ISSN 0120-4157

Biomédica

Revista del Instituto Nacional de Salud

PUBLICACIÓN ANTICIPADA EN LINEA

El Comité Editorial de *Biomédica* ya aprobó para publicación este manuscrito, teniendo en cuenta los conceptos de los pares académicos que lo evaluaron. Se publica anticipadamente en versión pdf en forma provisional con base en la última versión electrónica del manuscrito pero sin que aún haya sido diagramado ni se le haya hecho la corrección de estilo.

Siéntase libre de descargar, usar, distribuir y citar esta versión preliminar tal y como lo indicamos pero, por favor, recuerde que la versión impresa final y en formato pdf pueden ser diferentes.

Citación provisional:

Tobar J, Ramos-Sarmiento D, Tayupanta D, Rodríguez M, Aguilar F.

Microscopic and molecular evaluation of *Strongyloides venezuelensis* in an experimental life cycle using Wistar rats. Biomédica. 2021;41 (Supl. 1).

Recibido: 03-07-20

Aceptado: 14-12-20

Publicación en línea: 14-12-20

Microscopic and molecular evaluation of *Strongyloides venezuelensis* in an experimental life cycle using Wistar rats

Evaluación microscópica y molecular de *Strongyloides venezuelensis* en un ciclo de vida experimental utilizando ratas Wistar

Evaluation *S. venezuelensis* in Wistar rats

Jairo Tobar, Daniel Ramos-Sarmiento, Diana Tayupanta, Melina Rodríguez, Fabián Aguilar

Área de Parasitología y Bioterios, Gestión de Investigación, Desarrollo e Innovación, Instituto Nacional de Investigación en Salud Pública Leopoldo Izquieta Pérez, Quito, Ecuador

Correspondence:

Daniel Ramos-Sarmiento, Área de Parasitología y Bioterios, Gestión de Investigación, Desarrollo e Innovación, Instituto Nacional de Investigación en Salud Pública Leopoldo Izquieta Pérez, Iquique N14-285 y Yaguachi, Quito, Ecuador.

Teléfono: +593 96 197 2884

danielrs2012@gmail.com

Authors contributions

All authors actively participated in the conception and outline of the work, writing of the manuscript, critical review of content and approval of the final version of the manuscript.

Introduction: *Strongyloides venezuelensis* is a nematode whose natural host is rats. It is used as a model for the investigation of human strongyloidiasis caused by *S. stercoralis.* The latter is a Neglected Tropical Disease that affects Ecuador, where there are no specific plans to mitigate this parasitic illness.

Objective: To evaluated the stages of *S. venezuelensis* in an experimental life cycle using Wistar rats.

Materials and methods: Male Wistar rats were used to replicate the natural biological cycle of *S. venezuelensis* and describe characteristics, both morphometric and parasitic development. Furthermore, the production of eggs per gram of feces was quantified using two diagnostic techniques and assessment of parasite load: Kato-Katz and qPCR.

Results: Viable larval stages (L1, L2, L3) could be obtained until 96 hours through fecal culture. Parthenogenetic females were established in the duodenum at the 5th Day Post Infection. Fertile eggs were observed in the inspected intestinal tissue and fresh feces, where it was determined that the production peak was on the 8th Day Post Infection. Unlike Kato-Katz, qPCR detected parasitic DNA on days not typically reported.

Conclusions: The larval migration of *S. venezuelensis* within the murine host in an experimental environment was equivalent to that described in a natural biological cycle. The Kato-Katz formed an immediate and low-cost quantitative technique, but the qPCR had greater diagnostic precision. This experimental life cycle can be used as a tool for the study of strongyloidiasis or other similar nematodiasis.

Keywords: *Strongyloides*; Nematoda; life cycle stages; intestinal diseases, parasitic; Wistar rats; Ecuador.

Introducción: *Strongyloides venezuelensis* es un nematodo cuyo huésped natural son las ratas. Se utiliza como modelo para la investigación de la estrongiloidiasis humana producida por *S. stercoralis*. Esta última es una Enfermedad Tropical Desatendida que afecta al Ecuador, donde no existen planes específicos para mitigar esta parasitosis.

Objetivo: Evaluar los estadios de *S. venezuelensis* en un ciclo de vida experimental utilizando ratas Wistar.

Materiales y métodos: Se emplearon ratas Wistar macho para replicar el ciclo biológico natural de *S. venezuelensis* y describir características, tanto morfométricas como desarrollo parasitario. Además, se cuantificó la producción de huevos por gramo de heces mediante dos técnicas de diagnóstico y valoración de carga parasitaria: Kato-Katz y qPCR.

Resultados: Estadios larvarios viables (L1, L2, L3) pudieron obtenerse hasta las 96 horas a través del cultivo fecal. Hembras partenogenéticas se establecieron en el duodeno a partir del 5to Día Post Infección. Huevos fértiles fueron observados en el tejido intestinal inspeccionado y en heces frescas, en las cuales se determinó que el pico de producción fue al 8vo Día Post Infección. A diferencia del Kato-Katz, la qPCR detectó ADN parasitario en días no reportados típicamente.

Conclusiones: La migración larvaria de *S. venezuelensis* dentro del huésped murino en un ambiente experimental fue equivalente al descrito en un ciclo biológico natural. El Kato-Katz conformó una técnica cuantitativa inmediata y de bajo costo, pero la qPCR tuvo mayor precisión diagnóstica. Este ciclo de vida experimental puede usarse como una herramienta para el estudio de la estrongiloidiasis u otros nematodiasis similares.

Palabras clave: *Strongyloides*; Nematoda; estadios del ciclo de vida; parasitosis intestinales; ratas Wistar; Ecuador.

Strongyloides are a genus of helminths made up of about 50 species of which mainly two *S. stercoralis* and *S. fülleborni* are gastrointestinal parasites that may be affecting up to 3,700 million people across the world (1,2). In Latin America, the disease is underestimated due to that generally unreliable diagnosis methods are used. However, there are regions, including Ecuador, that have reported prevalence among 0.7% to 4.1%, depending on the different ecosystems studied (3,4). Thus, it is important to recognise this parasitic ill as a latent public health risk, especially for inhabitants of underdeveloped areas with precarious health conditions and immunosuppressed patients. For these reasons, the Strongyloidiasis has been recognized inside of the Tropical Neglected Diseases by the World Health Organization.

