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# *Meriones unguiculatus* infected by *Haemonchus contortus*: evaluation of different experimental protocols

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## Abstract

Many important studies on resistance reversion, anthelmintic efficacy and, especially, new molecules with antiparasitic effects are performed in laboratories using gerbils (*Meriones unguiculatus*) as the experimental model. This study aimed to evaluate the use of corticosteroids (dexamethasone and methylprednisolone acetate) in gerbils experimentally infected with different doses of infective larvae (sheathed or exsheathed) of *Haemonchus contortus*. In the first experiment, 28 gerbils were divided into seven groups infected by 2–6 × $10^3$ larvae, with or without immunosuppression using corticosteroids. In the second experiment, eight gerbils were divided into two groups infected by 2 × $10^3$ sheathed or exsheathed larvae. For the third assay, seven immunosuppressed gerbils were infected with 2 × $10^3$ sheathed larvae and were killed 15 days post infection (PI). The highest number of parasites was recovered from methylprednisolone-immunosuppressed animals. We observed red and white blood cell alterations and biochemical parameters in infected animals that had undergone immunosuppression with methylprednisolone. We highlight that in the first and second experiments a satisfactory number of worms was recovered using sheathed larvae and immunocompetent animals. When exsheathed larvae were used, the number of worms recovered was unsatisfactory. A considerable larval burden was recovered from immunosuppressed gerbils 15 days PI, and body weight did not influence establishment of larvae.

## Introduction

Gastrointestinal nematodes cause health problems and economic losses. The parasite *Haemonchus contortus* is a nematode of small ruminants, with high prevalence and pathogenicity (Arosemena *et al.*, 1999). Its haematophagous behaviour causes severe clinical symptoms, such as anaemia, and it has become the most pathogenic parasite of its host animals (Urquhart *et al.*, 1990). The increasing prevalence of helminths presenting multi-drug resistance has increased the need to better understand parasite resistance mechanisms and life cycles. Thus, *in vitro* and *in vivo* laboratory experiments have been conducted (Grando *et al.*, 2015, 2016).

Many studies have been conducted using gerbils (*Meriones unguiculatus*), and this experimental model has demonstrated much promise regarding reversion of resistance (Molento & Prichard, 1999), anthelmintic evaluations (Kates & Thompson, 1967) and investigations of new anti-parasitic compounds (Ribeiro *et al.*, 2013). Gerbils are susceptible to infections caused by several nematodes, including *Strongyloides stercoralis* (Nolan *et al.*, 1993), *Strongyloides venezuelensis*, *Nippostrongylus brasiliensis* (Horii *et al.*, 1993), *Trichostrongylus colubriformis* (Conder *et al.*, 1991; Ziam *et al.*, 1999), *H. contortus* (Conder *et al.*, 1990) and *Ostertagia circumcincta (Teladorsagia circumcincta)* (Court *et al.*, 1988).

Use of new experimental models and methodologies with high reliability and repeatability is important for comparing scientific results quickly and easily. Moreover, use of alternative protocols that cause less discomfort to animals has been recommended in order to improve animal welfare. Currently, some aspects of optimal protocols for infection of gerbils by *H. contortus* remain unclear, such as doubts regarding the use of sheathed or exsheathed larvae and the employment of corticosteroids to induce animal immunosuppression. This study therefore aimed to compare the infectivity of sheathed and exsheathed larvae of *H. contortus* in gerbils that were either immunocompetent or had undergone immunosuppression through various immunosuppression protocols.



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## Materials and methods

### *Isolation of* Haemonchus contortus

A multi-drug resistant *H. contortus* isolate (Almeida *et al.*, 2010) was used to infect a sheep. This animal was fed with hay and received water *ad libitum*. Initially, the sheep was treated with the anthelmintic monepantel (Zolvix®). Five days later, faecal examination was performed using the zinc sulphate centrifugation-flotation technique and no parasite was detected. Later, the animal was infected orally with $1.0 \times 10^4$ third-stage larvae at three different times: first, a dose of $4.0 \times 10^3$ larvae, and then another two doses of $3.0 \times 10^3$ larvae, at three-day intervals. The larvae that were used to infect the gerbils were recovered by means of coproculture, in accordance with the method described by Roberts & O'Sullivan (1950), as modified by Ueno & Gonçalves (1998). The larvae for infecting the animals were previously stored at room temperature for seven days.

