



## Secnidazole for control of giardiasis in dairy calves

Andreia Volpato <sup>a</sup>, Bruno F. Fortuoso <sup>b</sup>, Gabriela Campigotto <sup>b</sup>, Patrícia Glombowsky <sup>b</sup>, Nathieli B. Bottari <sup>c</sup>, Leandro S. Lopes <sup>a</sup>, Aleksandro Schafer Da Silva <sup>a, b, c, \*</sup>

<sup>a</sup> Graduate Program of Animal Science, Universidade do Estado de Santa Catarina (UDESC), C, Chapecó, 89815-630, Chapecó, SC, Brazil

<sup>b</sup> Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Rua Beloni Trombeta Zanin, Chapecó, 89815-630, Chapecó, SC, Brazil

<sup>c</sup> Biochemistry and Molecular Biology Department, Universidade Federal de Santa Maria (UFSM), Av. Roraima 1000, Santa Maria, RS 97105-900, Brazil

### HIGHLIGHTS

- Giardiasis is an important parasitic disease in calves.
- Secnidazole is 100% effective in the treatment of giardiasis in dairy heifers.
- Drug eliminates the excretion of *Giardia duodenalis* cysts.
- Effect persists for at least 30 days of secnidazole.

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### ABSTRACT

The aim of this study was to verify whether secnidazole, given in a single oral dose (10 mg/kg), decreases or eliminates the excretion of *Giardia duodenalis* cysts. Holstein calves were raised from birth to 15 ± 2 days of age in individual stalls. Subsequently, 12 calves were grouped and housed in collective stalls. After seven days (day of life 21), we collected stool samples directly from the rectal ampulla in order to determine the degree of parasitic infection. Fecal examination was performed by a centrifugal-flotation technique, which allows for visualization and quantification of *G. duodenalis* cysts. After division into control and treatment groups, six animals were treated with one 400 mg secnidazole capsule. The first stool collection following treatment was performed on day 5 and the second on day 30. This experiment was repeated at 15 days, with a total of 24 calves studied. Animals on the farm where the experiment was conducted often suffer from giardiasis, despite hygiene care (disinfection) and adequate facilities. All 24 calves were excreting *G. duodenalis* cysts prior to starting treatment. Five days after receiving the treatment, animals in the experiment group were *Giardia*-negative, i.e., they did not excrete parasite cysts, whereas calves in the control group continued to excrete cysts. After 30 days of treatment, the stool of most treated animals (83.3%) remained free of *G. duodenalis* cysts. Therefore, we believe that secnidazole was 100% effective in eliminating the excretion of *Giardia duodenalis* cysts.

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### 1. Introduction

The raising of heifers is very important to dairy cattle husbandry, as it ensures the replacement of cows, permits increases in herd size, and consequently, milk production. However, high rates of morbidity and mortality compromise production, although this phenomenon is not well-documented. Currently, the main cause of early death in

heifers is infectious diarrhea, mainly caused by bacteria and protozoa. Infections caused by these pathogens cause malabsorption of nutrients, weight loss, decreased food intake, and slow growth. Infectious diarrhea creates substantial economic burdens for producers because of costs associated with treatment, and in many cases, the cost of replacing dead calves. It is estimated that diarrhea is directly associated with death of calves during the first week of life and at 8 to 31 days of age. The disease accounts for more than 50% of calf mortality (Gulliksen et al., 2009; USDA, 2007).

Among the agents capable of provoking intestinal infections leading to diarrhea in animals, most prominent is *Giardia*, the protozoan responsible for giardiasis. Infections caused by *Giardia*

\* Corresponding author. Department of Animal Science, Centro de Educação Superior do Oeste (CEO), UDESC, Rua Beloni Trombeta Zanin, Chapecó, SC 89815-630, Brazil.

E-mail address: [aleksandro\\_ss@yahoo.com.br](mailto:aleksandro_ss@yahoo.com.br) (A.S. Da Silva).

*duodenalis* can be controlled with the use of paromomycin sulfate, an effective active ingredient when used for 5 consecutive days (Geurden et al., 2006). However, the most used and effective agent is the antiprotozoal fenbendazole, used for 3 consecutive days (Geurden et al., 2006; O’Handley et al., 2001). In other words, current treatments for giardiasis control in calves require continuous use, making management difficult for the animals and their caretakers.

Another potential treatment for giardiasis in calves is the anti-protozoal secnidazole. Single-dose therapy has been used in humans, but has never been studied in calves. Nevertheless, the drug showed promise in lambs (Ural et al., 2014), cats (Da Silva et al., 2011), and Swiss mice (Franco et al., 2015). The purpose of this study was to verify that secnidazole used in a single dose decreases or eliminates the excretion of *Giardia duodenalis* cysts in calves.

