

Electroacupuncture in rats infected with *Strongyloides venezuelensis*: effects on gastrointestinal transit and parasitological measurements

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ABSTRACT

Objective To investigate the effects of electroacupuncture (EA) at ST36 and CV12 on gastrointestinal transit and parasitological measurements during *Strongyloides venezuelensis* infection in rats.

Design Rats were infected with *S. venezuelensis* and allocated to one of three groups that were infected and remained untreated (SV group, n=8), infected and treated with EA at CV12 (SV+CV12 group, n=8) or infected and treated with EA at ST36 (SV+ST36 group, n=8). EA was performed every 3 days over a 21-day period, at 4 mA intensity and 15 Hz frequency for 20 min. At 2 and 20 days post-infection (dpi), body weight, food and water intake, and faecal characteristics were monitored over a 24-hour period. Gastric emptying, caecal arrival time, small intestinal transit and eggs per gram (EPG) of faeces were calculated at 3, 9, 15 and 21 dpi. At 21 dpi, intestinal worm recovery was counted.

Results EA at ST36 and CV12 slowed gastric emptying over the course of infection time. An accelerated intestinal transit was observed in the ST36 group, and after CV12 treatment the same effect was observed at 9 and 15 dpi. At 9 dpi, EPG was increased in the CV12 group. ST36 treatment decreased EPG at 9 and 15 dpi. At 21 dpi, both the ST36 and CV12 groups had increased EPG and worm numbers. No changes were observed in the other parameters analysed.

Conclusions EA at ST36 and CV12 provoked changes in gastrointestinal transit that may be beneficial to the host during *S. venezuelensis* infection; however, based on the number of worms and EPG at 21 dpi, the indication for EA in the treatment of strongyloidiasis needs to be carefully assessed.

be quantified in rats by measuring the propulsion of several non-absorbable materials within the gut at predetermined time intervals.^{3,4} However, a large number of animals are killed in these procedures.⁴ Alternating current biosusceptometry (ACB) has already been employed to follow-up GI transit and contractility and has advantages such as low cost, lack of radiation and non-invasiveness.^{5,6}

Electroacupuncture (EA) modifies contractility and restores GI motor disorders based on a well-characterised neural mechanism.^{7–10} Acupuncture has been shown to accelerate return of bowel function after GI surgery and relieve intestinal obstruction in humans.^{11,12} Acupuncture at the traditional points ST36 (*Zusanli*) and CV12 (*Zhongwan*) has been shown to improve GI motor abnormalities.^{13,14} EA at CV12 exerts a significant inhibitory effect on gastric motility^{10,14} by acting on the nucleus of the solitary tract (NTS) via somatosympathetic pathways, inducing muscle relaxation. Acupuncture at ST36 has a stimulatory effect on gastric motility, increasing intestinal myoelectric activity via a somatoparasympathetic pathway.^{8,10} Considering these opposite effects, the comparison of EA at CV12 versus ST36 represents an interesting model to study GI motility after acupuncture stimulation under different conditions. Although the use of EA is endorsed by the US National Institutes of Health and World Health Organization (WHO), its mechanisms of action in a large number of pathological conditions remain unknown,^{15,16} including its use for the treatment of intestinal parasitism.

INTRODUCTION

Gastrointestinal (GI) motility comprises both smooth muscle contractility and GI transit, and motor disorders are linked to several diseases.^{1,2} GI transit can