### In vivo *experiment number 1*

Twenty-eight male and female five-week-old outbred gerbils (*M. unguiculatus*), of average body weight 37 g, which were visually in a healthy and parasite-free condition, were obtained from the Animal Care Center of the Federal University of Santa Maria (UFSM), Brazil. The gerbils were kept in polypropylene boxes under controlled temperature and humidity (22°C ± 2°C; 40% relative humidity, RH) under a 12/12 h dark/light cycle and were fed with commercial feed and water *ad libitum*. After a week of adaptation, the animals were divided randomly according to body weight into seven groups (A to G) of four individuals each. They were infected orally on day 0, except for group G (uninfected), which received a placebo solution (0.9% NaCl). The gerbils were subjected to a 24-hour fasting period to enhance the chances of larval infection. The groups were composed as follows:

#### *Immunosuppression protocols*

*Protocol 1.* Immunosuppression applied to gerbils on days −2, −1, 0, 1 and 2 using 0.1 ml (0.2 mg) of dexamethasone (Azium®, Schering Plus; 2 mg/ml), intramuscularly (IM).

*Protocol 2.* Immunosuppression applied to gerbils on days −2, −1, 0, 2, 4, 6, 8 and 10 using 0.1 ml (0.2 mg) of dexamethasone (Azium®, Schering Plus; 2 mg/ml), IM.

*Protocol 3.* Immunosuppression applied to gerbils on days −2, −1, 0, 1 and 2 using 0.1 ml (4 mg) of methylprednisolone acetate (Depo-Medrol®, Pfizer; 40 mg/ml), IM.

#### *Method for obtaining exsheathed larvae*

Infective larvae were exsheathed in 0.9% sodium hypochlorite (NaClO), by adding 14 µl of NaClO per ml of water. When 90% of the larvae had become exsheathed, they were washed with distilled water, followed by centrifugation for 3 minutes at 2000 rpm. This procedure was repeated three times. Later, the larvae were placed on a mesh (25 µm) and those with high motility were selected.

#### *Groups formed*

*Group A.* Immunocompetent gerbils infected with $2 \times 10^3$ sheathed larvae.

*Group B.* Immunosuppressed gerbils (protocol 1) infected with $2 \times 10^3$ sheathed larvae.

*Group C.* Immunosuppressed gerbils (protocol 1) infected with $6 \times 10^3$ sheathed larvae.

*Group D.* Immunosuppressed gerbils (protocol 2) infected with $2 \times 10^3$ sheathed larvae.

*Group E.* Immunosuppressed gerbils (protocol 3) infected with $2 \times 10^3$ sheathed larvae.

*Group F.* Immunosuppressed gerbils (protocol 1) infected with $2 \times 10^3$ exsheathed larvae.

*Group G.* Immunocompetent gerbils that remained uninfected.

#### *Parasite recovery and sampling*

Ten days post infection (PI), the animals were anaesthetized with isoflurane and whole blood was collected for haematological and biochemical analyses. The stomach was removed, washed externally with 10 ml of warm distilled water (37°C), opened longitudinally in a Petri dish and incubated with 20 ml of 0.9% NaCl at 37°C in a chamber at 37°C for 5 h, following the method of Conder *et al.* (1990). After this period, the stomachs were washed with 0.9% NaCl and the larvae were placed in Falcon tubes with 50 ml of 4% buffered formaldehyde. The parasites were counted using an inverted optical microscope (40×).

#### *Haematological analysis*

Haematological parameters were assessed in whole blood that had been collected in tubes containing EDTA, using an automatic counter (Coulter T890®; Coulter Electronics, Inc., Hialeach, FL, USA). Total leukocytes (WBC), total erythrocytes (RBC), haematocrit (HCT), haemoglobin concentration (HGBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT) were determined. Blood smears were fixed in methanol and were stained with Instant-Prov (NewProv®) stain for differential WBC counts; in these, at least 200 WBCs were counted.

#### *Biochemical analysis*

Blood was collected, and the serum was separated by means of centrifugation (3000 rpm for 15 minutes) and stored at −20°C for biochemical analysis. Serum levels of albumin and total protein (TP) were analysed using Labtest kits (Labtest Diagnostica SA, Vista Alegre, MG, Brazil) through an automatic analyser (CELM SBA 200®; CELM, Barueri, SP, Brazil). Globulin values were obtained by subtracting the albumin from the total protein. All tests were carried out in duplicate.

