

GASTRIC ACID SECRETION RESPONSE IN THE *Cebus apella*. A MONKEY MODEL OF CHRONIC CHAGAS DISEASE

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SUMMARY

The objective was to study the secretory pattern, both basal and stimulated either by histamine (0.1 mg/kg) or pentagastrin (64 ug/kg) in eighteen *Cebus apella* monkeys chronically infected with different *T. cruzi* strains (CA1, n=10; Colombian, n=4 and Tulahuen, n=4) and to describe the morphological findings in the gastrointestinal tract in twelve infected (6 sacrificed and 6 spontaneously dead) and four healthy monkeys. All infected monkeys and 35 healthy ones were evaluated by contrast X-ray examination.

No differences were observed in basal acid output between control and infected groups. Animals infected with the Tulahuen and Colombian strains showed significant lower values of peak acid output in response to histamine or pentagastrin ($p < 0.01$ and $p < 0.05$ respectively; "t" test) in comparison to the controls.

Barium contrast studies showed enlargement and dilatation of the colon in three infected animals. Histopathological lesions were seen in 75% of the autopsied animals either in colon alone (33%) or both, in colon and esophagus (42%).

The normal secretion observed in the CA1 infected group could be due to a lower virulence of the strain, a lower esophagic tropism or the necessity of a longer post-infection time to cause lesions.

KEY WORDS: *Cebus* monkey; Chagas' disease; Gastric Acid Secretion.

INTRODUCTION

Chagas' disease or American Trypanosomiasis is a zoonosis, restricted to the American continent. In our country there are some 3 million infected subjects (10% of the population) and it is estimated that 16 people die per day as a result of chagasic cardiomyopathy²⁵ or have to be operated on for a complication of their megaviscera.

The decrease of the Auerbach's plexus cells and perineural infiltrates are the characteristic lesions observed in the gastrointestinal tract tube during the chronic phase of the disease: this results in disturbance of the motility and visceral dilata-

tion, especially of the esophagus and colon, the incidence ranging from 16% to 50% in the different countries⁽¹⁹⁾. Amastigotes are occasionally found in tissue lesions⁽⁷⁾.

The New World primate, *Cebus apella*, being native in an endemic area, may develop the disease by natural infection⁽¹⁶⁾; for the reason this species is a useful model to study the pathophysiology of chronic Chagas' disease^(8, 11, 12, 13, 15).

The aims of the present work were: a) to study the gastric acid secretion, both basal and after

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stimulation, either with histamine or pentagastrin, considering that in patients with chronic Chagas' disease it was reported to be lower than in healthy subjects and b) to describe the morphological findings observed in the gastrointestinal tract. Both objectives were achieved, in *Cebus* monkeys, experimentally infected with different *T. cruzi* strains, during the first five-year follow-up.

MATERIAL AND METHODS

Fifty three *Cebus apella* monkeys, having normal electrocardiographic, echocardiographic⁽¹⁰⁾ and hematological⁽¹⁷⁾ parameters and negative specific serology for Chagas' disease were selected from the breeding and rearing outdoor colony. They were lodged in our indoor colony, in individual cages, with water ad libitum and food provided according to a standard pellet diet (25% protein, 290 calories/100 gr., Cargill, Buenos Aires, Argentina) and supplemented with fresh fruit. Temperature, humidity and light conditions were adjusted to the needs of the experiments.

Table 1 shows a summary of the study design. Eighteen monkeys, of both sexes, were inoculated with three different *T. cruzi* strains: CA1 (n=10, conjunctival route, one single dose of 1×10^4 to 1×10^6 organisms) Tulahuen and Colombian (both with n=4, intraperitoneal route and several inoculations of 3×10^6 organisms).

The reinfections were performed to put the animals in similar conditions to those found by people living in endemic areas where the periods of natural reinfection vary along their lives.

The follow-up of the animals in the control (n=35) and infected groups (n=18), include the demonstration of parasites in the patient's blood (parasitemia), thick blood film, Strout's method⁽²⁹⁾, xenodiagnosis⁽³⁾, specific serology (indirect hemagglutination - IHA (Cellognost-Chagas Behringwerke) and enzyme-linked-immunosorbent assay-ELISA⁽³²⁾, electrocardiogram, echocardiogram and ⁹⁹Tc scintigraphy⁽²⁶⁾.