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According to the WHO,¹⁷ strongyloidiasis is a neglected tropical disease caused by *Strongyloides stercoralis*, which infects humans, primates, dogs and gerbils, and has a wide range of GI symptoms such as diarrhoea, constipation or intermittent vomiting.¹⁸ There are different experimental models that are capable of modelling some features of the *S. stercoralis* life cycle and pathology, thus improving our understanding of host defence mechanisms.¹⁹ Studies using gerbils infected with *S. stercoralis* are useful to analyse the hyperinfection syndrome, a severe complication of human strongyloidiasis that cannot be induced by rodent specific *Strongyloides* species.¹⁹ *Strongyloides venezuelensis*, which naturally infects rats, is frequently used in experimental studies to extend the analysis of the parasite's intestinal life stage.^{19,20} Similar to *S. stercoralis* in humans, *S. venezuelensis* larvae migrate through the host lungs before they become established in the duodenal mucosa.^{21,22} Completing its life cycle, the host excretes eggs produced by adult worms in the stool.²³ Rats spontaneously eliminate adult worms 20–30 days following infection^{24,25}; however, previous studies using ACB have indicated that intestinal transit remains slow 21 days after infection despite a reduction in oviposition and worm numbers.²⁶ Indeed, accelerated GI transit has been considered to be part of the host response mechanism to expel parasites, a condition that could be induced by acupuncture.^{1,27} In this study, our aim was to investigate the effects of EA at ST36 and CV12 on GI transit and parasitological measurements during *S. venezuelensis* infection in rats.

METHODS

Animals

Male Wistar rats (weighing 250–350 g) were housed individually in polypropylene cages under controlled lighting conditions (12/12 hours light/dark cycle) at room temperature (22–26°C) with free access to water and food. All experiments were approved by the Institutional Animal Care and Use Committee of the Federal University of Mato Grosso (protocol number 23108.002959/13–3) and were conducted in accordance with animal ethics guidelines consistent with the National Research Council 'Guide for the Care and Use of Laboratory Animals'. All animals (EA-treated rats and infected controls) were acclimatised to restraint for 2 weeks before starting the experiments by the same researcher.

Parasites

S. venezuelensis third-stage infective larvae (L3) were obtained from vermiculite cultures of the infected faeces of gerbils (*Meriones unguiculatus*). The cultures were incubated at 28°C for 72 hours, and the infective larvae were collected and concentrated by using a Baermann apparatus. *S. venezuelensis* larvae were recovered and washed in phosphate-buffered saline (PBS) to be counted. The rats were individually inoculated

via subcutaneous injection in the dorsal region with 2000 *S. venezuelensis* larvae (L3), as detailed by Anjos-Ramos *et al.*²⁶

Experimental design

Twenty-four rats were infected with *S. venezuelensis* and randomly allocated to one of three groups, which were infected and remained untreated (SV group, n=8), infected and treated with EA at ST36 (SV+ST36 group, n=8) or infected and treated with EA at CV12 (SV+CV12 group, n=8). At 2 and 20 days post-infection (dpi), each infected rat was placed individually on grids over clean absorbent paper, with food and water freely available, for a 24-hour period. After this, the food and water intake were measured and faeces were collected and weighed. The faeces were monitored and graded for degree of diarrhoea using the following score: 0, firm faeces; 1, malformed faeces; 2, watery faeces with perianal staining; and 3, severe perianal staining.²⁸ The body weight of each rat was also recorded and the body weight growth rate was calculated using the following formulae²⁹: body weight growth rate = (body weight on day 20 dpi – body weight on day 2 dpi)/body weight on day 2 dpi x 100%. Gastric emptying time, caecal arrival time, small intestinal transit time and eggs per gram (EPG) of faeces were analysed at 3, 9, 15 and 21 dpi. At 21 dpi, all experimental groups were euthanased by anaesthetic overdose administered intraperitoneally (240 mg/kg ketamine (Cetamin, Syntec, Brazil) plus 45 mg/kg xylazine chlorhydrate solution (Xilazin, Syntec, Brazil)) followed by decapitation. Laparotomy was performed to remove the small intestine and the number of worms recovered were counted.

Parasitological analysis

Rats infected with *S. venezuelensis* were placed individually on grids over clean moist absorbent paper on which the animals defecated. The faeces were then collected, diluted in saturated saline, homogenised and counted in triplicate under a microscope (Nikon, Tokyo, Japan) using the McMaster technique to calculate EPG of faeces.³⁰ The worm number refers to the number of female worms recovered from the small intestine. The small intestine was removed, cut longitudinally and incubated at 37°C for 3 hours, and the adult parthenogenetic females were then counted using stereomicroscopy (Nova Optical System, Piracicaba, Brazil).