Strongyloidiasis is a zoonotic disease produced by *S. stercoralis*, nematode that remains in the ground as part of its life cycle, and it can introduce through the skin, principally, when the person is barefoot. This parasite has been detected in species such as dogs and primates which circumstantially may be transmitted accidentally to humans upon contact with infected faeces (5,6). The disease may remain asymptomatic for a long time, but it may present symptoms such as itchiness or hives by the mobilization through the skin, cough, wheezing and chronic bronchitis in lungs infect stage or abdominal pain and diarrhea in the intestinal infect stage. Also, it may occur an autoinfection in the perianal skin or in the bowel. The adult larvae penetrate the mucosa, mature and produce eggs that enter into lymphatics and general circulation. These are distributing and hatching by all the body causing sepsis in various tissues (7). The infection may potentially be fatal in cases of immunodeficiency, and the diagnosis is developed through

genome amplification in stool samples, serologic tests and direct microscopy. Treatment is carried out using Ivermectin, Tiabendazol and Albendazol (8).

S. venezuelensis and *S. ratti*, which infects rats, present a lifecycle and migration pattern similar to *S. stercoralis*, except that do not exist autoinfection and larvae excretion in faeces. Both parasites are able to develop transmammary transmission in different phases. The most notorious difference among both species is that *S. venezuelensis* is less pathogenic than *S. ratti* because it needs a high larval concentration to present disease, and the larval developing of *S. ratti* is faster, such as we can detect free living mature female and male (9). Thus, *S. venezuelensis* makes it possible to develop inferring studies that would improve the strategies to aboard and control the strongyloidiasis. A detailing study of experimental biological cycle of *S. venezuelensis* optimises the molecular biology analyses, as well as parasite-host interactions, therapeutic assays, and the obtainment of heterologous antigens to develop immunological techniques (10-13).

In this study, we evaluated by microscopic and molecular analyses the *S. venezuelensis* in an experimental life cycle using Wistar rats housed in artificial tropical parameters in an Experimental Bioterium in Quito, Ecuador. The standardization of this parasite as a model to strongyloidiasis made it possible to describe morphological and morphometric characteristics of the parasite during its different stages both inside and outside the host. Additionally, we were able to evaluate the veracity of different diagnosis techniques to detect the parasite in faecal matter, and quantify the progress of the infection when evaluating the presence of eggs in stool.

Materials and methods

Animals

Male Wistar rats (*Rattus novergicus*), obtained from Charles River Laboratories, USA, were maintained in the Experimental Bioterium of the Instituto Nacional de Investigación en Salud Pública Dr. Leopoldo Izquieta Pérez in Quito, Ecuador. They were adapted to 29°C with 35% humidity, with a light-dark cycle of 12/12, at an altitude of 2,850 meters above sea level. For the development of the present study, 8 weeks old and weighed between 120 to 180 g specimens were selected. At the end of the experiment, the rats were sacrificed humanitarianly using deep sedation through the intraperitoneal route, with Xilazin 10mg/kg (Dormi-Xyl®2) Ketamina 60 mg/kg (Ket-A-100®) in combination and cervical dislocation after.

Infection of biomodels

Healthy biomodels were inoculated with 3000 infective L3 larvae (iL3) through a subcutaneous tissue in the inner side of the leg. The inoculated larvae presented a morphology typical of filariform with an acceptable motility to light and viability greater than 95%. These parasites were obtained from the Institute of Biological Sciences, National University of Minas Gerais, Brazil.

The rodents were maintained in a cage with wood chips, *ad libitum* access to water and a food ration based on 15 g/animal/day. On the 5th day post infection (DPI), the rats were transferred to a metabolic cage. The cages ground had 2 strips of absorbent paper moistened with distilled water, over which a wire net with an aperture of 1 cm^2 was placed that it was separating the faeces from the cage floor and the rats which were on the top of the net.

Bronchoalveolar lavage for parasite recovery

For the confirmation of the larval migration in lungs, bronchoalveolar lavage (BAL) was developed in a group of the infected animals on the 2nd DPI. After euthanasia, a dissection was carried out making a 2 cm incision throughout the middle line in the ventral area of the trachea at one-third cm from the entrance to the thorax. An Nº 18 catheter was introduced and fixed with a knot using silk thread. To develop the BAL, 5mL of phosphate buffered saline 1x (PBS) with 0.6 mm ethylenediaminetetraacetic acid (EDTA) was introduced through the catheter. The fluid obtained was transferred to polypropylene tubes placed on ice. The same procedure was repeated until a total volume of 15 mL was collected, and the tubes were centrifuged at 455 g for 15 min at 4°C. The supernatants were discarded, and the pellets were suspended in 3 mL of RPMI at 4°C to then be transferred into 24-well culture tissues plate. The cultured plate was taken to the inverted microscope to verify the presence of *S. venezuelensis* larvae.

Evaluation of eggs in faeces

From the 5th DPI, the faeces of the infected rats, which kept in the cages with dampened paper, were collected daily. Drawing from the pool of faeces, a 5 g previously homogenised sample was taken to count the number of eggs using the Kato-Katz method with a template for 41.7 mg (14,15). The slides were immediately observed with 100x magnification in an optical microscope (Motic, Hong Kong, China) coupled to the software Images Plus 2.0 (Motic, Hong Kong, China) to do the morphometric analyses. The total eggs observed were counted, and the final value was multiplied by 24 to calculate de number of eggs per gram of faeces (EPG).

Stool culture and larvae maturationThe hatching of the eggs and the larval maturing at the first, second and third stages was carried out using a culture of the faeces obtained in each cage. The faeces were mixed with fine-grained vermiculite and sterile water in a 28% of the initial stool weight. This culture was incubated at 28°C.