The electrocardiographic and echocardiographic studies were performed once a week during the first 3 months post-infection, twice a week during the first 2 years post-inoculation and from then on, once a month.

X Ray Examination

To evaluate if the parasite will produce any changes in the colon and esophagus, X ray studies were performed on the control and infected animal groups, previous to the inoculation and one and three years after infection.

The X Ray studies of the gastrointestinal tract were done using a CGR X-ray equipment, 1000

Table 1
Experimental Design

Strain	CA 1	Colombian	Tulahuen	Control
Material and Methods				
Number of animais	10	4	4	35
Sex	male	2 male - 2 female	male	male
Estimated age of first inoculation (years)	6 - 10	1,2 - 3	4 - 4,5	5 - 9
Weight (g)	2110 - 3320	940 - 1800	1660 - 1950	2250 - 2570
Date of first inoculation	06 - 80, 07 - 81	09 - 82, 10 - 82	11 - 82, 12 - 82	-
Number of inoculations at 06 - 84	1/2	17/18	10/11	-
Number of <i>T. cruzi</i> (each inoculation)	4×10^4 to 1×10^6	3×10^6	3×10^6	-
Route	conjunctival	intraperitoneal	intraperitoneal	-

mAmp, with a seriograph and image amplifier. The infusion pressure of the contrast material was controlled during the whole procedure to avoid mechanic dilatation. Neither spasmolytic drugs nor air were used.

Study of Gastric Acid Secretion

The gastric acid secretory pattern was studied in 18 infected *Cebus* monkeys and 6 controls, during the first five-years of follow-up.

After an overnight fast, they were placed in restraint chairs and then intubated with a radiopaque nasogastric tube (Levin No. 10) that was fluoroscopically positioned. A two hours accomodation time was allowed before starting with the test. The animals were kept without sedation, during the test.

Two secretagogues, histamine dihydrochloride and pentagastrin (Peptavlon-Ayerst), were given in two different days with one week interval. The gastric acid secretion stimulated by each secretagogue was studied twice in each monkey and the values were averaged. Fifteen minutes before the subcutaneous injection of histamine, the animals were given intravenously 10 mg/kg diphenhydramine-hydrochloride (Benadryl, Parke Davis).

During the stimulation with two injections, of either histamine (0.05 mg/kg) or pentagastrin (32 ug/kg), with a 30 min. interval, the secretion was collected every 15 min., during a 90 min. period.

Before starting the study the gastric contents were aspirated and discarded.

Volume was measured to the nearest 0.1 ml and acid concentration was determined by titration with 0.1 N NaOH to pH7 on an automatic titrator (Radiometer, Copenhagen, Denmark).

Basal acid output (BAO) was calculated by adding the four values obtained, during 60 minutes, before the administration of the secretagogue.

Peak acid output (PAO) was calculated by doubling the sum of the two highest consecutive values obtained after the administration of secretagogue. The results were expressed as X + SE and significant differences between groups were analysed by means of the "t" test.

Morphological Studies

The histological studies were performed in the animals that died spontaneously (n=6) and in six others sacrificed (n=6) at random from those inoculated previously and showing electrocardiographic and/or echocardiographic disturbances¹⁵. The results were compared to the observed in four control animals sacrificed at the same time.

A complete autopsy of the animals was made. The esophagus and colon were fixed in Zamboni's fluid for 24 hr. and embedded in paraffin. The sections were stained with hematoxylin-eosin, and Masson's trichrome.

RESULTS

Preliminary studies

In the three groups of animals infected with *T. cruzi* positive parasitemia was detected by the fresh drop, Strout's test and or xenodiagnosis. Specific serology determined by IHA and ELISA became positive at week 4, being lower from week 49 onwards¹¹.

In the chronic stage, alterations in the ECG patterns compatible with those described in human pathology^(4,28) were recorded in all the animals except in one inoculated with Tulahuen strain⁽¹³⁾. Echocardiographic disturbances were observed in the 100% of the animals⁽¹⁵⁾. The histopathological findings, in the heart, resembled those found in the human disease^(12, 19).

X Ray examination

No changes in the diameter, length and motility of the colon and esophagus were observed in the animals of the control group.

Three of the monkeys of the infected group showed enlargement and dilatation of the colon in the contrast studies compared with the controls (Fig. 1 and 2). The changes in the X-ray studies compared well with histological lesions of the Auerbach's plexus responsible for the organ dilatation, were found.