Electroacupuncture

All animals were treated every 3 days with EA during the 21-day period.³¹ All rats were manually immobilised to access both ST36 and CV12. ST36 was located at the anterior tibial muscle, 5 mm lateral to and below the anterior tubercle of the tibia¹⁰; CV12 was located in the centre of the abdomen, in the midline of the body.¹⁰ Two stainless-steel needles (0.25 × 15 mm,

DongBang Acupuncture, Inc, Chungnam, Korea) were inserted bilaterally to a depth of 0.5–1 mm deep at the two acupuncture points. The anode and cathode leads from an electrical stimulator (WQ IOD1; Donghua, China) were connected to the two acupuncture needles. A direct current with an intensity range of 4 mA and stimulation frequency of 15 Hz was applied for a 20-min period.

ACB technique and GI transit recordings

The ACB sensor is an assembly of induction coils used to monitor the response of magnetic materials to an externally applied magnetic field.³² The signal intensity depends on the amount of magnetic material as well as the distance between the sensor and the magnetic sample. Ferrite powder ($\text{MgZnFe}_2\text{O}_3$, Imag, Brazil) was used as a non-absorbable magnetic marker in the GI tract.³² The ACB sensor was recently improved for rodent studies and the operating procedure has been detailed elsewhere.^{5,6}

After an overnight fast, the animals ingested a solid magnetic pellet prepared with 0.5 g of ferrite powder mixed with 1.5 g of laboratory chow. Afterwards, they were handled gently by the neck in order to place the ACB sensor (Br4Science, Brazil) on the abdominal surface. The point of maximum magnetic signal intensity was recognised as the stomach and the data were registered. The sensor was also placed at the caecal projection (based on anatomical references) and the magnetic signal intensity was recorded.⁵ Subsequent measurements were performed in awake rats at these two points at regular 15-min intervals for at least 6 hours.

Magnetic data analysis

Data analyses were performed by an investigator blinded to treatment group allocation. All signals were analysed in MatLab (Mathworks, Inc, USA) by visual inspection and the statistical moment was calculated. The statistical moment was obtained through the temporal average pondered by magnetic intensity curves, normalised by area under the curve.⁵ Using this approach, the following measurements were quantified: mean gastric emptying time, defined as the time t (min) when a mean amount of magnetic meal was emptied from the stomach, and calculated by the area under the emptying curve; mean caecal arrival time, defined as the time t (min) when an increase occurred in the mean amount of magnetic meal that arrived in the caecum, and calculated by the area between the caecal arrival curve and the maximal cumulative values; and mean small intestinal transit time, which was quantified as the difference between the caecal arrival time and the gastric emptying time.⁵

Statistical analysis

The normality of continuous variables was evaluated using the Kolmogorov-Smirnov test. Overall difference

between groups was analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered significant. The data are expressed as mean \pm SD.

RESULTS

None of the animals presented with bristling hair, bleeding or aggression during the experimental procedures. There were no statistically significant differences in the food and water intake or body weight growth rates among the groups (figure 1). According to the criteria adopted, no alterations in faecal weight or diarrhoea following EA treatment were observed.

Gastric emptying was slowed over the course of infection in both the ST36 and CV12 groups compared with the SV group (figure 2). Treatment with EA at CV12 had greater inhibitory effects on gastric emptying at the peak of infection compared with treatment at ST36 ($p = 0.004$). Caecal arrival time in the SV+CV12 group was delayed only at 3 and 21 dpi compared with the SV and SV+ST36 groups (both $p < 0.001$). Accelerated small intestinal transit was observed after EA treatment at ST36. By contrast, after EA at CV12, the small intestinal transit was slower at 3 dpi ($p < 0.001$) and 21 dpi ($p = 0.0024$) compared with the SV+ST36 group, and faster at 9 and 15 dpi (both $p < 0.001$) compared with the untreated, infected control group.

