptomatic patients with normal anorectal manometry findings, 2 patients had megarectum. Thus, a slightly higher proportion of symptomatic patients with abnormal results from the anorectal manometry study also had a megarectum compared with the asymptomatic patients.

Relationships between symptomatic status and anorectal motility and colonic morphology abnormalities. Overall, the 27 symptomatic patients had a significantly higher proportion of anorectal motility abnormalities than the 42 asymptomatic patients (70.4% versus 21.4%, $P < 0.001$). Conversely, there was no appreciable difference in the presence of megarectum between symptomatic and asymptomatic patients (37% versus 19%, $P = 0.097$). Remarkably, the occurrence of an altered anorectal inhibitory reflex was virtually nil in our patient study population (1 of 69 patients, and the patient was asymptomatic).

Comparison between anorectal manometry results in patients with Chagas disease and in non-Chagas disease patients with functional constipation. A high proportion of non-Chagas disease patients with functional constipation had an abnormal anorectal manometry study with evidence of defecatory motility impairment (27 of 30 patients, 90%). This proportion was somewhat larger when compared with the 70.4% of symptomatic patients with Chagas disease in our study, but the difference did not reach statistical significance ($P = 0.061$).

Clinical follow-up. Nineteen Chagas disease patients with constipation and abnormal anorectal manometry results (17 patients with constipation, and 2 patients with irritable bowel syndrome with constipation), and the 27 non-Chagas disease patients with constipation and defecatory motility impairment underwent anorectal biofeedback (three sessions

for 1 month), with clinical improvement that was similar in both groups (71% versus 67%, respectively; $P = 0.901$).

DISCUSSION

In this study, we have investigated the prevalence of symptoms (mostly constipation with or without associated abdominal pain), morphological colon abnormalities (presence or absence of luminal dilation by barium enema), and anorectal motility disturbances in immigrants to Spain with Chagas disease, where *T. cruzi* infection is not endemic. The results show that, among symptomatic Chagas disease patients, anorectal motility disturbances are relatively common, whereas morphologic colon abnormalities as assessed radiologically are both uncommon and unrelated to the motor defects. The proportion of patients with symptoms and the proportion of patients with radiological abnormalities are similar to those described in other studies performed in non-endemic countries.³⁻⁵

The selection of patients for this study was based on positive serological results for *T. cruzi* infection. The possibility of this infection is routinely evaluated among immigrant population attending our Infectious Diseases Department outpatient services and coming from Latin American countries where Chagas disease is highly prevalent. This method of selection based on positive serology rather than clinical manifestations of Chagas disease implies that a relatively high proportion (two-thirds) of our study population were asymptomatic. This proportion of asymptomatic patients is similar to that reported in other series in both endemic and non-endemic countries for Chagas disease.³⁻⁶

Abnormalities in the barium enema have been described in asymptomatic patients with *T. cruzi* infection.¹⁴ In our study, 8 of 42 (19%) asymptomatic patients showed evidence of megarectum, and a similar proportion was found among symptomatic patients. Thus, the presence of a dilated bowel does not appear to be a determinant factor in whether a patient is symptomatic or asymptomatic. The explanation for this apparent discrepancy may be that bowel motor disturbances may induce constipation without necessarily causing simultaneous dilatation of the viscera. Therefore, radiological assessment seems unlikely to be a sensitive and specific approach to verify the presence or absence of digestive tract involvement in Chagas disease. Nevertheless, constipation in association with colonic dilatation shown by radiological studies has been reported previously by other authors.¹⁵

In contrast to barium enema, the anorectal manometry test yielded a much higher prevalence of abnormalities in symptomatic Chagas disease patients with constipation than in the asymptomatic ones, suggesting that such motility disturbances are pathogenetically relevant in the constipation associated with Chagas disease. Previous studies in Chagas disease patients have evidenced colonic hypomotility defined by low basal motility index and low wave frequency in the rectosigmoid, and impairment of the anorectal inhibitory reflex.^{16,17} The results of these previous studies agree only partially with our findings: we observed that 28 of 69 (40.6%) patients with Chagas disease showed manometric evidence of anorectal motor dysfunction, but an abnormal anorectal inhibitory reflex was only confirmed in one asymptomatic patient.