Gastric Secretion

BAO in the control group, were similar to those observed in the groups infected with CA1, Tulahuen and Colombian strains.

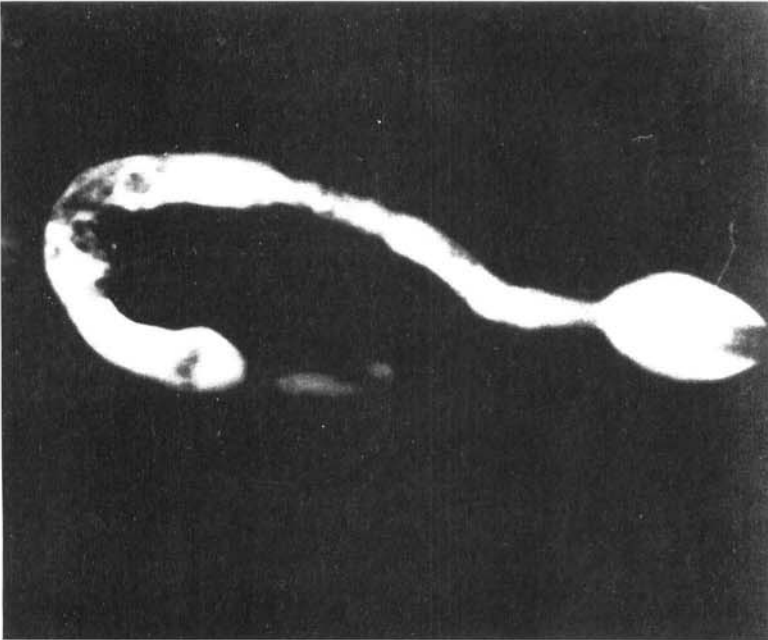


Figure 1: Contrast X ray of a control *Cebus* monkey showing the normal colonic diameter and length for this species.



Figure 2: Contrast X ray of a monkey infected with CA1 strain showing an image compatible with megavisceras.

PAO values in response to histamine and pentagastrin in the 4 groups were compared to the respective BAO values and the results expressed as $\text{mmol} \times \text{h}^{-1}$ are shown in Figure 3.

Mean PAO values in response to histamine and pentagastrin stimulation did not differ in the control group. In the animals infected either with the Tulahuen or Colombian strain, PAO values were significantly lower ($p < 0.01$ and $p < 0.05$, respectively) than in the controls for both secretagogues and than in the monkeys inoculated with the CA1 strain ($p < 0.05$) for histamine

Pentagastrin produced significantly lower PAO values ($p < 0.05$) than histamine in the Tulahuen group.

Morphological Findings

Enlargement of the whole colon with narrowing in the rectal segment was observed in three of the infected animals.

Focal infiltrates of lymphocytes and plasma cells were seen in the smooth muscle and Auerbach's plexus of the esophagus in 5 animals (Fig. 4) and of the colon in 3 animals (Fig. 5 and 6).

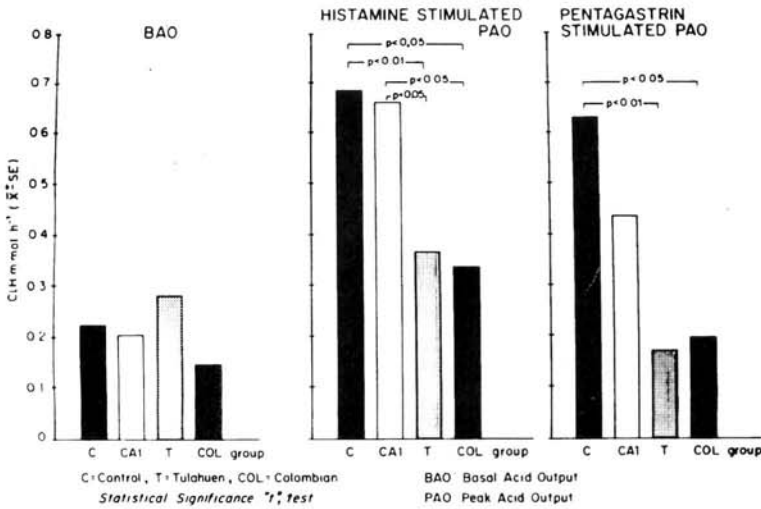


Figure 3: Acid secretion in response to two secretagogues in *Cebus* monkeys chronically infected with *T. cruzi*.

