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Research Brief

Impact of protein malnutrition on histological parameters of experimentally infected animals with *Giardia lamblia*

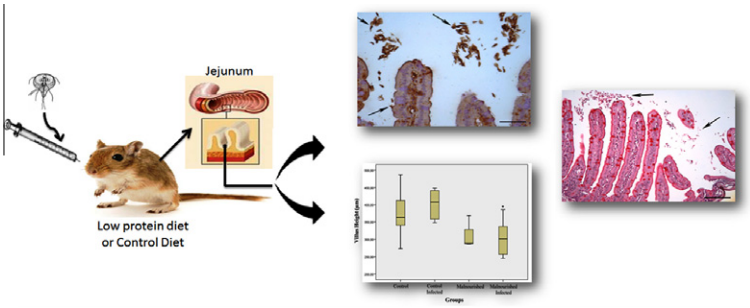
Luciana L.A. Ventura [a], Dirce R. Oliveira [b], João C. Viana [a], Joseph F.G. Santos [c], Marcelo V. Caliari [d], Maria A. Gomes [a,*]

[a] Dept. Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627 CEP: 31270-901 Belo Horizonte, MG, Brazil
[b] Dept. Nutrição, Escola de Enfermagem, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627 CEP: 31270-901 Belo Horizonte, MG, Brazil
[c] Hospital IPSEMG, Alameda Ezequiel Dias, 225, Belo Horizonte, Minas Gerais, Brazil
[d] Dept. Patologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627 CEP: 31270-901 Belo Horizonte, MG, Brazil

HIGHLIGHTS

- Histological parameters in giardiasis combined with low-protein diet was evaluated.
- Quantification of *G. lamblia* in mucosa was not significantly affected by diet.
- Villus height was significantly affected by diet.
- Mucus production was significantly affected by diet and infection.
- Malnutrition may contribute to disease severity in Giardiasis.

GRAPHICAL ABSTRACT



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ABSTRACT

Giardiasis is one of the most common parasitic diseases worldwide, and the disease is an important cause of diarrhoea and malabsorption in children and immunosuppressed individuals. However, there is no evidence that characterises malnutrition as an aggravating factor for this disease. We evaluated changes in villi structures to examine the association between malnutrition and *Giardia lamblia* infection.

We used 32 gerbils, divided into 4 groups: Control (CT) and Control Infected (CTIn), which each received a 20% protein diet, Malnourished (MN) and Malnourished Infected (MNIn), which each received a 5% protein diet. Groups CTIn and MNIn were inoculated with 1×10^6 trophozoites of *G. lamblia*, while the remaining groups were mock infected. Seven days post-infection, all groups were sacrificed, and the proximal portions of the small intestines were collected for the analysis of villus height, mucus area and extent of *Giardia* infection.

Gerbils fed with a low-protein diet had significantly lower body weights.

Malnourished infected animals presented significantly increased production of mucus, suggesting a synergism occurs between malnutrition and Giardiasis, potentially to control the adhesion of *Giardia* in the mucosa.

Villus height was significantly lower in group MNIn compared to CTIn. This work suggests that malnutrition contributes to severity of Giardiasis by decreasing the intestinal absorption capacity via shortening of the villi.

1. Introduction

Giardiasis, an intestinal infection caused by *Giardia lamblia*, is one of the most common parasitic diseases worldwide, present

* Corresponding author. Fax: +55 31 34092970.
E-mail address: magomes@icb.ufmg.br (M.A. Gomes).

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both in developed countries (CDC, 2010) and in those under development (Dib et al., 2008). It is estimated that each year there are approximately 280 million symptomatic cases (Lane and Lloyd, 2002), which can lead to cognitive deficits and severe growth defects in children (Berkman et al., 2003).

The transmission of this parasitic disease usually occurs indirectly following the ingestion of food or water contaminated with cysts or by person-person contact, particularly among institutionalised individuals living in conditions of poor hygiene and sanitation (Abe and Teramoto, 2012).

Clinical manifestations among individuals are highly variable, ranging from asymptomatic to symptomatic severe cases. The infection can cause diarrhoea, weight loss, dehydration, abdominal pain, malabsorption, maldigestion and steatorrhoea (Buret, 2008). Additionally, chronic Giardiasis, when undiagnosed and untreated, can lead to malabsorption of lipids (Bansal et al., 2005), fat-soluble vitamins (Saki et al., 2011), zinc and iron (Demirci et al., 2003), vitamin B12, and sodium (Buret, 2008) and to the reduced action of disaccharidases (Gomes et al., 2012; Solaymani-Mohammadi and Singer, 2011).

Individuals diagnosed with chronic Giardiasis may also exhibit a loss of intestinal barrier function due to apoptosis of enterocytes (Chin et al., 2002; Panaro et al., 2007). Consequently, there is a diffuse shortening of the microvilli epithelium in these patients, which potentially results in a reduction in the secretion of disaccharidases, contributing to the clinical manifestations of the disease (O'Hara and Buret, 2008).