The collection of the larval stages was carried out according to the Baermann technique (15,16), with some modifications. The stool cultures were wrapped in 6 layers of gauze. Each wrap was suspended over sterile water at 42°C that it arranged in cone-shaped crystal cups. They were maintained idle for 60 minutes for the mobilization by thermotropism of the larvae towards the cup bottom. With a suction pump, three-fourth parts of the supernatant were eliminated, and one-fourth of the remaining parts was homogenised and transferred to a 10 mL test tube. The tubes were centrifuged for 3 minutes at 600 g, and the exceeding liquid was eliminated leaving approximately 2 mL of the supernatant liquid with sediment. The content was prolongedly homogenized, and 2 µL were extended on a slide twice. The slide was visualised with 40x magnification in an optical microscope (Motic, Hong Kong, China) coupled to the software Images Plus 2.0 (Motic, Hong Kong, China) to do the morphometric study.

For the study of the larval stages, the modified Baermann technique was performed to 24, 48, 72 and 96 hours after making the stool culture. The percentage of larval stages was estimated by visual differentiation and the counting of larvae with motility, calculating a percentage relative frequency of each one for the determined time periods.

Analysis of parthenogenetic females

Wistar rats sacrificed humanitarianly were dissectionated in the abdominal cavity, and the duodenum was removed. It was opened lengthwise and carefully chopped. The intestinal tissues were placed over 6 layers of gauze, and the adult larvae was picked up after 3 hours by the modified Baermann method using NaCl saline solution at 0.9% as a medium. Having finished, the result fluid was discharged in tissue culture plates to visualise the presence of adult larvae with 40x magnification in an inverted microscope (Motic, Hong Kong, China) coupled to the software Images Plus 2.0 (Motic, Hong Kong, China) for do the morphological analyses.

Molecular assays

Samples of faeces from infected rats to 1st, 3rd, 4th, 5th, 7th, 8th, 11th, 15th, 21st, 28th and 31st DPI were collected in triplicate. It was conserved in 2.5% potassium dichromate at -80°C until the DNA extraction process. The genomic DNA was extracted using the MagaZorb® DNA Mini-Prep Kit (Promega, Madison, USA) following the protocols described by the manufacturer. The DNA was quantified in a Nanodrop 2000 (Thermo Fisher Scientific, Massachusetts, USA) with absorbance of 260–280 nm. A sample of *S. venezuelensis* eggs confirmed and quantified by Kato-Katz method was used as a positive control. DNA isolate from the tissues of *Ascaris suum, Trichuris trichiura* and *Taenia saginata* were used as a negative control.

The quantitative polymerase chain reaction (qPCR) primers (FW 5´-GAATTCCAAGTAAACGTAAGTCATTAGC-3´, RV 5´-TGCCTCTGGATATTGCTCAGTTC-3´ and FAM –ACACACCGGCCGTCGCTGC-BHQ1) was performed to amplify 101 bp of the 18S rRNA region of *S. stercoralis*

and *S. venezuelensis* (17) in a thermal cycler CFX96 Real-Time PCR Detection System (Bio-Rad, California, USA). Each reaction was prepared in a total volume of 20 µL which it contained 1 µL of the sample, 10 µL of the Master Mix 2X of the kit GoTaq® Probe qPCR Master Mix (Promega, Madison, USA), 18 µM of each primer and 0.8 µM of the FAM fluorophore. The qPCR included an initial denaturation at 95°C for 2 minutes, followed by 40 cycles (95 °C for 3 s, 60°C for 30 s, 72°C for 40 s) and a final elongation step at 72°C for 5 min. The data analysis was carried out using the CFX Manager Software, Version 3.1 (Bio-Rad, California, USA).

The qPCR results were considered negative if the values of the umbral cycle (Ct) were greater than 34 cycles. This value was the detectable limit of the serial dilutions. With these dilutions, the standard qPCR curve was constructed for the molecular quantification of the parasitic load.

Data analysis

Statistical analyses for estimate EPG values respect to days post infection and percentage relative frequency of larval stages in stool culture based to incubation hours were performed using the GraphPad Prism software version 6.01 (GraphPad Software Inc., California, USA).

Ethical Considerations

The methodology established in this study was certified and approved by the Bioterium Platform of the National Institute for Public Health Research Dr. Leopoldo Izquieta Pérez, Ecuador. All the experimental procedures were carried out according to bioethical manuals of experimentation and animal welfare, applying the Principles of the 3 R's, Supervision Protocols, Principle of the 5

freedoms and Criteria for Humanitarian Final Point, taking into account the other recommendations established in the Guide for the Care and Use of Laboratory Animals created by the Institute of Laboratory Animal Resources and the National Research Council, USA.

Results

Experimental life cycle and detection of the parasite in Wistar rats

After the subcutaneous inoculation to the experimental models, the iL3 larvae (figure 1a) migrated through the tissues toward cardiac blood. later, they were observed in the lungs afterward 2nd dpi (figure 1b). These larvae had similar morphology features than the infective stage. Then, the larvae were mobilizing to the trachea during 24 to 48 hours next, and the animal had been swallowing indirectly them through the feeding and hydration. Afterwards, they migrated to the small intestine until 5th DPI for matured to the adult form (figure 1c). in this stage, the parasites were hooked of the bowel tissue, and the female larvae released a large number of eggs by parthenogenesis in the stool (figure 1d).

Using the Kato-Katz technique, parasitic eggs were evidenced as of the 6th DPI, with a peak production in the 8th DPI, to then descend drastically on the 15th DPI (figure 2a) and completely disappearing on the 28th DPI. The molecular detection using qPCR allowed to amplify the DNA of the parasite between 5th DPI to 31st DPI (figure 2b). The eggs peak production was in the 8th DPI like the microscopical data. A positive correlation was found ($r=0.97$) ($p\text{-value} < 0.05$) upon correlating the results reflected by the calculation of the eggs per gram (EPG).

The stool of the infected animals was cultured for the maturation of the eggs throughout the different larval stages. As described in the figure 3, it was possible

to see 79% of L1 larvae, 18% of L2 larvae and a maximum of 3% of iL3 larvae within the first 24 hours of incubation. Continuing, 6% of L1 larvae, 46% of L2 and 48% of iL3 were observed at 48 hours. Next, 4% of L1, 5% of L2 and 91% of iL3 were picked at 72 hours. Finishing at 96 hours, it was possible to find 1% of L2 and 99% of iL3. For these reasons, the best time to recovery infective stage larvae were at the 96 hours of incubated the stool culture, and those larvae were the inoculate to preserved the experimental life cycle.