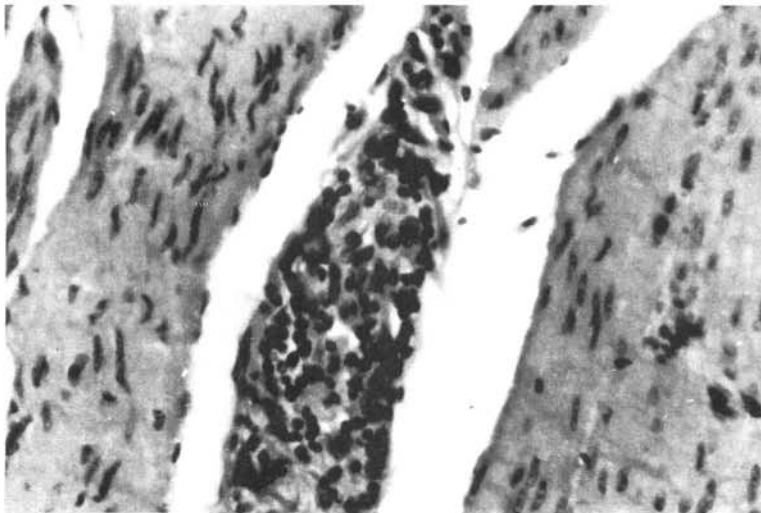


Figure 4: Lymphocytes surround the nerve cells and infiltrate the nerve fibers of the Auerbach's plexus in the esophagus of a monkey, 25 months after inoculation with Tulahuen strain (HE 100X).

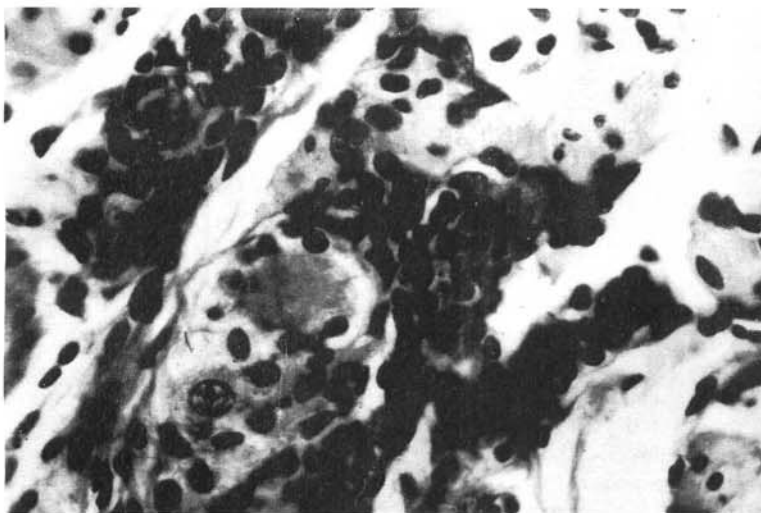


Figure 5: Auerbach's plexus in the colon of a monkey sacrificed 25 months after inoculation with Tulahuen strain and in which neurons and nerve fibers are surrounded by lymphocytes (HE 400X).