The oviposition peak occurred at 9 dpi for all three groups; however, EA at ST36 and CV12 decreased and increased, respectively, this EPG peak in comparison with the infected untreated (control) group (both $p < 0.001$). After the peak, the EPG values started to decrease and EA at ST36 accentuated this decrease compared with the infected control group ($p < 0.001$). Compared to the SV (control) group, at 21 dpi, EPG and worm number were increased in both SV+ST36 and SV+CV12 ($p \leq 0.001$, figure 3).

DISCUSSION

In our study, EA applied at ST36 and CV12 was able to modify gastric emptying, intestinal transit, the number of eggs eliminated in the faeces, and worm number compared with untreated infected animals. EA at both acupuncture points slowed gastric emptying and had dual effects on intestinal transit time in infected rats. In the ST36 group, intestinal transit was faster at all stages of infection, whereas in the CV12 group, transit was accelerated only at 9 and 15 dpi. It seems that at the peak of infection, EA exerted regulatory effects on gastric emptying and intestinal transit, bringing them to levels comparable with those observed in uninfected control animals.²⁶ On the other hand, EA did not impact parameters such as stool weight, water and food intake, or weight growth rate throughout the experiment.

A number of studies have already shown the positive effect of acupuncture on GI function in humans,^{11,12}

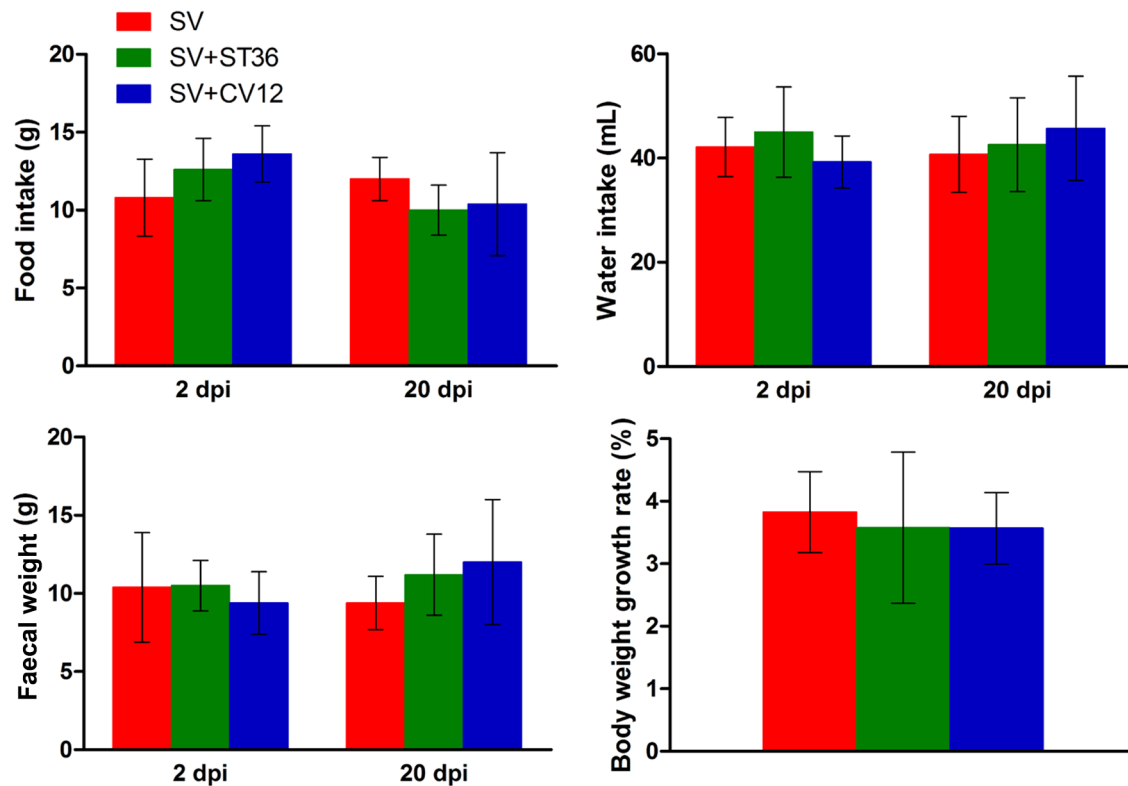


Figure 1 Food and water intake, faecal weight and body weight growth rate at 2 and 20 days post-infection (dpi) in 24 Wistar rats inoculated with *Strongyloides venezuelensis* that remained untreated (SV group, n=8) or received electroacupuncture (EA) treatment at ST36 (SV+ST36 group, n=8) or CV12 (SV+CV12 group, n=8). Data are presented as mean±SD.

and its regulatory effects.^{13 33 34} The effects of EA on GI motility depend on various factors such as species, acupuncture procedure, activation pathways, and baseline motor activities.³³ Clinical studies have indicated that manual acupuncture (MA) inhibits excessive intestinal motility induced by drugs, whereas motility is enhanced under inhibitory conditions. It is important to emphasise that these effects were not observed under normal conditions—that is, in the absence of a baseline disturbance.^{35 36} In this context, studies in normal rats have shown that acupuncture at ST36 enhances GI motility via a somatoparasymphathetic pathway.