## 2. Material and methods

### 2.1. Treatment

The active ingredient secnidazole used in this study was purchased from Medley. Subsequently, 400 mg secnidazole capsules were produced in a compounding pharmacy.

### 2.2. Animals and experimental design

Newborn Holstein calves, weighing  $38 \pm 2.5$  kg at birth, were housed in individual stalls and received 2 L of colostrum within the first 2 h after birth. After ingestion of colostrum, the animals received 3 L of milk twice a day ( $40 \pm 3.2$  kg at 15 days of life), in addition to food and water *ad libitum*. Animals on the farm where the experiment was conducted often suffer from giardiasis, despite hygiene care (disinfection) and adequate facilities. This history of the disease was the reason for choosing this farm for the experiments.

After approximately 15 days, twelve calves were transferred and grouped in a collective stall, with automatic feeding (6 L/day), in addition to feed, water and hay *ad libitum*. The ration was produced on the same farm. It consisted of corn bran (47%), soybean meal (31%), soybean husk (17%), and mineral-vitamin commercial premix (5%), containing 22% protein. Hay was produced from Tifton 85, offered *ad libitum*.

At 15 days of life, the calves were divided into two groups: the treatment group received a capsule containing 400 mg of secnidazole orally, corresponding to approximately 10 mg/kg; and other group was used as a control, without receiving secnidazole. This experiment was performed in duplicate; 24 calves were divided into two stages (Step I and II), with a 15-day interval between the beginning of each stage.

### 2.3. Sample collection

The first fecal collection was performed seven days after grouping the animals in a collective stall and before the beginning of the experiment, which was designated as day 0. New fecal samples were collected on days 5 and 30 after secnidazole treatment. Fecal collections were performed directly from the rectal ampulla of the animals in order to verify the number of cysts of *G. duodenalis* per gram of feces.

### 2.4. Parasitological examination of feces

The parasitological examination of feces aimed to determine the presence and degree of infection of each animal with *G. duodenalis* cysts using a centrifugal-flotation technique (Faust et al., 1938),

according to a methodology described in detail by Monteiro (2010). We used hypersaturated sugar solution, and counted cysts according to the methodology of Barbier et al. (1990).

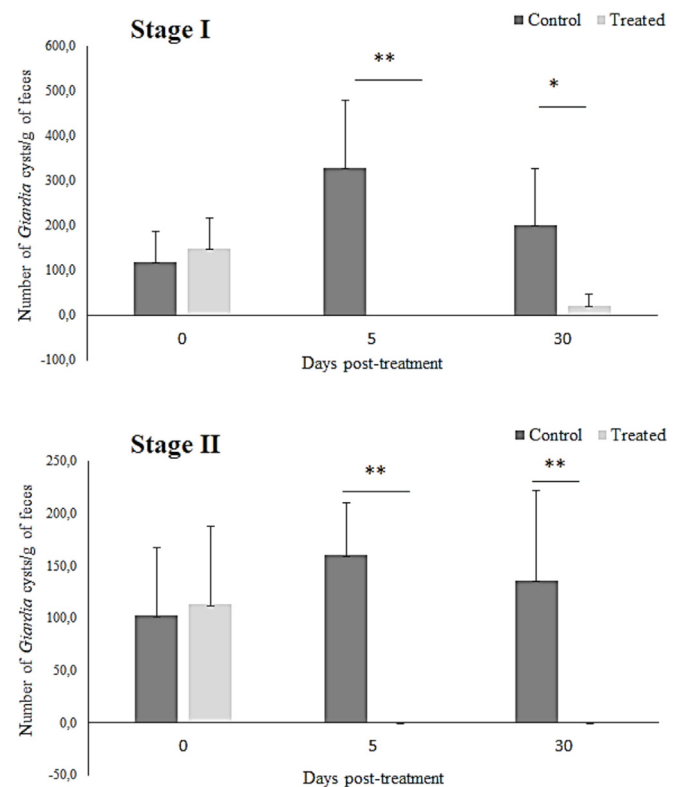
### 2.5. Statistical analysis

Number of cysts were first analyzed descriptively. We computed measures of central tendency (median) and data dispersion (range-stand for the interval between the minimum and maximum values in the data). The variable was further subjected to Shapiro Wilk’s W-test for normal distribution verification. Since most of the variable did not meet criteria for parametric testing, we used a nonparametric test (Kruskal-Wallis). We define statistical significance as P value < 0.05.

## 3. Results

For the 24 calves excreting *G. duodenalis* cysts before starting treatment, the results obtained in Step I were similar to those obtained in Step II. After administration of secnidazole, no *G. duodenalis* cysts were found in feces on day 5 (0%), and we saw a reduction of cysts at 30 days following treatment (83.3%, Fig. 1). Therefore, we observed a significant difference ( $P < 0.05$ ) between groups with respect to cyst count on days 5 and 30 following treatment (Fig. 2).