### In vivo *experiment number 2*

A second experiment was performed using two groups: group A, infected with $2 \times 10^3$ sheathed larvae, and group B, infected with $2 \times 10^3$ exsheathed larvae. Eight six-week-old male gerbils, of body weight 44.5 g, were divided into two groups of four individuals each. The animals were infected orally on day 0. Ten days later, the stomachs were removed for larval recovery.

### In vivo *experiment number 3*

The correlation between the establishment of third-stage larvae of *H. contortus* 15 days PI and body weight was investigated. Seven five-week-old gerbils, males and females, of body weight 35 g, were subjected to immunosuppression using three doses of Depo-Medrol® (Pfizer) (2–4 mg IM, according to body weight) on days −2, −1 and 7 PI. The gerbils were subjected to fasting for 24 hours (18 h prior to infection and 6 h PI), to enhance

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**Table 1.** Mean (± SD) numbers of *Haemonchus contortus* larvae recovered from the stomachs of gerbils on day 10 post infection (PI), and mean (± SD) body weights pre-infection (day 0) and 10 days PI. Groups: A, infected with $2 \times 10^3$ larvae; B, infected with $2 \times 10^3$ larvae and immunosuppressed (protocol 1); C, infected with $6 \times 10^3$ larvae and immunosuppressed (protocol 1); D, infected with $2 \times 10^3$ larvae and immunosuppressed (protocol 2); E, infected with $2 \times 10^3$ larvae and immunosuppressed (protocol 3); F, infected with $2 \times 10^3$ larvae (exsheathed) and immunosuppressed (protocol 1); G, uninfected. N/A, not applicable.

| Groups (n = 4) | Mean number of worms (± SD) | Mean weight (± SD) | |
|---|---|---|---|
| | | Day 0 | Day 10 |
| A | 169.25 ± 40.63[a] | 39.5 ± 6.6 | 49.02 ± 6.63 |
| B | 43.24 ± 29.06[b] | 40.0 ± 5.41 | 51.02 ± 5.25 |
| C | 184.75 ± 56.94[a] | 35.5 ± 6.80 | 43.07 ± 3.98 |
| D | 247.0 ± 30.11[a] | 39.5 ± 8.69 | 49.1 ± 7.0 |
| E | 442.5 ± 57.81[c] | 39.5 ± 8.38 | 37.82 ± 8.72 |
| F | 0.75 ± 1.5[b] | 34.0 ± 8.48 | 42.1 ± 6.5 |
| G | N/A | 40.6 ± 7.19 | 49.75 ± 4.71 |

Different letters indicate significantly different mean values ($P < 0.05$).

the chances of infection by $2 \times 10^3$ sheathed larvae on day 0. The animals were sacrificed on day 15 PI in an isoflurane chamber and their stomachs were removed and washed. The larval content of their stomachs was saved as described in the section *Parasite recovery and sampling*, with minor modifications.

### *Data analysis*

For *in vivo* experiment 1, data were compared using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test. For experiment 2, the Mann–Whitney test was used to compare means. In experiment 3, Pearson's correlation coefficient was used to determine the strength of the correlations among the variables: number of larvae recovered and body weight of the gerbils. All analyses were performed with the significance level taken to be $P < 0.05$.

## Results

### In vivo *experiment number 1*

The number of parasites recovered during the necropsy, and the body weight of the gerbils on days 0 and 10 are shown in table 1. A higher number of parasites was found in group A than in group B. Groups B and F had significantly fewer parasites than groups A, C and D. The highest number of parasites was recovered in group E (using the immunosuppressive drug Depo-Medrol® and sheathed larvae), and the lowest number of parasites was found in group F (using exsheathed larvae and immunosuppressed animals). All the gerbils had higher body weight 10 days PI except those in group E, which were treated with methylprednisolone. This drug possibly reduced the mean body weight.

Table 2 shows the mean red and white blood cell counts and biochemical parameters of gerbils infected by *H. contortus* and the control group (uninfected). For group E, significant alterations to RBC, HCT, PLT, MCHC, WBC, lymphocyte and neutrophil counts can be observed. There were also significant differences in TP and total globulins in all groups, compared with the uninfected control group (group G), with the highest alterations in group E (treated with methylprednisolone). Other parameters of the red blood series (HGBC) and white blood series (rod neutrophils, eosinophils and monocytes) were also measured (data not shown).