Eggs morphometric evaluation

The eggs observed in the faeces had different stages of development. In sample of stool, it was possible to differentiate granulated embryo (figure 4a,4c), and larval eggs (figure 4b, 4d) which it presented a larva moving internally. Both phases had an oval shape with symmetric polar points. They presented a thin chitinous cortex with a smooth surface. Additionally, they (n=92) had an average length of 43.22 µm (standard error=0.23 µm) and width of 28.8 µm (standard error=0.15 µm).

Morphometric analysis of larval stages

The L1 larvae (figure 5a) were characterized by having a rounded shape on the front end, followed by a rhabditiform oesophagus, and an intestine that it made up approximately half of the total length. Additionally, a genital primordium was presented in the central segment, and the larvae ended in a pointed tail. They (n=58) were found to be 294.99 µm (standard error=0.23 µm) in length and 17.09 µm in width (standard error=0.16 µm) in the oesophagus-intestine divide.

The change in L2 larvae produced an enlargement of the entire body. L2 larvae (figure 5b) presented a rhabditiform oesophagus which it made up approximately 30% of the length of the parasite, and it was jointed with the intestine. Additionally,

they contained a nervous ring in the central part of the oesophagus, and a genital primordium located in the middle (18,19). L2 larvae (n=29) presented a total length of 429.56 µm (standard error=6.85 µm) and a width in the oesophagus-intestine division of 17.23 µm (standard error=0.29 µm).

Larvae in the third stage or iL3 (figure 5c) presented a rounded front end, followed by a long and filariform oesophagus, which it made up the half of the entire parasite dimension. The oesophagus was connected to the intestine, and they ended in a typically sharpened tail. The L3 (n=84) had a total length of 547.14 µm (standard error=3.74 µm) and width of the oesophagus-intestine division of 18.54 µm (standard error=0.08 µm).

Parthenogenetic females' morphometric study

The adult parasites were found in the mucus of the small intestine of infected rats. Morphologically, the parasitic females (figure 6a) presented a rounded front end with chitinous projections like teeth (figure 6b). A cylindrical filariform oesophagus was observed, which made up approximately one-third of the body length, and it was connected to the intestine that was extended together with the ovary in a spiral shape, throughout the parasite (figure 6c). Additionally, the uterus contained granulated embryo eggs, following the vulva located on the ventral midline of the parasitic body (figure 6d). The tail was sharp, and the anal hole was viewed aside of its terminal area (Figure 6e). The parthenogenic females (n=49), which collected on the 8th DPI, had a total length of 2.67 mm (standard error=26.21 µm) and a width at the oesophagus-intestine division of 29.81 µm (standard error=0.23 µm).

Discussion

In this study, the experimental life cycle of *S. venezuelensis* was modelled in Wistar rats maintained on artificial tropical parameters in the Andean region of Ecuador at 2,850 meters above sea level. Biological cycle features, microscopic and molecular diagnosis analysis comparation, morphometric relations and own characteristics of the species were able to observe during their different stages both inside and outside the host.

The methods of parasitic detection detailed in this article could be used for the diagnosis of parasite homologous like *S. stercoralis* in humans. Besides, it exists other qualitative tests such as direct swabbed and coproparasitic with lugol staining, and quantitative assays such as McMaster quantification, serodiagnosis tests (ELISA, IFAT and immunoblot) or molecular amplification (10,11,20-22). The Kato-Katz method was used as an immediate quantitative measurement technique, given that the *S. venezuelensis* eggs present a thin chitinous membrane, easily degradable, common in the Rhabditidae family (8,23). Using this technique, it was possible to observe eggs in the faeces as of the 6th DPI, reaching a maximum peak on the 8th DPI and a progressive reduction since 9th to 28th DPI which presented a total absence. These data agree with other research where a peak exists between 6th to 8th DPI, with a duration in the expulsion of eggs of less than one month (10,11,22). The reduction observed in egg production as of the 9th DPI may be attributed to the action of the immune system of the host, whereby it is possible to highlight the activity of the eosinophils present in the mucosa of the intestine (24); lymphocytes B (25), and the activity of the mastocytes activated by cytokine stimulus, such as IL-3, IL-9, IL-18 and immunoglobulins IgE & IgG (26). In

addition, it could not be observed out a subclinical coinfection with *Syphacia muris* in any evaluation day. The parasite mentioned is an oxyurid nematode that occurs in the gastrointestinal tract of rats, normally (22).

qPCR test was used to amplify a specific region of the 18s rRNA gene for the *Strongyloides* genus. Although, the Kato-Katz technique did not manage to observe eggs on the 5th DPI, the results observed by qPCR at this point indicated a small increase (ΔCt=3.68 equal to 1.71 EPG) compared to the negative control. Besides, although the shape of the production curve of the eggs by qPCR was similar to the obtained by Kato-Katz technique, the concentrations calculated by qPCR were markedly higher, especially on the umbral days (7th and 8th DPI). These values could be explained by the diagnostic potential between microscopical and molecular tests. Moreover, immature parasites could be detected in the small intestine from 60 hours of infection (27) where presumably maturated into adult parasites in a progressive manner, such as the one seen for the in vitro larvae production. While in this study the 6th DPI was not analysed using qPCR, the microscopic observation of eggs at this point confirmed the presence of the parasite, and the establishment of a biological cycle in the infected animals. However, on the last day of the study (31st DPI), a small increase was observed in the relative quantity of eggs, compared to the previous point (28th DPI). This may have indicated that the adult parasites housed in the intestine slightly increased the oviposition, but not until the necessary limit so as to be detected by direct microscopy. Or, at the same time, the detected levels could have corresponded to the adult parasites that were eliminated in the faeces, a factor attributed to the immune response by increasing the contraction of the intestinal walls, favouring

their removal (28). Given that this study analysed the dynamic of the biological cycle until 31st DPI, it would be interesting to see the results of the analysis by qPCR after this point, to determine the minimum possible amplification levels. While it is true that the Kato-Katz method was less responsive than the qPCR, its ease of implementation, low cost, and the fact that it does not require sophisticated equipment which they are factors that allow its daily use in simple laboratories. Additionally, since this technique showed a high potential of use as a screening test for the diagnosis of different nematodes (29,30) and schistosomiasis (15), it could be used in vulnerable areas that do not have the technological capacity or sufficient resources.