Other authors have looked into the question of anorectal inhibitory reflex in Chagas disease and shown that with the use of larger-volume distending balloons, an impaired anorectal inhibitory reflex is demonstrable in 43.6% of 39 patients with chagasic megacolon.¹⁸ However, even excluding patients with megarectum (in whom the standard distending balloon may have failed to stimulate sufficiently the rectal pouch), we were unable to find impaired anorectal inhibitory reflex. Others have postulated that an impaired anorectal inhibitory reflex is a pathogenetic factor in chagasic megacolon; but it has been also observed that not all patients with megarectum present an impaired anorectal inhibitory reflex, which suggests that other abnormalities may also play a role in the development of megarectum and megacolon.¹⁹

The high proportion of symptomatic Chagas disease patients with anorectal motility abnormalities would appear to suggest that chronic *T. cruzi* infection has induced neuronal damage in these individuals, as observed by other authors.^{20,21} However, in selected groups of constipated individuals it has been previously shown that the majority of them have impaired relaxation of the anal sphincter during the defecatory maneuvers. A similar pattern was observed in our control population of constipated non-Chagas disease individuals, about 90% of whom showed abnormal anorectal manometry. Thus, although in our selected population of symptomatic Chagas disease patients the proportion of abnormal anorectal manometry was substantial, we cannot exclude the possibility that the motor abnormalities detected by our test were not specific and, in fact, unrelated to Chagas disease.

One limitation of the study is that no formal sample size was previously calculated for the study population, and this must be taken into account when interpreting the results. Another issue is that the control group was not similarly evaluated, because barium enema was not performed in this group.

In summary, our study has shown that in a sample population of chronically infected patients with *T. cruzi* there is a relatively high proportion of anorectal motor disturbances in symptomatic patients compared with asymptomatic ones. We caution that some of these motor abnormalities may be non-specific for Chagas disease, as they are also quite common in non-Chagas disease patients with chronic constipation; further prospective case-control studies are needed to solve this issue. Furthermore, megarectum was observed in about one-third of our study population, although it was not found exclusively in symptomatic patients, suggesting that megarectum may develop without perceptible defecation impairment.

Received August 30, 2014. Accepted for publication February 8, 2015.

Published online March 16, 2015.

Authors' addresses: Fernando Salvador, Adrián Sánchez-Montalvá, and Israel Molina, Department of Infectious Diseases, Vall d'Hebron University Hospital, Barcelona, Spain, and PROSICS, Barcelona, Spain, E-mails: fmsalvad@vhebron.net, adsanche@vhebron.net, and imolina@vhebron.net. Marianela Mego, María Morís, Ana Accarino, Juan-Ramon Malagelada, and Fernando Azpiroz, Digestive System Research Unit, University Hospital Vall d'Hebron, Barcelona, Spain, and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain, E-mails: mmego@vhebron.net, morismaria@gmail.com, aaccarino@telefonica.net, jrmalage@vhebron.net, and fazpiroz@vhebron.net. Kathleen Ramírez, Department of Radiology, Vall d'Hebron University Hospital, Barcelona, Spain, E-mail: kramirez@vhebron.net.