^{2 16 29} By contrast, the effect of acupuncture on colonic motility appears to be different in certain diseases.²⁹ Dual effects of EA at ST36 have been demonstrated in different GI segments by stimulation of gastric emptying and inhibition of hastened colonic transit in rats.¹³ In addition, regulatory effects have also been observed on gastric motility, since both effects (stimulatory and inhibitory) were observed in rats after treatment at the same acupuncture point.^{7 33}

Stimulatory effects are mediated via cholinergic pathways, while inhibitory effects may be independent of sympathetic mechanisms in the disease state.^{7 13} This may be attributed to the activation of γ -aminobutyric acid (GABA) and glutamate in the dorsal motor nucleus of the vagus, which is adjacent to the NTS, playing a role in the regulation of GI function.^{13 29} Moreover, EA increases levels of serotonin (5-hydroxytryptamine, 5-HT), an important

neurotransmitter associated with the enteric nervous system (ENS).^{29 37–39} The ENS coordinates GI motility and may be stimulated by EA, although such a link has not been fully established yet.⁴⁰

Assessment of transit through the GI tract *in vivo* provides useful information concerning the physiology and pathophysiology of different segments. Evaluation of isolated gut segments does not always reproduce the physiology as a whole.⁴¹ Differing from other approaches, ACB allows evaluation of GI transit *in vivo*, embracing the influence of gut hormone levels and, most importantly, an intact ENS.

Acupuncture has been widely used for the treatment of several GI disorders¹⁰; however, there is little information on its effects in parasitic infections. To our knowledge, this is the first study employing EA treatment in an experimental model of strongyloidiasis. Rats spontaneously eliminate adult worms, which can be observed and assessed by EPG of faeces and worm number.^{24 25} In the present study, EPG of faeces decreased at peak infection (9 and 15 dpi) after ST36 treatment, compared with infected control rats. By contrast, EPG of faeces after CV12 treatment was greater than in the untreated, infected control group and after ST36 treatment on the same dpi. Treatment at both acupuncture points increases the number of worms at 21 dpi and does not contribute to eradication of the infection, since the profile after EA is similar to that observed in untreated rats.²⁶ It is possible that