On the other hand, it was possible to study the morphologic changes of *S. venezuelensis* stages during the experimental life cycle previously described. *In vivo*, the iL3 followed a typical migration where could be penetrated blood vessels after subcutaneous administration (2,9). After, iL3 was found in lung fluid through bronchoalveolar lavage developed on the 2nd DPI, and as such, it was confirmed that iL3 reached the alveoli, breaking the capillary membrane and bronchial epithelium. Due to this aggregation, the larvae caused small haemorrhages, known as pulmonary petechiae (18). Subsequently, they mobilized toward outside of the organism across the trachea until the pharynx where the larvae were swallowed involuntary by the animals in the food and water. Then, they migrated typically across the digestive tract and maturated into adult parasites in the duodenal mucosa and the upper part of the jejunum. The eggs were produced via parthenogenesis by the female larvae, and they were expelled in the intestinal content (2,9,31). Thus, the migration of *S. venezuelensis* was comparable to other

parasites of Secernentea class and similar to other species of *Strongyloides* genus, to some extent since, the most important difference between *S. stercoralis* and *S ratti* was the capability to develop infective larvae in the large intestinal, which will be able to disseminate and create autoinfection and/or hyper infections in the same small intestine or other organs (7,9). *Ex vivo*, the production of larvae in vitro was related to the number of EPG of faeces, for which a larger obtainment of larvae with stool samples was found on the 8th DPI. The larval development was asynchronous before the 96 hours of stool culture incubation due to progressive maturation of eggs produced by adult females (20). At that point, it was possible to find a greater proportion of L3 larvae, and it became an ideal collection point of infective larvae that may be used in different studies.

In conclusion, this study describes the implementation of an experimental model of *S. venezuelensis* in a manageable and reproducible system. The biological cycle standardized creates a study tool for parasitic biology, toxicology, host-parasite interactions and development of new technologies or therapies for the boarding of strongyloidiasis or other regional impetus helminthiasis caused by nematodes.

Acknowledgments

The authors would like to thank Dr. Alan de Melo (Departamento de Parasitologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, Brazil) for his support and kind donation of *S. venezuelensis* iL3 larvae for this study. To the colleagues from Programa Nacional para el Abordaje Multidsciplinario de las Parasitosis Desatendidas en el Ecuador (PROPAD), who have contributed to the accomplishment of this project. To José Manuel Pico, specialist of Bioteriums from INSPI LIP, for his revision and support in all

procedures in the context of care and use of laboratory animals. In addition, we would like to express our gratefulness to Karen Llerena for the revision of this article.

Conflict of interest

None

Financial Support

This study was supported by the Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT), Ecuador (grant number 20130372, 2013).

References

1. Buonfrate D, Mena MA, Angheben A, Requema-Mendez A, Muñoz J, Gobbi F, *et al*. Prevalence of strongyloidiasis in Latin America: a systematic review of the literature. Epidemiol Infect. 2015;143:452-60. https://doi.org/10.1017/S0950268814001563
2. Viney ME, Lok JB. The biology of *Strongyloides* spp. Wormbook: The Online Review of *C. Elegans* Biology. 2015:1-17. Fecha de consulta: incluir día, mes y año. https://doi.org/10.1895/wormbook.1.141.2
3. Jacobsen KH, Ribeiro PS, Quist BK, Rydbeck BV. Prevalence of intestinal parasites in young Quichua children in the highlands of rural Ecuador. J Health Popul Nutr. 2007;25:399-405.
4. Reyes-Chacón JA. Evaluación del RT-PCR en el diagnóstico de 6 parásitos intestinales en un área con parasitismo de baja intensidad en el Trópico, Ecuador. Quito: Universidad San Francisco De Quito; 2012.

5. Júnior AF, Gonçalves-Pires MR, Silva DA, Gonçalves ALR, Costa-Cruz JM. Parasitological and serological diagnosis of *Strongyloides stercoralis* in domesticated dogs from southeastern Brazil. Vet Parasitol. 2006;136:137-45. https://doi.org/10.1016/j.vetpar.2005.10.022
6. Mati VLT, Raso P, de Melo AL. *Strongyloides stercoralis* infection in marmosets: replication of complicated and uncomplicated human disease and parasite biology. Parasit Vectors. 2014;7:579. https://doi.org/10.1186/s13071-014-0579-2
7. Nutman TB. Human infection with *Strongyloides stercoralis* and other related *Strongyloides* species. Parasitology. 2017;144:263-73. https://doi.org/10.1017/S0031182016000834
8. Ruano AL. Óxido nítrico como modulador de la estrongiloidosis. Salamanca: Universidad de Salamanca; 2008.
9. Viney M, Kikuchi T. *Strongyloides ratti* and *S. venezuelensis* - rodent models of *Strongyloides* infection. Parasitology. 2017;144:285-94. https://doi.org/10.1017/S0031182016000020
10. Chaves LA., Gonçalves ALR, Paula FM, Silva NM, Silva CV, Costa-Cruz JM, *et al*. Comparison of parasitological, immunological and molecular methods for evaluation of faecal samples of immunosuppressed rats experimentally infected with *Strongyloides venezuelensis*. Parasitology. 2015;142:1715-21. https://doi.org/10.1017/S0031182015001298
11. Marques PD, Malta FM, Meisel DMCL, Corral MA, Pinho JR, Costa-Cruz JM, *et al*. Diagnosis of the strongyloid nematode *Strongyloides venezuelensis* in

experimentally infected rats. J Helminthol. 2016;90:422-7. https://doi.org/10.1017/S0022149X15000528

