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Male Swiss mice (*Mus musculus*) as a most suitable experimental model for the study of *Giardia duodenalis* BIV

Camundongo (*Mus musculus*) Suíço macho como melhor modelo experimental para estudo de *Giardia duodenalis* BIV

Ratón (*Mus musculus*) Suizo macho como mejor modelo experimental para el estudio de *Giardia duodenalis* BIV

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Abstract
In this study, we proposed to verify the most suitable murine experimental model for studying human giardiasis. In total 150 animals were used. Fifty mice (*Mus musculus*) from each lineage (Swiss, Balb/c and C57BL/6), 25 females and 25 males, were divided into 5 groups with 5 animals each, according to the lineage/sex. Three groups were infected with 10^4 cysts of *Giardia duodenalis* of assemblage BIV and 2 negative control groups. The animals were followed and evaluated for 15 days after receiving the inoculum. The clinical parameters evaluated were body weight, water and feed intake, excretion, appearance of fur and feces, elimination of *Giardia* spp cysts and behavioral assessment. The clinical parameters of the groups infected with *G. duodenalis* were compared with the non-infected groups within their own lineage/sex. In the 15 days of monitoring, only the male Swiss mice presented differences in these parameters. The infected animals consumed more feed, water and eliminated more excreta than the non-infected group. There was no difference in the general average of the weight of the animals or in the behavioral assessment in any group. Only the infected male Swiss mice eliminated *G. duodenalis* cysts in the feces, which was confirmed by the molecular diagnosis and by observing the presence of trophozoites in the intestinal mucosa. The results demonstrate that the most suitable animal model for the study of human giardiasis is the male Swiss mice, since it was the only one capable of developing infection by *G. duodenalis* cysts.
Keywords: Balb/c; C57BL/6; Experimental model; *Giardia duodenalis*; Giardiasis.

Resumo
Neste trabalho foi proposto verificar o modelo experimental murino mais adequado para o estudo da giardíase humana. No total, 150 animais foram utilizados. Cinquenta camundongos (*Mus musculus*) de cada linhagem (Suíços, Balb/c e C57BL/6), 25 fêmeas e 25 machos, foram divididos em 5 grupos com 5 animais cada, de acordo com a linhagem/sexo. Três grupos foram infectados com 10^4 cistos de *Giardia duodenalis* da *assemblage* BIV e 2 grupos controle negativo. Os animais foram acompanhados e avaliados durante 15 dias após receberem o inóculo. Os parâmetros clínicos avaliados foram peso corporal, ingestão de água e ração, excreção, aspecto dos pelos e fezes, eliminação de cistos de *Giardia* spp e avaliação comportamental. Os parâmetros clínicos dos grupos infectados com *G. duodenalis* foram comparados com os grupos não-infectados de sua própria linhagem/sexo. Nos 15 dias de acompanhamento, apenas os camundongos Suíços machos apresentaram diferenças nesses parâmetros. Os animais

infectados consumiram mais ração, água e eliminaram mais excretas do que o grupo não-infectado. Não houve diferença na média geral do peso dos animais ou na avaliação comportamental em nenhum grupo. Apenas os camundongos Suíços machos infectados eliminaram cistos de *G. duodenalis* nas fezes, confirmado pelo diagnóstico molecular e pela observação da presença de trofozoítos na mucosa intestinal. Os resultados demonstram que o modelo animal mais adequado para o estudo da giardíase humana é o camundongo Suíço macho, uma vez que foi o único capaz de desenvolver infecção por cistos de *G. duodenalis*.
Palavras-chave: Balb/c; C57BL/6; Modelo experimental; *Giardia duodenalis*; Giardíase.

Resumen

En este estudio nos propusimos verificar el modelo experimental murino más adecuado para el análisis de la giardiasis humana. En total se utilizaron 150 animales. Cincuenta ratones (*Mus musculus*) de cada linaje (Suizos, Balb/c y C57BL/6), 25 hembras y 25 machos, se dividieron en 5 grupos con 5 animales cada uno, según el linaje/sexo. Se infectaron tres grupos con 10^4 quistes de *Giardia duodenalis* del *assemblage* BIV y 2 grupos control negativo. Los animales fueron seguidos y evaluados durante 15 días después de recibir el inóculo. Los parámetros clínicos evaluados fueron peso corporal, consumo de agua y comida, excreción, aparición de pelo y heces, eliminación de quistes de *Giardia* spp y valoración del comportamiento. Los parámetros clínicos de los grupos infectados por *G. duodenalis* se compararon con los de los grupos no infectados dentro de su propio linaje/sexo. En los 15 días de seguimiento, solo los ratones Suizos machos presentaron diferencias en estos parámetros. Los animales infectados consumieron más comida, agua y eliminaron más excrementos que el grupo no infectado. No hubo diferencia en el promedio general del peso de los animales o en la evaluación del comportamiento en ningún grupo. Solo los ratones suizos machos infectados eliminaron los quistes de *G. duodenalis* en las heces, lo que se confirmó mediante el diagnóstico molecular y al observar la presencia de trofozoítos en la mucosa intestinal. Los resultados demuestran que el modelo animal más adecuado para el estudio de la giardiasis humana es el ratón Suizo macho, ya que es el único capaz de desarrollar la infección por quistes de *G. duodenalis*.
Palabras clave: Balb/c; C57BL/6; Modelo experimental; *Giardia duodenalis*; Giardiasis.

1. Introduction

The etiologic agent of giardiasis, *Giardia duodenalis* (synonyms: *Giardia lamblia* and *Giardia intestinalis*) is one of the most prevalent protozoa in the human gastrointestinal tract (Colli et al., 2015; Hooshyar et al., 2019), and the most associated with cases of infectious diarrhea (Cock et al., 2020), which are an important cause of morbimortality in children under five years old (Baker & Alonso, 2018). Transmission is via the fecal-oral route and occurs mainly by accidental ingestion of cysts in contaminated water and food, being favored by the absence of water and sewage treatment systems, and by the conglomerate of people (Fantinatti et al., 2016).

Giardiasis has a global distribution, reaching an average of between 2 and 7% of the population in developed countries, and might reach 30% in developing or underdeveloped countries (Fantinatti et al., 2016), totaling more than 200 million cases diagnosed by year (Hooshyar et al., 2019). It can be asymptomatic or symptomatic with chronic diarrhea and intestinal malabsorption, leading to disorders in growth and intelectual/cognitive development in children (Lima et al., 2019).