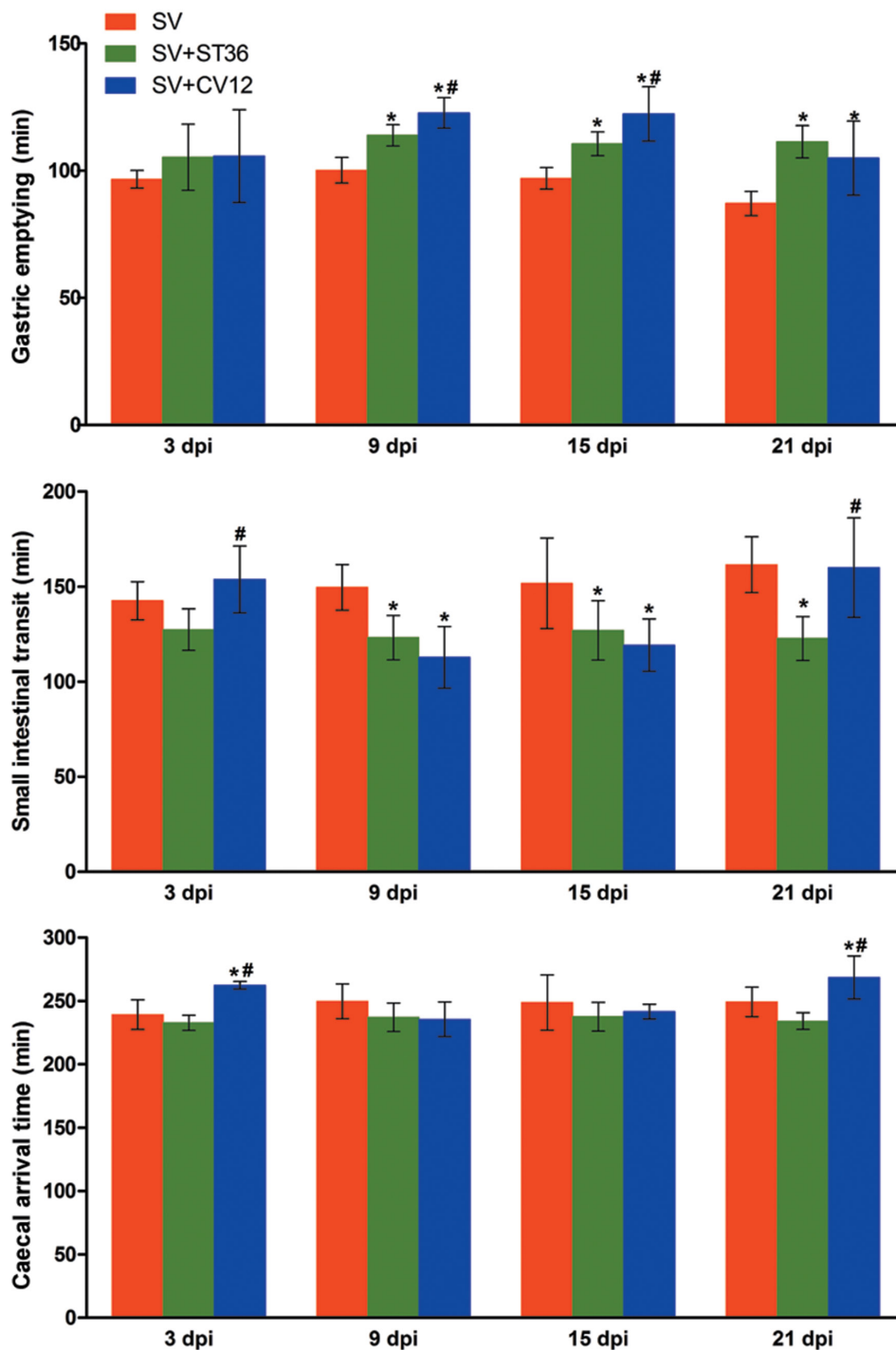


Figure 2 Gastric emptying, caecal arrival and small intestinal transit at 3, 9, 15 and 21 days post-infection (dpi) in 24 Wistar rats inoculated with *Strongyloides venezuelensis* that remained untreated (SV group, n=8) or received electroacupuncture (EA) treatment at ST36 (SV+ST36 group, n=8) or CV12 (SV+CV12 group, n=8). Data are presented as mean±SD *p<0.05 versus SV, #p<0.05 versus SV+ST36 group.