Even after 30 days of secnidazole capsule intake, most calves remained *Giardia* negative, unlike those in the control group who continued to excrete *Giardia* cysts. It is important to emphasize that animals in both groups shared the same bay. Therefore, animals in the treatment group were exposed daily to environmental contamination by protozoan cysts.



**Fig. 1.** Number of *Giardia* cysts at day 0 and post treatment (days 5 and 30) with a single oral dose of secnidazole (10 mg/kg) at the two stages of the experiment (Step I (n = 12) and Step II (n = 12)).

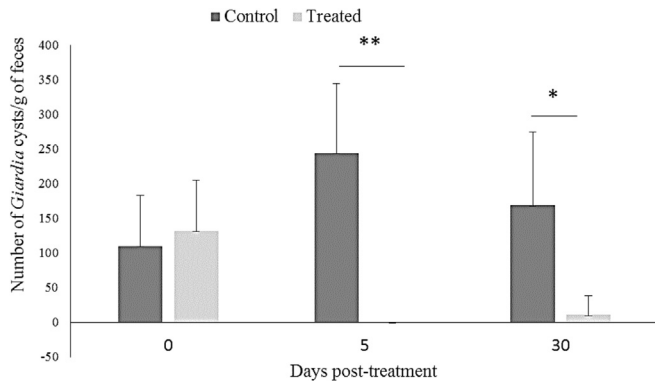


Fig. 2. Response to secnidazole by *Giardia* cysts. Data plotted together (Stage I and II) (n = 24).

#### 4. Discussion

Transmission of the parasite in domestic cattle is particularly concerning because of the high concentration of animals in a small area of land, and the large output of feces. We suggest that a major factor is dissemination among calves. Previous studies examining *Giardia* in cattle in North America revealed a point prevalence of 6.5–11% in adult cattle, but up to 100% cumulative prevalence in calves under 6 months of age (Hoar et al., 2001; Ralston et al., 2002). Despite recognition of the prevalence *G. duodenalis*, only a handful of agents have been used in therapy, and the agents that are available may have adverse effects or may be contraindicated in certain clinical situations.

We observed no *G. duodenalis* cysts in calves treated with a single dose of secnidazole at 10 mg/kg. This result is similar to those seen in other studies in lambs (Ural et al., 2014), cats (Da Silva et al., 2011) and mice (Franco et al., 2015). Secnidazole is a nitroimidazole class antiprotozoal derived from 5-nitroimidazole. Like other drugs of this class, enters microorganisms through passive diffusion and is activated by reduction of the nitro group (Gardner and Hill, 2001), effectively killing *Giardia*. In anaerobic microorganisms, such as *G. duodenalis*, this intracellular reduction occurs through pyruvate ferredoxin oxidoreductase and results in a concentration gradient across the cell membrane, which, in turn, increases the transport of the original drug into the cell. Since the electron affinity of 5-nitroimidazoles is greater than the reduced ferredoxin, the drug disrupts the normal flow of electrons, aerobic microorganisms have a more positive redox potential (ie, more efficient electron acceptors) than secnidazole and other 5-nitroimidazoles, which explains the selective toxicity of these drugs against anaerobic microorganisms. However, DNA is the intracellular target of 5-nitroimidazoles and when induced by the drug results in tape rupture, loss of helical structure and impaired template function. However, the lethal effect of 5-nitroimidazoles on the microorganism is attributed to a short-term reduction product, probably a nitro radical anion (Gardner and Hill, 2001). Although the mechanism of action of secnidazole in infections caused by these microorganisms has apparently not been investigated, it would have been expected to be similar to that of metronidazole.

In addition to combating infection, secnidazole treatment prevented reinfection in more than 80% of the animals. This effect is likely due to secnidazole being a pharmacological substance derived from long-acting 5-nitroimidazole. The drug is absorbed rapidly and completely, and has a half-life of 17–29 h to humans (Gillis and Wiseman, 1996). The half-life of secnidazole in ruminants is unknown. It is important to remember that, in some countries, the use of drugs derived from nitroimidazoles is

prohibited in food animals. However, since the half-life of the drug is short, it would have no negative consequences for food production when used in young animals such as dairy calves, as these would only have started their productive life as cows when they are more than 2 years old.

#### 5. Conclusion

Therefore, we conclude that secnidazole is 100% effective in the treatment of giardiasis in dairy heifers. It eliminates the excretion of *Giardia duodenalis* cysts; it is available in convenient single-dose form; and the effect persists for at least 30 days. Since giardiasis is a persistent infection, a single dose treatment option is attractive. Because of this, studies on half-life, excretion and possible side effects should be undertaken to validate that treatment with secnidazole is a safe option in combating giardiasis in young ruminants.

#### Ethics committee

This study was approved by the Ethics Committee on the Use of Animals of the State University of Santa Catarina (CEUA/UESC), protocol n° 4964301116.

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