12. Viney M. Strongyloides. Parasitology. 2017;144:259-62. https://doi.org/10.1017/S0031182016001773
13. Bosqui LR, Marques PD, de Melo GB, Gonçalves-Pires MR, Malta FM, Pavanelli WR, *et al*. Molecular and immune diagnosis: further testing for human strongyloidiasis. Mol Diagn Ther. 2018;22:485-91. https://doi.org/10.1007/s40291-018-0340-1
14. World Health Organization. Medios Auxiliares para el Diagnóstico de las Parasitosis Intestinales. Ginebra: WHO; 1994.
15. Girard de Kaminsky R. Métodos para laboratorios de atención primaria de salud. 2nd ed. Tegucigalpa: Universidad Nacional Autónoma de Honduras y Hospital-Escuela; 2003.
16. Rugai E, Mattos T, Brisola AP. A new technic for the isolation of nematode larvae from faeces; modification of Baermann's method. Rev Inst Adolfo Lutz. 1954;14:5-8.
17. Verweij JJ, Canales M, Polman K, Ziem J, Brienen EAT, Polderman AM, *et al.* Molecular diagnosis of *Strongyloides stercoralis* in faecal samples using real-time PCR. Trans R Soc Trop Med Hyg. 2009;103:342-46. https://doi.org/10.1016/j.trstmh.2008.12.001
18. Beaver PCh, Jung RC, Cupp EW. Rhabditida: *Strongyloides* y formas relacionadas. Parasitología clínica de Craig Faust. 3rd ed. México DF: Masson Doyma S. A.; 2003.
19. Mehlhorn H. Encyclopedia of parasitology. 3rd ed. New York: Springer. 2008.

20. Marra NM, Chiuso-Minicucci F, Machado GC, Zorzella-Pezavento SF, França TG, Ishikawa LL, *et al*. Migratory route of *Strongyloides venezuelensis* in Lewis rats: comparison of histological analyses and PCR. Exp Parasitol. 2011;127:334-9. https://doi.org/10.1016/j.exppara.2010.08.006
21. Gonçalves ALR, Silva CV, Ueta MT, Costa-Cruz JM. Antigen, antibody and immune complex detection in serum samples from rats experimentally infected with *Strongyloides venezuelensis*. Exp Parasitol. 2012;130:205-8. https://doi.org/10.1016/j.exppara.2012.01.007
22. Paula FM, Sitta RB, Malta FM, Gottardi M, Corral MA, Gryschek RCB, *et al*. Parasitological and molecular diagnosis in experimental *Strongyloides venezuelensis* infection. Rev Inst Med Trop São Paulo. 2013;55:141-3. https://doi.org/10.1590/S0036-46652013000200015
23. Vieira FM, Lima SS, Bessa EC. Morfologia e biometria de ovos e larvas de S*trongyloides* sp. grassi, 1879 (*Rhabditoidea: Strongyloididae*) parasito gastrointestinal de *Hydrochaeris hydrochaeris* (Linnaeus, 1766) (Rodentia: Hydrochaeridae), no Município de Juiz de Fora, Minas Gerais. Rev Bras Parasitol Vet. 2006;15:7-12.
24. El-Malky M, Maruyama H, Hirabayashi Y, Shimada S, Yoshida A, Amano T, *et al*. Intraepithelial infiltration of eosinophils and their contribution to the elimination of adult intestinal nematode, *Strongyloides venezuelensis* in mice. Parasitol Int. 2003;52:71-9. https://doi.org/10.1016/s1383-5769(02)00086-7
25. El-Malky MA, Maruyama H, Al-Harthi SA, El-Beshbishi SN, Ohta N. The role of B-cells in immunity against adult *Strongyloides venezuelensis*. Parasit Vectors. 2013;6:148. https://doi.org/10.1186/1756-3305-6-148

26. Sasaki Y, Yoshimoto T, Maruyama H, Tegoshi T, Ohta N, Arizono N, *et al*. IL-18 with IL-2 protects against *Strongyloides venezuelensis* infection by activating mucosal mast cell-dependent type 2 innate immunity. J Exp Med. 2005;202:607–16. https://doi.org/10.1084/jem.20042202
27. Takamure A. Migration route of *Strongyloides venezuelensis* in rodents. Int J Parasitol. 1995;25:907-11. https://doi.org/10.1016/0020-7519(95)00014-s
28. Wynn TA. IL-13 effector functions. Annu Rev Immunol. 2003;21:425-56. https://doi.org/10.1146/annurev.immunol.21.120601.141142
29. Gabrie JA, Rueda MM, Canales M, Sánchez A. Utilidad del método Kato-Katz para diagnóstico de uncinarias: experiencia en una zona rural de Honduras, 2011. Rev Med Hondur. 2012;80:96-101.
30. Easton AV, Oliveira RG, Walker M, O'Connell EM, Njenga SM, Mwandawiro ChS, *et al*. Sources of variability in the measurement of *Ascaris lumbricoides* infection intensity by Kato-Katz and qPCR. Parasit Vectors. 2017;10:256. https://doi.org/10.1186/s13071-017-2164-y
31. Gonçalves ALR, Silva CV, Carvalho EFG, Ueta MT, Costa-Cruz JM. Transmammary transmission of strongyloidiasis in immunosuppressed rats. Neotrop Helminthol. 2013;7:195-200. https://doi.org/10.2307/3285443