Until now, eight genetic assemblages of *G. duodenalis* (A–H) have been described (Thompson et al., 2000; Lasek-Nesselquist et al., 2010; Feng & Xiao, 2011; Colli et al., 2015, Qi et al., 2020). Assemblages A and B are considered zoonotic and are responsible for the majority of known human cases of the disease (Matsuchita et al., 2017). The assemblage A can be subdivided into subassemblages AI and AII, and assemblage B can be subdivided into BIII and BIV. In human infection, some authors report that assemblage B is the most commom (Cacciò & Ryan, 2008; Feng & Xiao, 2011). In the southern region of Brazil, there is a predominance of the assemblage BIV (Colli et al., 2015).

Due to its unique and remarkable cellular characteristics, *G. duodenalis* has been intensively studied (Mayol et al., 2019); however, it is still unclear which is the most suitable experimental murine model for the study of this pathology. Rodents are the most used experimental models in scientific research (Chorilli et al., 2007; Guénet, 2011). In general, for the study of human pathologies, mice of the species *Mus musculus* are predominant (Ehret et al., 2017), due to their great genotypic homology and physiological similarities to human beings. These animals are susceptible to infections (Pavanelli et al., 2018), are easy to maintain and observe, have a high reproduction rate and have a large amount of basic information

available (Chorilli, et al., 2007).

Due to their different genotypes, the lineages of experimental animals can behave differently when infected by the same pathogen (Ehret et al., 2017). Among the lineages of *Mus musculus* species, the following stand out: Swiss, heterogeneous animals, resulting from random mating, in which each animal from the same colony responds in a variable way when subjected to the same experiment; Balb/c and C57BL/6, isogenic animals, resulting from inbreeding crosses with a homozygosity index of 99%, which makes the animals from the same colony to respond similarly to the same experiment (Massinori, 2009). In this context, the aim of our study was to suggest, among Swiss, Balb/c and C57BL/6 mice, the most suitable animal model for the study of human giardiasis, based on the evaluation of parasitological, molecular and clinical parameters.

2. Methodology

An unprecedented experimental animal study was carried out. In total 150 animals were used. Fifty mice (*Mus musculus*) from each lineage (Swiss, Balb/c and C57BL/6), 25 females and 25 males, were divided into 5 groups with 5 animals each, according to the lineage/sex. The experiment consisted of groups of control animals and groups of infected animals. Three groups were infected with 10^4 cysts of *Giardia duodenalis* of assemblage BIV and 2 negative control groups. The variables observed were: initial parasitological and molecular evaluation, to ensure that the animals were free of previous infections, and monitoring of the pre-patency period - period ranging from inoculation of the cysts to the development of infection with elimination of the cysts in the feces, in which quantitative parameters (body weight, water and feed, intake and elimination of excreta) and qualitative (appearance of fur and feces), elimination of cysts in feces and behavioral testing were evaluated. All variables from the groups of infected animals were compared with the negative control groups according to their lineage/sex. The experimental groups, the steps and chronology of the experiments are described in the following sub-items.

2.1 Ethical aspects

This study was approved by the Ethics Committee on the Use of Animals (CEUA/UEM) of the State University of Maringá - Brazil (process number 9375170816) and all the guidelines of the Brazilian Society of Sciences in Laboratory Animals were followed.

2.2 Animals

Were used 150 mice (*Mus musculus*), 50 from each lineage (Swiss, Balb/c and C57BL/6), being 25 females and 25 males, 21 days old, with no statistical difference in the average weight of the animals in each group. The Swiss and Balb/c animals were from the Central Vivarium of the State University of Maringá (Universidade Estadual de Maringá - UEM) and the C57BL/6 mice were from the Central Vivarium of the State University of Western Paraná (Universidade Estadual do Oeste do Paraná - Unioeste).

The animals were maintained in the vivarium of the Environmental and Food Parasitology Laboratory at the State University of Maringá (LPAA/UEM) and placed into micro-acclimated cages (Alesco®; polysulfone cages of 20 cm width x 32 cm length x 21 cm height). During the experiments, there was control of temperature (22.7 ± 1.2 ºC), 12h/12h light/dark cycle, with filtered/boiled water and feed (Nuvital® Nuvilab Cr-1) offered *ad libitum*.

2.3 Experimental groups

The mice were distributed into 30 groups with five animals each, according to the lineage and sex. The experiments were conducted in three different periods, as described in Table 1.

Table 1. Experiment number and experimental groups according to sex and lineage of animals.

Experiment	Groups	Sex	Lineage
1	**Infected animals** MS1, MS2 e MS3 **Negative control*** MS4 e MS5	Male (N = 25)	**Swiss**
	Infected animals FS1, FS2 e FS3 **Negative control*** FS4 e FS5	Female (N = 25)	
2	**Infecteds animals** MB1, MB2, MB3 **Controle negativo*** MB4 e MB5	Male (N = 25)	**Balb/c**
	Infected animals FB1, FB2, FB3 **Negative control*** FB4 e FB5	Female (N = 25)	
3	**Infected animals** MC1, MC2, MC3 **Negative control*** MC4 e MC5	Male (N = 25)	**C57BL/6**
	Infected animals FC1, FC2, FC3 **Negative control*** FC4 e FC5	Female (N = 25)	

*Negative control for *Giardia duodenalis* infection;
MS: Male Swiss mice; FS: Female Swiss mice; MB: Male Balb/c mice; FB: Female Balb/c mice; MC: Male C57BL/6 mice; FC: Female C57BL/6 mice; N: numbers of animals.
The experiments were divided according to mouse lineage. Each group consisted of 5 animals, totaling 25 for each lineage/sex. The infected groups received inoculum of 10^4 cysts of *G. duodenalis*, whereas the negative control groups only received saline solution.
Source: Authors.

2.4 Parasitological evaluation and molecular diagnosis

Feces from each animal were collected individually. The samples were processed by the method of Faust et al. (1938), in order to analiser the presence of pathogens in the intestinal tract, such as, cysts of *Giardia muris*. The analysis were performed for three consecutive days taking into account the intermittent elimination of cysts in the feces, to verify the absence or presence of *Giardia*.

A pool of fecal samples (1 g) from each group was processed using the modified Ritchie method (Bezagio et al. 2020). The recovered *Giardia* cysts were quantified in a Neubauer chamber, in triplicate, and the results were obtained by calculating the simple average. The samples were classified as low, medium or high parasitic load according to the standards established by Uda-Shimoda et al. (2014).