acupuncture could modulate the adaptive immune response by altering the balance of Th1/Th2 T helper cells in disease states to maintain homeostasis.⁴² The immune response to *Strongyloides* species is characterised by the production of Th2-type cytokines²¹ and it has been suggested that sequential EA stimulation at ST36 suppresses the production of these cytokines.⁴³ Mice infected with *Leishmania major* did not present

significant differences in regard to the Th1 profile or parasite burden after acupuncture at ST36 combined with other acupuncture points.⁴⁴ *In vitro* analysis showed that EA at ST36 increased susceptibility to this parasite.⁴⁵ However, few studies have been conducted to evaluate the effect of acupuncture at CV12 on immune function, which contributes to our lack of knowledge concerning such effects.

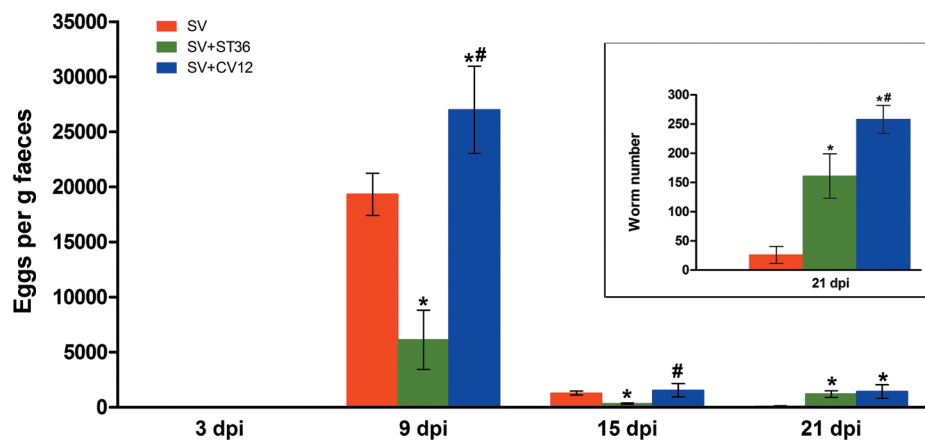


Figure 3 Eggs per gram (EPG) of faeces obtained at 3, 9, 15 and 21 days post-infection (dpi) in 24 Wistar rats inoculated with *Strongyloides venezuelensis* that remained untreated (SV group, n=8) or received electroacupuncture (EA) treatment at ST36 (SV+ST36 group, n=8) or CV12 (SV+CV12 group, n=8). Worm numbers were determined at 21 dpi for all groups (right inset). Data are presented as mean±SD *p<0.05 versus SV, #p<0.05 versus SV+ST36 group.

Acupuncture point selection, time course, treatment duration and EA intensity/frequency should be taken into account for the purposes of clinical practice and must be specifically established for the possible treatment of parasitic infection.^{39 46 47} Previous studies have shown that, when the frequency of twirling MA manipulation is increased, the therapeutic effects on gastric motility initially appear to increase, and then decrease.⁴⁷ The frequency adopted in our study is within the range that has already been used successfully.^{13 29 37 39}

It is important to emphasise the difference between *S. stercoralis* and *S. venezuelensis*, considering that hyperinfection and extremely chronic infections, which are the hallmarks of infection in humans, are absent with *S. venezuelensis*.¹⁹ Meanwhile, experimental infections provide the opportunity of studying the immune response and pathological processes involved in the host–parasite relationship, including the mechanisms of eradication of parasitic adults from the intestine.^{19 48} The therapeutic effects of EA for the treatment of each pathological condition should be observed and, regarding parasites, the potential for EA-induced increases in contractility to aid their expulsion needs to be investigated further.^{27 43 44}

In summary, EA at ST36 and CV12 provoked changes in GI transit that may be beneficial to the host during *S. venezuelensis* infection; however, based on the number of worms and EPG, the potential use of EA in the treatment of strongyloidiasis still needs to be carefully assessed.

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