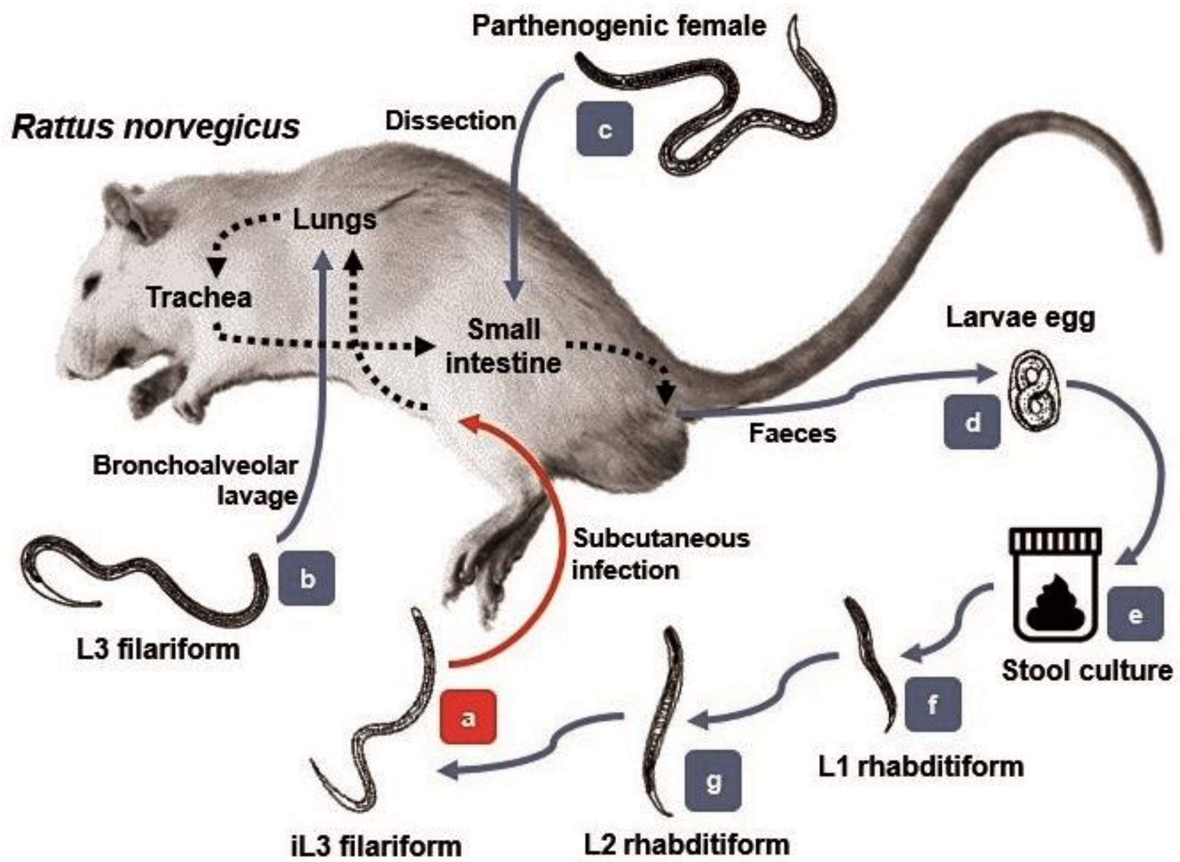

Figure 1. Experimental life cycle of *S. venezuelensis* in Wistar rat, *R. norvegicus.*

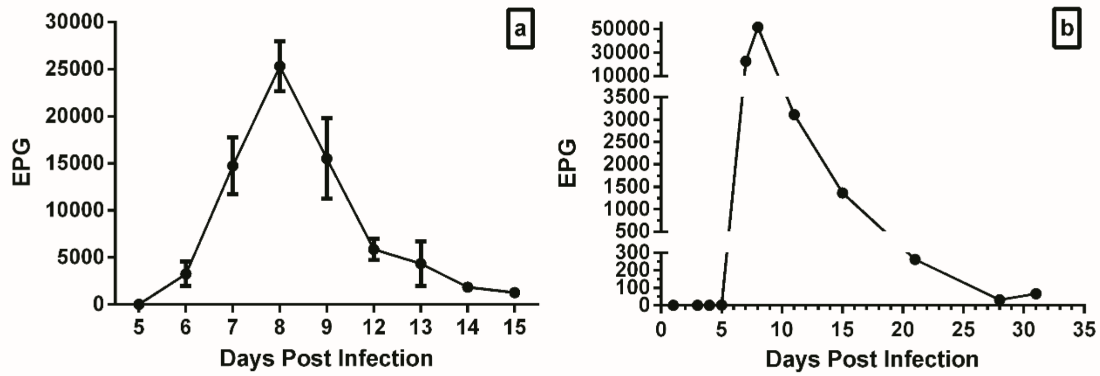

Figure 2. EPG values of *S. venezuelensis* respect to days post infection. a: Kato-Katz technique analysis (Average ± Standard Error, n=18), b: qPCR assay results (EPG: Eggs per gram of faeces).

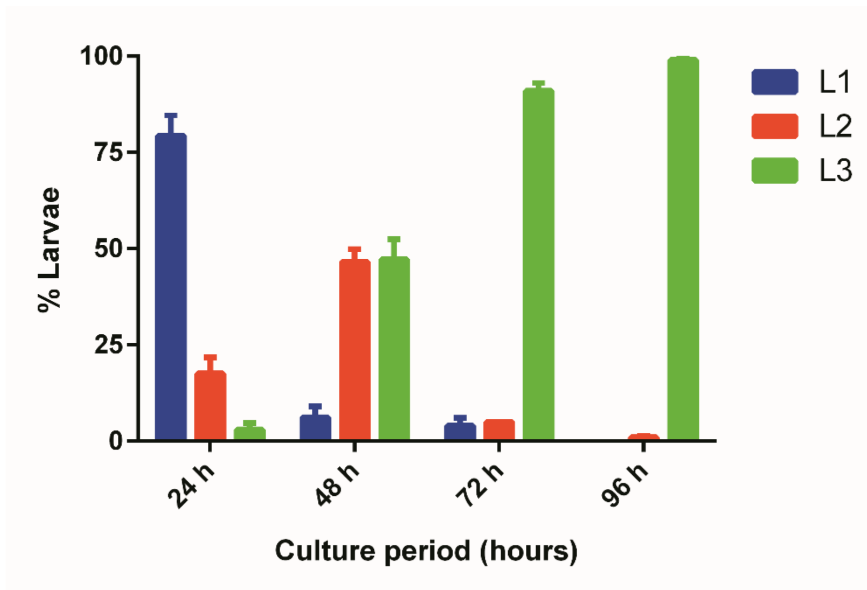

Figure 3. Bar graph of percentage relative frequency of larval stages of *S. venezuelensis* in stool culture based to incubation hours.