The presence of *G. muris* was also investigated through Polymerase Chain Reaction (PCR) using primers G18S2 and G18S3, which amplify a fragment of 470 base of pairs (bp) of the 18S ribosomal RNA gene, according to Monis et al. (1999): 94°C for 2 minutes; 35 cycles 94°C for 30 seconds; 55°C for 30 seconds; 72°C for 1 minute; ending with 55°C for 7 minutes. The amplified product was visualized in polyacrylamide gel (4.5%) and revealed by silver salts.

The animals with positive parasitological/molecular results for *G. muris* (including negatives control groups) were etiologically treated according to the protocol established by Bezagio et al. (2017): Fembendazole 50 mg/kg - 1× only on the first day and metronidazole 500 mg/kg - 1×/day/7 days. The treatment was administered intragastrically using a gavage probe.

The materials which came into contact with the animals were sterilized daily and the animals were maintained in micro-acclimated cages on an acrylic support with holes that avoided contact with their own feces.

After the end of treatment, feces samples were individually collected for analysis during three consecutive days. Animals with negative results by the method of Faust et al. (1938) and by the molecular method were considered cured.

2.5 Inoculum and infection with cysts of *G. duodenalis*

The cysts of *G. duodenalis* used in this study were isolated from recently eliminated human fecal samples, with approval from the Research Ethics Committee Involving Human Beings (COPEP / UEM-439/2009), which were genotyped by PCR-RFLP as assemblage BIV and preserved at - 20 ºC in the LPAA/UEM.

In order to determine the viability of *G. duodenalis* cysts, the Trypan Blue test was performed. This dye at 0.4% was added to a concentrate of cysts previously quantified in a 1:1 ratio (Strober, 2015).

The inoculum was prepared with 0.85% saline solution so that the concentration was 10^4 viable *G. duodenalis* cysts (Nakada et al. 2018) in a volume of 300 µl/animal. The animals in the negative-control group received the same volume (300 µl/animal) of saline solution. Administration was performed intragastrically, using a gavage probe.

2.6 Monitoring of pre-patent period

2.6.1 Assessment of clinical signs

During the 15 days following the infection, the animals were monitored and evaluated daily for the parameters: quantitative (body weight, water and feed intake and elimination of excreta) and qualitative (appearance of fur and feces).

2.6.2 Elimination of cysts

A pool of feces samples from each group of infected animals were daily collected and processed by the method of Faust et al. (1938), until cysts of *Giardia* spp. were observed by microscopy and positive molecular method, or during the 15 days following infection.

DNA was extracted from the cysts, using the fragment kit PureLink PCR Purification® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations and Uda-Shimoda et al. (2014). The glutamate dehydrogenase gene (GDH) was amplified by the Semi-Nested PCR method with modifications proposed by Colli et al. (2015), for identification of *G. duodenalis*. The external forward primer GDHeF, internal forward primer GDHiF and reverse primer GDHiR were used to amplify a fragment of approximately 432 bp. The amplified product was visualized in polyacrylamide gel (4.5%) and revealed with silver salts.

2.6.3 Behavioral testing - Open Field Test (OFT)

It was performed on the 7th day postinfection (dpi) (Pavanelli et al., 2018). The open field consisted of a circular arena of 30 cm in diameter surrounded with opaque walls of 30 cm high, composed of a central area of 15 cm in diameter divided into four quadrants and a peripheral area divided into eight quadrants. Individually, the animals were placed in the center of the arena and freely explored the open field for a period of 5 minutes.

The sessions were recorded on video (Webcam LifeCam Cinema HD 720p Microsoft® using Microsoft LifeCam software version 3.22) and blindly analyzed using Open Field software. The analysis included central locomotion, peripheral locomotion, total locomotion and time spent in the central region and time spent in the peripheral region.

2.7 Necropsy and assessment of intestinal mucosa

After evaluating the pre-patent period, all mice were anesthetized with inhalable isuflurane and euthanized by craniocervical dislocation. The small intestine of two infected animals from each group, randomly chosen, was removed by opening the peritoneal cavity with a median insertion and washed with 0.85% saline. The intestines were longitudinally sectioned, dipped into 25 ml of 0.85% saline and scraped using a glass slide. A portion of fresh sample was examined by Direct Microscopy for searching of trophozoites in the intestinal mucosa.

2.8 Statistical analysis

The data were tabulated and analyzed using the GraphPad Prism software version 7.0 (GraphPad Software Inc., La Jolla, CA, USA). The analysis was performed by bidirectional ANOVA. When statistical differences were identified by two-way ANOVA, Bonferroni's post hoc test was performed to assess specific differences between the experimental groups. A *p*-value was considered significant when $p < 0.05$.

3. Results

In the experiment #01, all 50 Swiss mice (100%), male and female, presented positive results for *G. muris* at the beginning, classified as high parasite load (more than 2 cysts/field on the 20x objective) as confirmed by amplification the minor ribosomal subunit gene (G18S). After the etiological treatment, only the male Swiss animals achieved the cure criteria, in which *G. muris* cysts were not observed in the parasitological examination and amplification of the parasite's DNA by the molecular method did not occur. In the experiments #02 and #03, the Balb/c and C57BL/6 mice had negative parasitological results for the three days of analysis. In the three experiments, the results obtained by PCR confirm 100% of the results obtained by the parasitological exams performed initially, as shown in Table 2.

Table 2. Result of parasitological and molecular analysis for *G. muris* in an animal stool sample pool for three consecutive days.

Animals	**Parasitological[1]**			**Molecular[2]**		
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
Female Swiss	+	-	+	+	+	+
Female Balb/c	-	-	-	-	-	-
Female C57BL/6	-	-	-	-	-	-
Male Swiss	+	+	+	+	+	+
Male Balb/c	-	-	-	-	-	-
Male C57BL/6	-	-	-	-	-	-

[1]Microscopic visualization of *G. muris* cysts in stool samples; [2]Amplification a 470 bp fragment of the 18S ribosomal RNA gene;
(+): positives samples; (-): negative samples.
Female Swiss stools eliminated *Giardia* cysts on days 1 and 3, and PCR positive on three consecutive days. Male Swiss eliminated stool cysts and positive PCR during the three days. The other groups did not eliminate cysts and were not PCR positive.
Source: Authors.

During the monitoring of pre-patent period, male Swiss groups (MS1 and MS2) started to eliminate *Giardia* cysts in the feces on the 6th dpi and MS3 group, on the 8th dpi, whereas MS4 and MS5 groups (negative controls) did not eliminate cysts in any of the days. In the five groups of female Swiss mice (FS1, FS2, FS3, FS4 and FS5), *Giardia* cysts were observed every day during the monitoring. No elimination of cysts was observed in the animals Balb/c and C57BL/6 during the 15-day

follow-up. GDH gene was amplified only in samples MS1, MS2 and MS3, with the visualization of a 432 bp fragment, confirming infection with *G. duodenalis* only in the male Swiss mice and showing that female Swiss mice did not really respond to the etiological treatment and continued to be infected with *G. muris*.