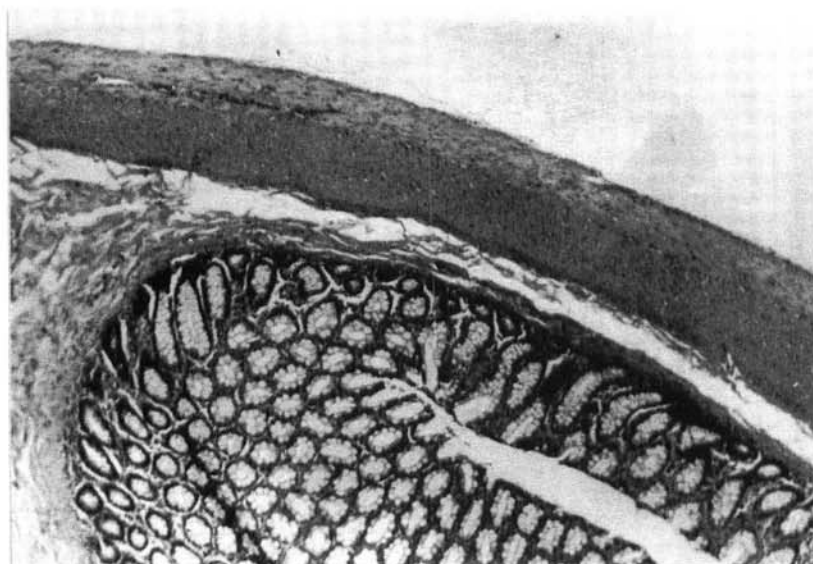


Figure 6: The elements of the Auerbach's plexus are diminished in the colon of an animal, 49 months after inoculation with Tulahuen strain (HE 40X).

Table 2
Anatomopathological Lesions in Cebus Monkeys chronically infected with *T. cruzi*

Strain	Months after first Inoculation	Esophagus	Colon	Heart
CA1	46°	—	a	mf
	46°	—	a	mf
	46°	—	—	mf
	53*	—	a	mf
	58*	—	a	mf
	67*	i	a	mf
Tulahuen	25°	i	i	ig
	40*	mi	a	mf
	49*	mi	a+i	mf
Colombian	21°	—	—	ig
	21°	mi	—	ig
	48*	—	—	mf

° Sacrificed
 • Spontaneous Death
 a = agangliosis
 i = infiltrates
 ig = infiltrative granulomatosis
 mi = muscular infiltrates
 mf = myocytolytic fibrosis

A decrease in the size of the Auerbach's plexus with slight increase of the connective tissue when compared with the control group, could be observed in 7 animals.

The analysis of the distribution of the lesions according to the *T. cruzi* strain inoculated is shown in Table 2. This table shows that heart lesions were present in all the animals at the time they were autopsied⁽¹²⁾.

Animals dying spontaneously showed a larger number of lesions in the colon and in the esophagus than those sacrificed; gastrointestinal lesions were not uniform, varying in type and onset from one strain to the other.

DISCUSSION

The digestive tract, as well as the heart, are particularly damaged by Chagas' disease in Cebus

monkey^(11, 12, 13, 15) as well as in humans^(20, 21). The esophagus and colon are the organs of the digestive tract more frequently involved.

ETZEL⁽⁹⁾, studying basal acid secretion in Chagas' disease observed that their patients with achlorhydria were highly frequent and that their pattern of secretion was not uniform. This fact was confirmed by others authors and a possible explanation could be that destruction of intramural nerves varies from one patient to another^(6, 31).

According to GROSSMAN⁽¹⁸⁾ the parietal cells have a membrane receptor locus for acetylcholine, another for gastrin and a third one for histamine. As a consequence of nerve destruction vagal stimulation can be impaired in patients with Chagas' disease⁽³⁰⁾.

In our work, no significant differences were observed in basal acid output between infected and control animals. In the contrary peak acid output, on response either to histamine or pentagastrin was significantly lower ($p < 0.01$ and $p < 0.05$, respectively) in animals infected with the Tulahuen and Colombian strain, in comparison to the controls.

The reason why PAO values in animals infected with the CA1 strain, did not differ from those of the controls, could not be determined, but a lesser virulence of the *T. cruzi*, the use of a smaller inoculum, a lower frequency of inoculation (only 1 to 2) or that the time after first inoculation and the sacrifice of the animals was too short to injure the esophagus and alter the secretory pattern could explain the difference. To favour these hypothesis, only in the animal dying spontaneously 67 months after infection, could esophageal lesions be demonstrated.

In contrast, the Tulahuen strain produced infiltrative lesions in the esophagus and in the colon of all the animals, and in two of them a mild colonic aganglionosis; coincidentally a marked effect on the secretory pattern, was observed.

Looking at the distribution of the histological lesions according to the *T. cruzi* strain inoculated (Table 2), we can see that in the 75% of the infected animals (9 out of 12) autopsied, lesions were present in the colon alone in 33% and in the colon and esophagus in 42%.

The CA1 strain caused predominantly

aganglionic lesions in the colon and in the animal with the longest time of evolution (67 months), we have observed lymphocyte and plasma cell infiltrates in the esophagus.

Although PAO values were lower in animals inoculated with the Colombian strain in only one of them we were able to show histological lesions in the esophagus and in the colon. Probably this *Trypanosoma* strain needs a longer time than the Tulahuen strain, to injure the gastrointestinal tract.