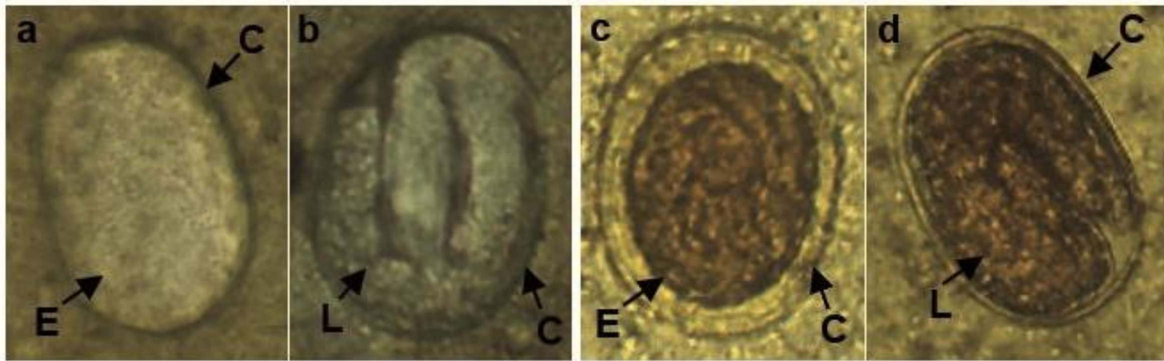

Figure 4. Development stages of *S. venezuelensis* eggs obtained in faeces of infected Wistar rats. a,c: Granulated embryo eggs, b,d: Larval eggs, a,b: without staining, c,d: lugol staining (E: embryo, C: cortex, L: larva).

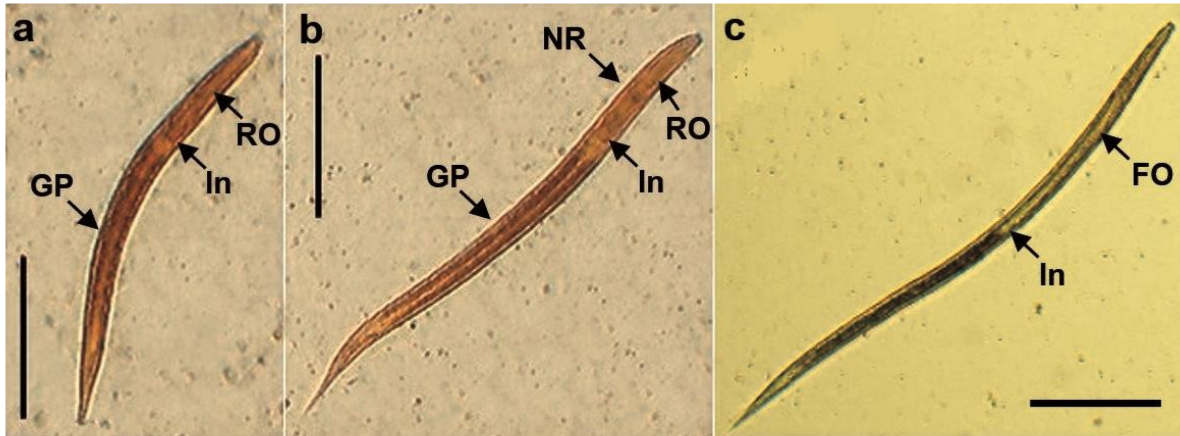

Figure 5. Stages of *S. venezuelensis* larvae obtained through culture of infected Wistar rats' stool. a: First stage larva (L1), b: Second stage larva (L2), c: Third stage larva (iL3). Scale bars: 100 μm (RO: rhabditiform oesophagus, FO: filariform oesophagus, NR: nervous ring, In: Intestine, GP: genital primordium).

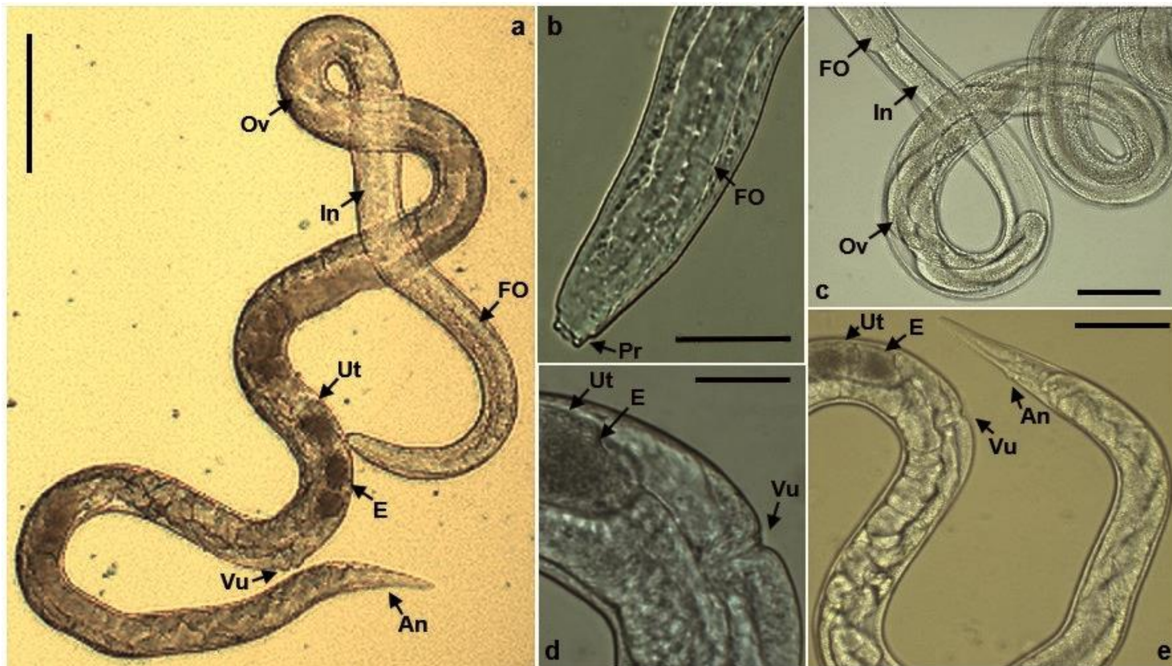

Figure 6. Parthenogenic female of *S. venezuelensis*. a: Entire view (Scale bars: 100 µm), b: head portion approach (Scale bars: 20 µm), c: middle portion view (Scale bars: 50 µm), d: sexual portion approach (Scale bars: 20 µm), e: last portion view (Scale bars: 50 µm) (FO: filariform oesophagus, In: Intestine, Ov: ovary, Ut: uterus, E: egg, Vu: vulva, An: anus, Pr: projections).