The averages of feed (Table 3) and water (Table 4) consumption, elimination of excreta (Table 5) and body weight, per animal, were calculated on days 1, 3, 6, 9, 12 and 15. For the general average of days, there was a difference only for the male Swiss mice, in which the infected animals ate more ($p < 0.0001$), and eliminated a greater amount of excreta ($p < 0.0001$) when compared with the non-infected animals. The overall mean water consumption of infected male Swiss was not significantly higher, but a significant increase was noted on days 9,12 and 15. Regarding the average body weight, the male Swiss mice were the only groups with a significant difference, in which the non-infected animals had an average weight greater than the infected ones on the 15th day ($p = 0.0004$). No diarrheal feces were observed. Some male and female Swiss mice, both infected and non-infected, had bristly hair in the first 4 days after infection.

Table 3. Average amount of feed (g/animal) ingested during the 15-day period after infection.

Animals	**Day**	**Negative control (g)**	**Infected animals (g)**	**Difference of means**	***p*-value***
Female Swiss	1	5.29	5.447	- 0.1567	> 0.9999
	3	**3.91**	**5.307**	**- 1.397**	**< 0.0001****
	6	6.045	5.773	0.2717	> 0.9999
	9	6.6	6.073	0.5267	0.1978
	12	6.76	7.083	- 0.3233	> 0.9999
	15	6.46	6.28	0.18	> 0.9999
Female Balb/c	1	4.505	4.54	- 0.035	> 0.9999
	3	3.995	3.87	0.125	> 0.9999
	6	4.36	4.383	- 0.02333	> 0.9999
	9	4.64	4.357	0.2833	> 0.9999
	12	3.975	3.557	0.4183	0.3835
	15	3.945	3.89	0.055	> 0.9999
Female C57BL/6	1	3.15	2.81	0.34	0.1105
	3	3.315	3.15	0.165	> 0.9999
	6	**4.02**	**3.577**	**0.4433**	**0.0136****
	9	4.17	3.92	0.25	0.4898
	12	**3.96**	**4.773**	**- 0.8133**	**< 0.0001****
	15	5.04	4.987	0.05333	> 0.9999
Male Swiss	**1**	**5.47**	**7.267**	**- 1.797**	**< 0.0001**
	3	**5.81**	**7.737**	**- 1.927**	**< 0.0001**
	6	**6.2**	**7.653**	**- 1.453**	**< 0.0001****
	9	**6.83**	**7.643**	**- 0.8133**	**0.0001****
	12	7.115	7.537	- 0.4217	0.1468
	15	**6.635**	**8.657**	**- 2.022**	**< 0.0001****
Male Balb/c	1	4.58	4.163	0.4167	0.1786
	3	4.64	4.283	0,3567	0.3575
	6	4.43	4.547	- 0.1167	> 0.9999
	9	5.5	5.03	0.47	0.0867
	12	5.435	5.397	0.03833	> 0.9999
	15	5.83	5.67	0.16	> 0.9999
Male C57BL/6	1	4.32	3.79	0.53	0.2390
	3	3.98	3.81	0.17	> 0.9999
	6	**3.77**	**4.47**	**- 0.7**	**0.0417****
	9	5.15	5.097	0.05333	> 0.9999
	12	5.32	5.483	- 0.1633	> 0.9999
	15	5.3	4.893	0.4067	0.6818

**p*-value is considered significant when $p < 0.05$. All *p*-value were adjusted according to Bonferroni's multiple comparisons test.
**The difference in means between the negative control and infected groups is significant
The average feed consumption corresponds to the overall average consumption of each animal according to the day, individually, expressed in grams of feed/animal.
It is important to note that on a few isolated days, the feed consumption of the infected and non-infected groups, within the lineage/sex itself, showed a significant difference, in which on some days the infected group consumed more and on others the group negative control, however, the difference in the

overall average consumption of all days was not significant. In the group of male Swiss animals, the feed consumption of the infected group was significantly higher than the negative control both in general average and in the days 1, 3, 6, 9 and 15.
Source: Authors.

Table 4. Average amount of water (ml/animal) generated during the 15-day period after infection.

Animals	Day	Negative control (ml)	Infected animals (ml)	Difference of means	*p*-value*
Female Swiss	1	9.9	10.1	- 0.20	0.5945
	3	**10.65**	**11.4**	**- 0.75**	**< 0.0001****
	6	11.35	11.47	- 0.1167	0,9473
	9	11.4	11.27	0.1333	0,9043
	12	**11.35**	**12.27**	**- 0.9167**	**< 0.0001****
	15	10.8	11.07	- 0.2667	0.2631
Female Balb/c	**1**	**6.35**	**5.6**	**0.75**	**0.0266****
	3	5.85	6.1	- 0.25	0.9153
	6	5.8	6.033	- 0.2333	0.9377
	9	5.35	5.567	- 0.2167	0.9558
	12	5.6	5.967	- 0.3667	0.6490
	15	**5.9**	**5.133**	**0.7667**	**0.0220****
Female C57BL/6	**1**	**4.6**	**5.267**	**- 0.6667**	**< 0.0001****
	3	**5.9**	**4.667**	**1.233**	**< 0.0001****
	6	4.45	4.667	- 0.2167	0.3153
	9	4.5	4.3	0.2	0.4080
	12	4.95	4.967	- 0.01667	> 0.9999
	15	5.05	5.1	- 0.05	0.9986
Male Swiss	1	11.45	11.6	- 0.15	0.9963
	3	11.2	11.13	0.06667	> 0.9999
	6	11.5	11.07	0.4333	0.5932
	9	**9.1**	**10.77**	**- 1.667**	**< 0.0001****
	12	**10.15**	**12**	**- 1.85**	**< 0.0001****
	15	**10.2**	**12.73**	**- 2.533**	**< 0.0001****
Male Balb/c	1	6.15	5.967	0,1833	0.9818
	3	**7.15**	**6.267**	**0,8833**	**0.0061****
	6	5.65	5.567	0,08333	0.9998
	9	5.6	6.233	- 0.6333	0.1001
	12	5.8	6.067	- 0.2667	0.8496
	15	6.20	6.3	- 0.1	0.9993
Male C57BL/6	1	6.55	6.333	0.2167	0.9321
	3	6.5	6.533	- 0.03333	> 0.9999
	6	6.3	5.767	0.5333	0.1447
	9	6.2	5.6	0.6	0.0716
	12	**6.15**	**5.433**	**0.7167**	**0.0174****
	15	6.2	5.773	0.4667	0.2671

**p*-value is considered significant when $p < 0.05$. All *p*-value were adjusted according to Bonferroni's multiple comparisons test.
**The difference in means between the negative control and infected groups is significant
The average water consumption corresponds to the general average consumption of each animal according to the day, individually, expressed in milliliters of water/animal.
It is important to note that on a few isolated days, the water consumption of the infected and non-infected groups, within the lineage/sex itself, showed a significant difference, in which on some days the infected group consumed more and on others the group negative control, however, the difference in the overall average consumption of all days was not significant. In the group of male Swiss animals, the water consumption of the infected group was significantly higher than the negative control both in general average and in the days 9, 12 and 15.
Source: Authors.