The cellular infiltration observed in the Auerbach's plexus, of the esophagus and colon, in the two animals with a short follow up, (21 and 25 months post infection), and the occurrence of megacolon radiographically detected and histologically confirmed in other 3 animals (46 to 67 months after the first inoculation) with a marked decrease of the neural elements of the plexus, are a clear demonstration that the autonomous nervous system is damaged.

The destruction of the plexuses in humans is not always followed by megavisera, which is reported as occurring in 3 to 59% of the cases of cardiomyopathy⁽⁵⁾. A higher percentage may show neuronal lesions with a decrease of ganglion cells.

The involvement of the ganglion cells of the esophagus in the *Cebus*, provides a unique model of chronic denervation of the organ which is suitable for studying the influence of the intrinsic innervation on gastric functions.

It is possible that as a result of the changes in the autonomous nervous system innervation, the megavisera caused by the infection with *T. cruzi* could be the consequence of the alteration in the peptidergic nerve endings producing VIP and substance P, that are markedly reduced in the areas of hypoganglionosis or aganglionosis in patients with Hirschsprung's disease⁽¹⁾. We should consider the possibility that the alteration of the receptors in the gastrointestinal cells, could be the first element involved in the pathogenesis of the disease, decreasing either motility⁽²³⁾ or gastric secretion, a subject that has not yet been studied.

CONCLUSIONS

Unlike other animal models, the *Cebus* sp monkeys showed, during the course of the infection, regardless from the *T. cruzi* strain utilized,

age and sex, serological conversion with positive titers for specific antibodies, detected either by IHA or ELISA. In all of them positive parasitemia could be detected by fresh drop, the Strout's method or the xenodiagnosis, during the acute phase of the disease, but not during the chronic phase, as it happens in humans⁽⁵⁾.

These results observed during this five-year-follow-up, convinced us that this animal is particularly different from other experimental models commonly used^(14, 22, 23, 27).

Consequently and according to Brener's requirements⁽²⁾ this New World non human primate, native to an endemic area, reproducing well in captivity, adapted to the conditions of an indoor colony and that can be kept at a reasonable cost, is an experimental model suitable for the study not only of cardiac lesions, but also of the gastrointestinal disorders, of chronic Chagas' disease once they reproduce the human clinical and histological gastrointestinal changes.

The use of this primate, that is readily available to all researchers in the endemic areas, together with a multidisciplinary and coordinate approach, will allow us to increase our knowledge of the pathogenesis and immunopathology of this disease, in its indeterminate and chronic phase. It will also permit us to evaluate new immunogenic and chemotherapeutic effects of drugs.

RESUMEN

Respuesta secretora acida gastrica en el *Cebus apella*, un modelo animal de la enfermedad de Chagas cronica.

El objetivo fue estudiar la respuesta secretora basal y estimulada por histamina (0.1 mg/kg) o pentagastrina (64 ug/kg) en 18 monos *Cebus apella* cronicamente infectados con diferentes cepas de *T. cruzi* (CA1 n=10; Colombiana n=4 y Tulahuén n=4). Además se describieron los hallazgos morfológicos en el tracto gastrointestinal de 12 animales muertos (6 sacrificados y 6 espontaneamente).

Se evaluó radiológicamente el tracto gastrointestinal de 35 *Cebus sp.* sanos (controles) y 18 infectados crónicamente. Se estudiaron histopatológicamente 4 controles y 12 infectados.

No hubo diferencias en la secreción basal ácida entre los grupos control e infectado.

Los animales infectados con la cepa Tulahuén y Colombiana tuvieron valores más bajos en el pico de secreción ácida, como respuesta a la histamina o a la pentagastrina ($p < 0.01$ y $p < 0.05$ respectivamente; test "t") con respecto a los controles.

Los estudios con contraste de bario mostraron agrandamiento y dilatación del colon en 3 animales infectados. En las autopsias de los chagásicos se encontraron lesiones histológicas en el 75% de los casos (solo en colon, 33%; en colon y esofago, 42%).

En el grupo CA1, la secreción ácida estuvo dentro de los parámetros normales y esto se podría deber a una baja virulencia de la cepa, a un menor tropismo esofágico o a que el tiempo post-infección fue demasiado corto para causar lesiones.

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