REFERENCES

1. World Health Organization, Chagas disease (American trypanosomiasis). Available at: <http://www.who.int>. Accessed August 1, 2014.
2. Schmunis GA, Yadon ZE, 2010. Chagas disease: a Latin American health problem becoming a world health problem. *Acta Trop* 115: 14–21.
3. Salvador F, Treviño B, Sulleiro E, Pou D, Sánchez-Montalvá A, Cabezas J, Soriano A, Serre N, Gómez i Prat J, Pahissa A, Molina I, 2014. *Trypanosoma cruzi* infection in a non-endemic country: epidemiological and clinical profile. *Clin Microbiol Infect* 20: 706–712.
4. Pérez-Ayala A, Pérez-Molina J, Norman F, Navarro M, Monge-Maillo B, Díaz-Menéndez M, Peris-García J, Flores M, Cañavate C, López-Vélez R, 2011. Chagas disease in Latin American migrants: a Spanish challenge. *Clin Microbiol Infect* 17: 1108–1113.
5. Muñoz J, Gómez I, Prat J, Gállego M, Gimeno F, Treviño B, López-Chéjade P, Ribera O, Molina L, Sanz S, Pinazo MJ, Riera C, Posada EJ, Sanz G, Portús M, Gascón J, 2009. Clinical profile of *Trypanosoma cruzi* infection in a non-endemic setting: immigration and Chagas disease in Barcelona (Spain). *Acta Trop* 111: 51–55.
6. Coura JR, Anunziato N, Willcox HP, 1983. Chagas disease morbidity. I- Study of cases originating in various states of Brazil, observed in Rio de Janeiro. *Mem Inst Oswaldo Cruz* 78: 363–372.
7. Pless M, Juranek D, Kozarsky P, Steurer F, Tapia G, Bermudez H, 1992. The epidemiology of Chagas' disease in a hyperendemic area of Cochabamba, Bolivia: a clinical study including electrocardiography, seroreactivity to *Trypanosoma cruzi*, xenodiagnosis, and domiciliary triatomine distribution. *Am J Trop Med Hyg* 47: 539–546.
8. Brenière SF, Carrasco R, Revollo S, Aparicio G, Desieux P, Tibayrenc M, 1989. Chagas' disease in Bolivia: clinical and epidemiological features and zymodeme variability of *Trypanosoma cruzi* strains isolated from patients. *Am J Trop Med Hyg* 41: 521–529.
9. Sánchez-Guillén MC, López-Colombo A, Ordóñez-Toquero G, Gómez-Albino I, Ramos-Jiménez J, Torres-Rasgado E, Salgado-Rosas H, Romero-Díaz M, Pulido-Pérez P, Pérez-Fuentes R, 2006. Clinical forms of *Trypanosoma cruzi* infected individuals in the chronic phase of Chagas disease in Puebla, Mexico. *Mem Inst Oswaldo Cruz* 101: 733–740.
10. Lescure FX, Le Loup G, Freilij H, Develoux M, Paris L, Brutus L, Pialoux G, 2010. Chagas disease: changes in knowledge and management. *Lancet Infect Dis* 10: 556–570.
11. Riera C, Verges M, Iniesta L, Fisa R, Gállego M, Tebar S, Portús M, 2012. Identification of a Western blot pattern for the specific diagnosis of *Trypanosoma cruzi* infection in human sera. *Am J Trop Med Hyg* 86: 412–416.
12. Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders. Available at: http://www.romecriteria.org/assets/pdf/19_RomeIII_apA_885-898.pdf. Accessed August 1, 2014.
13. Ximenes CA, 1984. Técnica simplificada para o diagnóstico radiológico do megacolo chagásico. *Rev Soc Bras Med Trop* 17: 23.
14. Pérez-Ayala A, Pérez-Molina JA, Norman F, Monge-Maillo B, Faro MV, López-Vélez R, 2011. Gastro-intestinal Chagas disease in migrants to Spain: prevalence and methods for early diagnosis. *Ann Trop Med Parasitol* 105: 25–29.
15. De Oliveira RB, Troncon LE, Dantas RO, Menghelli UG, 1998. Gastrointestinal manifestations of Chagas disease. *Am J Gastroenterol* 93: 884–889.
16. Meneghelli UG, 1985. Chagas disease: a model of denervation in the study of digestive tract motility. *Braz J Med Biol Res* 18: 255–264.
17. Meneghelli UG, de Godoy RA, Macedo JF, de Oliveira RB, Troncon LE, Dantas RO, 1982. Basal motility of dilated and non-dilated sigmoid colon and rectum in Chagas disease. *Arq Gastroenterol* 19: 127–132.
18. Cavenaghi S, Felicio OC, Ronchi LS, Cunrath GS, Melo MM, Netinho JG, 2008. Prevalence of rectoanal inhibitory reflex in chagasic megacolon. *Arq Gastroenterol* 45: 128–131.

19. Netinho JG, Cunrath GS, Ronchi LS, 2002. Rectosigmoidectomy with ileal loop interposition: a new surgical method for the treatment of chagasic megacolon. *Dis Colon Rectum* 45: 1387–1392.
20. Iantorno G, Bassotti G, Kogan Z, Lumi CM, Cabanne AM, Fisogni S, Varrica LM, Bilder CR, Muñoz JP, Liserre B, Morelli A, Villanacci V, 2007. The enteric nervous system in chagasic and idiopathic megacolon. *Am J Surg Pathol* 31: 460–468.
21. Da Silveira AB, D'Avila Reis D, de Oliveira EC, Neto SG, Luquetti AO, Poole D, Correa-Oliveira R, Furness JB, 2007. Neurochemical coding of the enteric nervous system in chagasic patients with megacolon. *Dig Dis Sci* 52: 2877–2883.