Table 5. Average amount of excreta (g/animal) eliminated during the 15-day period after infection.

Animals	Day	Negative control (g)	Infected animals (g)	Difference of means	*p*-value*
Female Swiss	1	6.04	6.453	- 0.4133	0.5587
	3	7.725	7.847	- 0.1217	0.9982
	6	8.34	7.94	0.4	0.5957
	9	8.055	7.743	0.3117	0.8214
	12	7.865	7.883	- 0.01833	> 0.9999
	15	7.69	8.05	- 0.36	0.7045
Female Balb/c	**1**	**3.365**	**2.49**	**0.875**	**0.0050****
	3	**3.085**	**2.393**	**0.6917**	**0.0459****
	6	2.945	2.71	0.235	0.9316
	9	3.135	2.207	- 0.1717	0.9851
	12	2.53	2.577	- 0.04667	> 0.9999
	15	3.45	2.847	0.6033	0.1137
Female C57BL/6	1	2.235	2.4	- 0.165	0.8587
	3	2.29	2.143	0.1467	0.9135
	6	2.355	2.15	0.205	0.6925
	9	**3.21**	**2.617**	**0.5933**	**0.0009****
	12	**1.95**	**3.333**	**- 1.383**	**< 0.0001****
	15	2.86	3.237	- 0.3767	0.0820
Male Swiss	**1**	**4.905**	**6.553**	**- 1.648**	**< 0.0001****
	3	**6.895**	**7.813**	**- 0.9183**	**< 0.0001****
	6	8.115	8.533	- 0.4183	0.2317
	9	**7.1**	**7.983**	**- 0.8833**	**0.0002****
	12	**6.19**	**7.543**	**- 1.353**	**< 0.0001****
	15	**6.33**	**8.883**	**- 2.553**	**< 0.0001****
Male Balb/c	**1**	**3.61**	**2.68**	**0.93**	**0.0012****
	3	2.57	2.36	0.21	0.9484
	6	2.945	3.197	- 0.2517	0.8853
	9	4.1	4.24	- 0.14	0.9933
	12	3.57	4.03	- 0.46	0.3139
	15	4.4	4.287	0.1133	0.9979
Male C57BL/6	1	3.19	3.133	0.0567	> 0.9999
	3	2.95	2.977	- 0.02667	> 0.9999
	6	**5.155**	**3.49**	**1.665**	**< 0.0001****
	9	4.57	4.3	0.27	0.8200
	12	**3.835**	**4.713**	**- 0.8783**	**0.0015****
	15	5.625	5.113	0.5117	0.1659

**p*-value is considered significant when $p < 0.05$. All *p*-value were adjusted according to Bonferroni's multiple comparisons test.
**The difference in means between the negative control and infected groups is significant
The average excreta elimination corresponds to the general average of excretion of each animal according to the day, individually, expressed in grams of excreta/animal.
It is important to note that on a few isolated days, the elimination of excreta of the infected and non-infected groups, within the lineage/sex itself, showed a significant difference, in which on some days the infected group eliminated more and on others the group negative control, however, the difference in the overall average excreta elimination of all days was not significant. In the group of male Swiss animals, the elimination of excreta of the infected group was significantly higher than the negative control both in general average and in the days 1, 3, 9, 12 and 15.
Source: Authors.

The OFT performed on the 7th dpi revealed that there was no difference in the behavioral parameters between the groups of infected animals when compared with the non-infected ones, for the studied lineages.

At the end of the experiments, the analysis of the intestinal mucosa of the animals evidenced trophozoites of *Giardia* spp. in both male and female Swiss mice, with more than 10 trophozoites/field, being classified as high parasitic load. In both male and female Balb/c and C57BL/6 mice, no trophozoites were observed in the intestinal mucosa.

4. Discussion

As far as we know, this is the first work that aimed to investigate which is the most suitable murine experimental model for studying human giardiasis, by comparing parasitological, molecular and clinical parameters and the pre-patent period of infection with *G. duodenalis* BIV in the lineages of *Mus musculus* (Swiss, Balb/c and C57BL/6). Only male Swiss mice acquired the infection, which was confirmed by parasitological and molecular methods, with a pre-patency period that ranged from the 6th to the 8th dpi, with significantly greater consumption of feed, water and excreta elimination when compared with non-infected animals. This indicates that infection with *G. duodenalis* alters the animal's physiology, and is consistent with what can also occur in humans infected with this parasite, especially children.

The elimination of the first cysts of *G. duodenalis* in MS1 and MS2 groups occurred on the 6th dpi. This result is in agreement with Buret et al. (1991), who also reported this pre-patency period; however, it differs from other studies that report elimination of cysts from the 3rd dpi (Pavanelli et al., 2018; Ware & Villegas, 2019). Some studies show that this period can vary from one to two weeks (Astiazarán-García et al., 2000; Soares et al., 2008). In MS3 group, the elimination of the first cysts occurred on the 8th dpi. This difference in the pre-patent period may be associated with host factors, such as immune response (Scott et al., 2000).

The alterations in clinical parameters regarding the consumption of feed, water, and greater elimination of excreta found in groups of animals that developed infection with *G. duodenalis*, are in agreement with the findings of Bezagio et al. (2017), when Swiss mice infected with *G. muris* were evaluated. Animals from groups that did not develop the infection also presented alterations in these parameters, raising the hypothesis that the animals' physiology and immune response may also be involved in this process. Since there was no specific pattern of differences between the groups with infected and non-infected animals, and as in most cases the alterations in these animals were not significant and when they were, they occurred on isolated days, it makes suggesting that the simple presence of the antigen could have led to these alterations.