Due to these factors, this disease has commonly been implicated as a cause of stunted growth in children, and the occurrence and frequency of diarrhoea, the duration of infection and the chance of reinfection have each been considered essential factors contributing to physical and mental deficiencies in this group (Simsek et al., 2004).

Although the clinical impact of Giardiasis is well known in children (Carvalho-Costa et al., 2007; Júlio et al., 2012; Matos et al., 2008) and immunosuppressed individuals (Feitosa et al., 2001; Gonçalves et al., 2009), the impact of malnutrition on this disease is speculative, and there is no evidence to establish the aggravating factor of malnutrition in Giardiasis. Considering the above, this study aims to assess the histological effects of infection with *G. lamblia* in a malnourished animal model.

2. Materials and methods

2.1. Experimental model and group divisions

We used 32 female gerbils (*Meriones unguiculatus*), aged 4–6 weeks. Animals were maintained under standard laboratory conditions with a 12:12 h light/dark cycle and controlled temperatures (23 ± 3 °C), receiving filtered water and a diet kept under refrigeration prior to feedings *ad libitum*. Special care with the feed and water ensured that no other sources of infection were introduced to these animals throughout the course of the study. The animals were divided evenly according to body weight into four groups of eight animals each: Control (CT) and Control Infected (CTIn), with a diet containing 20% protein, and Malnourished (MN) and Malnourished Infected (MNIn), which received a 5% protein diet. The experiments were performed in compliance with the guidelines of the Institutional Animal Care and Committee on Ethics of Animal Experimentation (Ethics Committee on Animal Experiments – CETEA, national guidelines Law 11.794, dated October 8, 2008) from Universidade Federal de Minas Gerais (UFMG); protocol number 070/2010.

2.2. Cultures and growth conditions

Trophozoites from the Portland-1 isolate (ATCC 30888), Assemblage A, were kept in culture in TYI-S-33 medium modified by Keister (1983) and supplemented with bovine serum.

2.3. Experimental design and inoculation

The experiments were performed following the protocol of Gomes et al. (2012) with modifications. From the first day of the experiment (Day 0), groups MN and MNIn were maintained on a low-protein diet for 4 weeks. During the same period, the remaining groups received the control diet for the maintenance of nutritional status. Following this period (28 days), animals from groups CTIn and MNIn were infected orally by gavage with 1×10^6 trophozoites contained in 0.8 ml Phosphate Buffered Saline (PBS), while the CT and MN groups received 0.8 ml of PBS. Seven days post-inoculation, the animals were euthanized. The animals were weighed each week individually using a balance (BL320H, Shimadzu).

2.4. Morphometric analysis

After sacrifice, the proximal portion of the small intestine (6 cm in length) was collected and fixed in 10% buffered formaldehyde (pH 7.2). After processing in alcohol and xylene, fragments were embedded in paraffin, and 4-μm thick sections were obtained and processed for Hematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS) and immunohistochemistry. After processing, 30 villi were randomly scanned with a JVC TK-1270/RGB microcamera (Tokyo, Japan), using up to 10× magnification for analysing villus height. Thirty additional images were scanned in the same manner but with an increased magnification of 40× for the examination of mucus area. Villus height, in μm, as well as the mucus area, in μm^2, were calculated using the software contained in the KS400 image analyser (Carl Zeiss). To identify and quantify *G. lamblia* trophozoites in histological sections, the following immunohistological procedure was performed. Paraffin-embedded intestinal sections from infected animals were deparaffinised, hydrated, and treated with 3.5% PBS/H_2O_2 solution to block endogenous peroxidases. Unspecific binding was blocked by goat serum diluted 1:40. Sections were incubated with polyclonal anti-*G. lamblia* serum diluted 1:1000 (produced in the laboratory of amoebiasis and intestinal protozoans, ICB-UFMG), followed by biotinylated goat IgG diluted 1:50 (Zymed Laboratories Inc., San Francisco, Calif.) and streptavidin diluted 1:100 (Zymed Laboratories Inc.). Colour was detected using 0.05% diaminobenzidine and 0.2% H_2O_2 solution, and sections were counterstained with diluted Harris's Haematoxylin. Primary antiserum was substituted with PBS for some sections for negative control purposes. After the reaction, the entire mucosal surface of the proximal portion of the small intestine was scanned at 10× magnification and analysed using the same software described above. All pixels marked with shades of brown by immunohistochemistry (indicating trophozoites) were selected to create a binary image and to calculate the total area of brown markings.

2.5. Statistical analysis

The data are expressed as the means ± standard error. We used the Shapiro–Wilk test to verify the normality of the sample and to compare the experimental groups and Analysis of Variance (ANOVA) followed by the Tukey test. All tests and analyses were performed in SPSS version 20.0, and a significance level of 5% ($p < 0.05$) was used.