Changes in these parameters, more intensely in animals that developed the infection, are probably related to the pathogenic action of the parasite. The trophozoites attached to the mucosa form a carpet and act as a physical barrier that hinders the absorption of water and nutrients. It can be aggravated by injury or atrophy of intestinal microvilli (Pavanelli et al., 2018), as it leads to a significant decrease in enzyme activities (Scott et al., 2000; Halliez & Buret, 2013). In an attempt to supply the nutritional deficiency caused by the difficulty of absorption, the animals increase the intake of water and feed, and due to the reduced absorption, the amount of excreta becomes greater. Change in the composition of the intestinal microbiota, lesions in the intestinal epithelium of the host and increase in the mucus production of male Swiss mice infected with assemblages AII and BIV of *G. duodenalis* (Pavanelli et al., 2018) reinforce a pathogenic action of the presence of the parasite and confirm the idea of using this sex and lineage as an experimental model for human giardiasis.

Cysts of *G. duodenalis* BIV did not infect Balb/c and C57BL/6 mice, even with inoculum of 10^4 parasites. Infection with cysts in these two lineages was reported in malnourished C57BL/6 (Bartelt et al., 2013), in BALB/cAnUnib Specified Pathogen Free (SPF) (Nakada et al., 2018), and also in immunosuppressed CF1 heterogeneous mice (Ware & Villegas, 2019), indicating that nutritional status and other environmental factors during the maintenance of mice directly influence their capacity to resist to pathogens (Mizutani et al., 2017; Vaidya, 2017). The host's immune response is also a determining factor for infection. The intestinal microbiota is an efficient defense mechanism of the mice's innate immune system and commensal bacteria have an anti-Giardia effect through competition, direct toxicity or modulation of the immune response (Goyal et al., 2013). Infection can be favored if, for example, SPF mice have absence or deficiency in their first line of defense. These findings corroborate the idea that differences in the composition of the hosts' microbiota and in the genetics of the parasites may help to clarify the variability in the course of the infection, ranging from the host's susceptibility to the occurrence or not of clinical manifestations (Solaymani-Mohommadi & Singer, 2010).

Infection with *G. duodenalis* in C57BL/6 mice was also reported when 10^6 trophozoites were administered by gavage (Li et al., 2014), or when these animals had concomitant infection with other parasite (Von-Allmen et al., 2006). Some authors report that cysts of *G. duodenalis* of assemblages A and B are not able to naturally infect mice and rats (Lebbad et al., 2010; Zhao, 2015), but the inoculation of trophozoites can lead to infection (Lemée et al., 2000). In this work, we opted for inoculation of cysts because this is the infectious form in humans, and according to Pavanelli et al. (2018), the conditions for testing the parasite in an animal model are different from those that occur in culture.

For the behavioral parameters, there was no difference between the groups of infected animals and the non-infected ones. Increased exploratory behavior in animals can occur in response to pain (Pavanelli et al., 2018). Baker (2006) describes that when the parasitic load in the animal is very high, some degree of inappetence and lethargy may occur, indicating the need for further studies with regard to animal behavior throughout the infection.

One difficulty and limitation of our study is that all Swiss mice, both male and female, came from the vivarium of the institution of origin naturally infected with *G. muris*, presenting a high parasitic load according to the classification of Uda-Shimoda et al. (2014). High infection rate with this parasite has also been reported in mice and rats produced in vivariums of Brazilian institutions (Bicalho et al., 2007; Bezagio et al., 2017; Almeida et al., 2021). As this parasite can cause an inflammatory response in the intestinal mucosa with increased mucus production and alterations in the animal's imune response, as well as alterations in clinical signs (Bezagio et al., 2017), the animals were etiologically treated before being infected with *G. duodenalis*. On the other hand, this treatment increases the experimentation time, making the animals to come into contact with the anti parasitic drugs, previously to the experimental infection, but this seems to be a reality in the vivariums of Brazilian institutions. However, it was possible to obtain cure and infection with *G. duodenalis* in the male Swiss animals, indicating that they are the most adequate experimental model for studies of human giardiasis. On the other hand, the Balb/c mice, which had the same origin as the Swiss mice, and the C57BL/6 mice from another institution, were not infected with *G. muris*, and did not get infected with *G. duodenalis*, presenting a lower susceptibility to infection, which varies according to sex, age, weight, lineage, among other factors inherent to the host.

5. Conclusion

It is concluded that male Swiss (*Mus musculus*) is the most suitable experimental model for the study of human giardiasis assemblage BIV, as it is the only lineage, compared with the other lineages studied, capable of becoming infected and eliminating *G. duodenalis* cysts in the feces. In this model, significant alterations in clinical parameters were observed in infected animals when compared with uninfected animals, signaling that important mucosal lesions, similar to the lesions present in human infection, may be occurring.

Once we were able to observe the resistance of Balb/c and C57BL/6 mice to develop infection by *G. duodenalis* from the intragastric inoculation of the cysts, new research and study possibilities arise, aiming to elucidate the resistance mechanisms presented by these animals. Knowing the mechanisms by which these mice destroy the parasite and prevent the infection from taking hold can be of great use, especially for researching new drugs for the treatment of human giardiasis.

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References

Almeida, C. R., Bezagio, R. C., Colli, C. M., Romera, L. I. L. & Gomes, M. L. (2021). Elimination of *Giardia muris* in naturally infected murine experimental model: Complementary treatment. *Research, Society and Development*, 10(7):e60010716996. https://doi.org/10.33448/rsd-v10i7.16996

Astiazarán-Garcia, H., Espinosa-Cantellano, M., Castañón, G., Chavéz-Munguía, B. & Martínez-Palomo, A. (2000). *Giardia lamblia*: effect of infection with symptomatic and asymptomatic isolates on growth of gerbils (*Meriones unguiculatus*). *Experimental Parasitology*, 95:128-135. https://doi.org/10.1006/expr.2000.4514

Baker, D. G. (2006). *Parasitic diseases.* In: Suckow MA, Weisbroth SH, Franklin CL (ed) The laboratory rat. Elsevier, 453-478.

Baker, J. M. & Alonso, W. J. (2018). Rotavirus vaccination takes seasonal signature of childhood diarrhea back to pre-sanitation era in Brazil. J*ournal of Infection*, 76:68–77. https://doi.org/10.1016/j.jinf.2017.10.001

Baltert, L. A., Roche, J., Kolling, G., Bolick, D., Noronha, F., Naylor, C., Hoffman, P, Warren, C., Singer, S. & Guerrant, R. (2013). Persistent *G. lamblia* impairs growth in a murine malnutrition model. *The Journal of Clinical Investigation*, 123:2672–2684. https://doi.org/10.1172/JCI67294

Bezagio, R. C., Colli, C. M., Romera, L. I. L., Almeida, C. R., Ferreira, É. C., Mattia, S., & Gomes, M. L. (2020). Improvement in cyst recovery and molecular detection of *Giardia duodenalis* from stool samples. *Molecular Biology Reports*, 47(2), 1233–1239. https://doi.org/10.1007/s11033-019-05224-5

Bezagio, R. C., Colli, C. M., Romera, L. I. L., Ferreira, É. C., Falavigna-Guilherme, A. L. & Gomes, M. L. (2017). Synergistic effects of fenbendazole and metronidazole against *Giardia muris* in Swiss mice naturally infected. *Parasitology Research*, 116:939- 944. https://doi.org/ 10.1007/s00436-016-5367-9

Bicalho, K. A., Araujo, F. T. M., Rocha, R. S. & Carvalho, O. (2007). Perfil sanitário de colônias de camundongos e ratos de biotérios de Minas Gerais: I - Endo e ectoparasitos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 59:1478-1484. https://doi.org/10.1590/S0102-09352007000600020

Buret, A., Gall, D. G. & Olson, M. E. (1991). Growth, activities of enzymes in the small intestines and ultrastructure of microvillous border in gerbils infected with *Giardia duodenalis*. *Parasitology Research*, 77:109-114. https://doi.org/10.1007/bf00935423

Cacciò, S. M. & Ryan, U. (2008). Molecular epidemiology of giardiasis. *Molecular and Biochemical Parasitology*, 160:75–80. https://doi.org/10.1016/j.molbiopara.2008.04.006

Chorilli, M., Michelin, D. C. & Salgado, H. R. N. (2007). Animais de laboratório: o camundongo. *Revista de Ciências Farmacêuticas Básica e Aplicada*, 28:1123. http://servbib.fcfar.unesp.br/seer/index.php/Cien_Farm/article/viewArticle/340

Cock, I. E. & Rayan, P. (2020). Ascorbic acid potentiates the *Giardia duodenalis* growth inhibitory activity of pure Terminalia ferdinandiana Exell compounds. *Parasitology Research*. https://doi.org/10.1007/s00436-019-06579-1

Colli, C. M., Bezagio, R. C., Nishi, L., Bignotto, T. S., Ferreira, É. C., Falavigna-Guilherme, A. L. & Gomes, M. L. (2015). Identical Assemblage of *Giardia duodenalis* in Humans, Animals and Vegetables in an Urban Area in Southern Brazil Indicates a Relationship among Them. *Plos One*, 10:e0118065. https://doi.org/10.1371/journal.pone.0118065

Dressen, L., Bosscher, K., Grit, G., Staels, B., Lubberts, E., Bauge, E. & Geldhof, P. (2014). *Giardia muris* infection in mice is associated with a protective interleukin 17A response and induction of peroxisome proliferator activated receptor alpha. *Infection and Immunology*, 82:3333-3340. https://doi.org/10.1128/IAI.01536-14

Ehret, T., Torelli, F., Klotz, C., Pedersen, A. B. & Seeber, F. (2017). Translational Rodent Models for Research on Parasitic Protozoa - A Review of Confounders and Possibilities. *Frontiers in Cellular and Infection Microbiology*, 7:238. https://doi.org/10.3389/fcimb.2017.00238

Fantinatti, M., Bello, A. R., Fernandes, O. & Da-Cruz, A. M. (2016). Identification of *Giardia lamblia* Assemblage E in Humans Points to a New Anthropozoonotic Cycle. *Journal of Infectious Diseases*, 214:1256–1259. https://doi.org/10.1093/infdis/jiw361

Faust, E. C., D´Antoni, J. S., Odom, V., Miller, M. J., Peres, C., Sawitz, W., Thomen, I. F., Tobie, J. & Walker, J. H. (1938). A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. *The American Journal of Tropical Medicine and Hygiene*, 18:169-183. https://doi.org/10.4269/ajtmh.1938.s1-18.169

Feng, Y. & Xiao, L. (2011). Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clinical Microbiology Reviews*, 24:110–140. https://doi.org/10.1128/CMR.00033-10

Guénet, J. L. (2011). Animal models of human genetic disesases: do they need to be faithful to be useful? *Molecular Genetics and Genomics*, 286:1-20. https://doi.org/10.1007/s00438-011-0627-y

Goyal, N., Rishi, P. & Shukla, G. (2013). Lactobacillus rhamnosus GG antagonizes *Giardia intestinalis* induced oxidative stress and intestinal disaccharidases: an experimental study. *World Journal of Microbiology and Biotechnology*, 29:1049-1057. https://doi.org/10.1007/s11274-013-1268-6

Hooshyar, H., Rostamkhani, P., Arbabi, M. & Delavari, M. (2019). *Giardia lamblia* infection: review of current diagnostic strategies. *Gastroenterology and Hepatology from Bed to Bench*, 12:3-12. https://doi.org/10.22037/ghfbb.v0i0.1414

Lasek-Nesselquist, E., Welch, D. M. & Sogin, M. L. (2010). The identification of a new *Giardia duodenalis* assemblage in marine vertebrates and a preliminar analysis of *G. duodenalis* population biology in marine system. *International Journal for Parasitology*, 40:1063–1074. https://doi.org/437 10.1016/j.ijpara.2010.02.015

Lebbad, M., Mattsson, J. G., Christensson, B., Ljungström, B., Backhans, A., Andersson, J. O. & Svärd, S. G. (2010). From mouse to moose: multilocus genotyping of *Giardia* isolates from various animal species. *Veterinary Parasitology*, 168:231–239. https://doi.org/10.1016/j.vetpar.2009.11.003

Lemée, V., Zaharia, I., Nevez, G., Rabodonirina, M., Brasseur, P., Ballet, J. J. & Favannec, L. (2000). Metronidazole and albendazole susceptibility of 11 clinical isolates of *Giardia duodenalis* from France. *Journal of Antimicrobial Chemotherapy*, 46:819–821. https://doi.org/10.1093/jac/46.5.819

Li, E., Liu, M. & Singer, S. M. (2014). Resistance to reinfection in mice as a vaccine model for giardiasis. *Human Vaccines & Immunotherapeutics*, 10:1536-1543. https://doi.org/10.4161/hv.29116

Lima, A. A. M., Oliveira, D. B., Quetz, J. S., Havt, A., Prata, M. M. G., Lima, I. F. N., Soares, A. M., Filho, J. Q., Lima, N. L., Medeiros, P. H. Q. S., Santos, A. K. S., Veras, H. N., Gondim, R. N. D. G., Pankov, R. C., Bona, M. D., Rodrigues, F. A. P., Moreira, R. A., Moreira, A. C. O. M., Bertolini, M., Bertolini, L. R., Freitas, V. J. F., Houpt, E. R. & Guerrant, R. L. (2019). Etiology and severity of diarrheal diseases in infants at the semiarid region of Brazil: A case-control study. *PLoS Neglected Tropical Diseases*, 13:e0007154. https://doi.org/10.1371/journal.pntd.0007154

Massironi, S. M. G. (2009). Padrão genético. In: Lapchik VBV, Mattaraia VGM, Ko GM (ed) *Cuidados e Manejo de Animais de Laboratório*, (2rd ed.) Editora Atheneu, 385 – 398.

Matsuchita, H. L. P., Pitz, A. F., Melanda, F. N., Bregano, R. M., Oliveira, F. J. A., Mori, F. M. R. L., Menezes, M. C. N. D., Costa, I. N., Pavanelli, W. R., Gomes, M. L., Colli, C. M., Venancio, E. J. & Conchon-Costa, I. (2017). Descriptive molecular epidemiology study of *Giardia duodenalis* in children of Parana State, Brazil. *International Journal of Epidemiology Research*, 4:1–9. http://ijer.skums.ac.ir/article_22927.html

Mayol, G. F., Revuelta, M. V., Salusso, A., Touz, M. C. & Rópolo, A. S. (2019). Evidence of nuclear transport mechanisms in the protozoan parasite *Giardia lamblia*. *Biochimica et biophysica acta - Molecular Cell Research*, 1867:118566. https://doi.org/10.1016/j.bbamcr.2019.118566

Mizutani, H., Tamagawa-Mineoka, R., Minami, Y., Yagita, K. & Katoh, N. (2017). Constant light exposure impairs immune tolerance development in mice. *Journal of Dermatological Science*, 86:63–70. https://doi.org/10.1016/j.jdermsci.2016.12.016

Monis, P. T., Andrews, R. H., Mayrhofer, G. & Ey, P. L. (1999). Molecular systematics of the parasitic protozoan *Giardia intestinalis*. *Molecular Biology Evolution*, 16:1135-1144. https://doi.org/10.1093/oxfordjournals.molbev.a026204

Nakada, L. Y. K., Franco, R. M. B., Fiuza, V. R. S., Santos, L. U., Branco, N. & Guimarães, J. R. (2018). Pre-ozonation of source water: assessment of efficacy against *Giardia duodenalis* cysts and effects on natural organic matter. *Chemosphere*. https://doi.org/10.1016/j.chemosphere.2018.09.164

Pavanelli, M. F., Colli, C. M., Gomes, M. L., Góis, M. B., Alcântara-Nogueira, G. M., Almeida-Araújo, E. J. & de Gonçales-Sant'Ana, D. M. (2018). Comparative study of effects of assemblages AII and BIV of *Giardia duodenalis* on mucosa and microbiota of the small intestine in mice. *Biomedicine & Pharmacotherapy*, 101:563–571. https://doi.org/doi:10.1016/j.biopha.2018.02.141

Qi, M., Ji, X., Zhang, Y., Wei, Z., Jing, B., Zhang, L., Lin, X., Karim, M. R., Wang, H. & Sun, M. (2020). Prevalence and multilocus analysis of *Giardia duodenalis* in racehorses in China. *Parasitology Research*. https://doi.org/doi:10.1007/s00436-019-06594-2

Scott, K. G. E., Logan, M. R., Klammer, G. M., Teoh, D. A. & Buret, A. G. (2000). Jejunal brush border microvillous alterations in *Giardia muris* - infected mice: role of T lymphocytes and interleukin-6. *Infection and Immunity*, 68:3412-3418. https://doi.org/ 10.1128/ iai.68.6.3412-3418.2000

Soares, J. F., Silva, A. S., Oliveira, C. B., Silva, M. K., Mariscano, G., Salomão, E. L. & Monteiro, S. G. (2008). Parasitismo por *Giardia* sp. e Cryptosporidium sp. em Coendou villosus. *Ciência Rural*, 38:548–550. https://doi.org/10.1590/s0103-84782008000200043

Solaymani-Mohammadi, S. & Singer, S. M. (2010). *Giardia duodenalis*: the double-edged sword of immune responses in Giardiasis. *Experimental Parasitology*, 126:292–297. https://doi.org/10.1016/j.exppara.2010.06.014

Strober, W. (2015). Trypan Blue Exclusion Test of Cell Viability. *Current Protocols in Immunology*. https://doi.org/10.1002/0471142735.ima03bs111

Thompson, R. C. A., Hopkins, R. M. & Homan, W. L. (2000). Nomenclature and genetic groupings of *Giardia* infecting mammals. *Parasitology Today*, 16:210-213. https://doi.org/10.1016/s0169-4758(99)01624-5

Uda-Shimoda, C. F., Colli, C. M., Pavanelli, M. F., Falavigna-Guilherme, A. L. & Gomes, M. L. (2014). Simplified protocol for DNA extraction and amplification of 2 molecular markers to detect and type *Giardia duodenalis*. *Diagnostic Microbiology and Infectious Disease*, 78(1):53-58. https://doi.org/10.1016/j.diagmicrobio.2013.09.008

Vaidya, H. B., Gangadaran, S. & Cheema, S. K. (2017). A high fat-high sucrose diet enriched in blue mussels protects against systemic inflammation, metabolic dysregulation and weight gain in C57BL/6 mice. *Food Research International*, 100:78–85. https://doi.org/10.1016/j.foodres.2017.08.033

Von-Allmen, N., Christen, S., Forster, U., Gottstein, B., Welle, M. & Muller, N. (2006). Acute trichinellosis increases susceptibility to *Giardia lamblia* infection in the mouse model. *Parasitology*, 133:139-149. https://doi.org/10.1017/S0031182006000230

Zhao, Z., Wang, R., Zhao, W., Qi, M., Zhao, J., Zhang, L., Li, J. & Liu, A. (2015). Genotyping and subtyping of *Giardia* and *Cryptosporidium* isolates from commensal rodents in China. *Parasitology*, 142:800–806. https://doi.org/10.1017/S0031182014001929

Ware, M. W. & Villegas, E. N. (2019). Propagation of *Giardia duodenalis* cysts in immunosuppressed CF-1 mice. *Veterinary Parasitology*, 268:32-35. https://doi.org/10.1016/j.vetpar.2019.02.010