### In vivo *experiment number 2*

More worms were recovered from gerbils infected by sheathed larvae (189.33 ± 126.26) than from those infected by exsheathed

**Table 2.** Mean (± SD) red and white blood cell counts and biochemical indicators of gerbils with or without infection by *H. contortus* on day 10 PI. Groups: A, infected with $2 \times 10^3$ larvae; B, infected with $2 \times 10^3$ larvae and immunosuppressed (protocol 1); C, infected with $6 \times 10^3$ larvae and immunosuppressed (protocol 1); D, infected with $2 \times 10^3$ larvae and immunosuppressed (protocol 2); E, infected with $2 \times 10^3$ larvae and immunosuppressed (protocol 3); F, infected with $2 \times 10^3$ larvae (exsheathed) and immunosuppressed (protocol 1); G, uninfected.

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| Red blood cells | | | | | | | |
| RBC (× $10^6$/µl) | 6.6 ± 0.4[a] | 6.7 ± 0.8[a] | 6.8 ± 0.5[a] | 6.5 ± 0.7[a] | 4.2 ± 0.3[b] | 6.7 ± 0.4[a] | 6.39 ± 0.32[a] |
| HCT (%) | 36.2 ± 1.7[a] | 36.6 ± 4.1[a] | 37.7 ± 1.6[a] | 36.1 ± 2.8[a] | 23.2 ± 1.3[b] | 36.7 ± 1.2[a] | 38 ± 1.84[a] |
| PLT (× $10^3$/µl) | 45.2 ± 5.1[a] | 47.2 ± 20.8[a] | 40.0 ± 8.1[a] | 64.0 ± 24.5[a] | 185.2 ± 25.5[b] | 48.25 ± 12.0[a] | 53.5 ± 14.84[a] |
| MCV (fl) | 54.3 ± 1.8[a] | 54.4 ± 1.3[a] | 54.9 ± 2.11[a] | 55.9 ± 2.4[a] | 54.8 ± 1.6[a] | 55.4 ± 1.5[a] | 55.4 ± 1.5[a] |
| MCHC (%) | 37.1 ± 0.9[a] | 35.9 ± 1.0[a] | 36.6 ± 1.7[a] | 36.9 ± 1.3[a] | 52.4 ± 2.8[b] | 35.5 ± 0.1[a] | 30.22 ± 0.29[c] |
| White blood cells | | | | | | | |
| WBC (× $10^3$/µl) | 4.6 ± 0.9[a] | 3.8 ± 1.9[a] | 3.3 ± 0.42[a] | 2.2 ± 1.2[a] | 17.2 ± 7.1[b] | 1.7 ± 0.3[a] | 3.7 ± 2.01[a] |
| Lymphocytes (%) | 71.5 ± 6.8[a] | 63.2 ± 5.9[a] | 68.5 ± 5.8[a] | 65.0 ± 6.4[a] | 91.7 ± 2.0[b] | 64.5 ± 4.4[a] | 72 ± 1.82[a] |
| Neutrophils (%) | 25.7 ± 6.6[a] | 34.0 ± 5.4[a] | 28.5 ± 6.5[a] | 32.7 ± 6.7[a] | 6.2 ± 1.9[b] | 35.2 ± 2.7[a] | 25 ± 1.41[a] |
| Biochemical indicators | | | | | | | |
| Total protein (mg/dl) | 5.5 ± 0.4[a] | 5.2 ± 0.6[a] | 5.3 ± 2.1[a] | 6.1 ± 0.3[a] | 9.0 ± 2.2[b] | 5.5 ± 0.13[a] | 4.82 ± 0.26[c] |
| Total globulins (mg/dl) | 2.7 ± 0.2[a] | 2.5 ± 0.3[a] | 2.8 ± 0.8[a] | 2.9 ± 0.5[a] | 6.2 ± 2.9[b] | 2.7 ± 0.23[a] | 2.22 ± 0.09[c] |
| Albumin (mg/dl) | 2.8 ± 0.2[a] | 2.7 ± 0.3[a] | 2.4 ± 1.2[a] | 3.1 ± 0.2[a] | 2.8 ± 0.8[a] | 2.8 ± 0.2[a] | 2.6 ± 0.18[a] |

Each treatment was compared with the control. The letters compare means in the columns, and different letters indicate significantly different mean values ($P < 0.05$).

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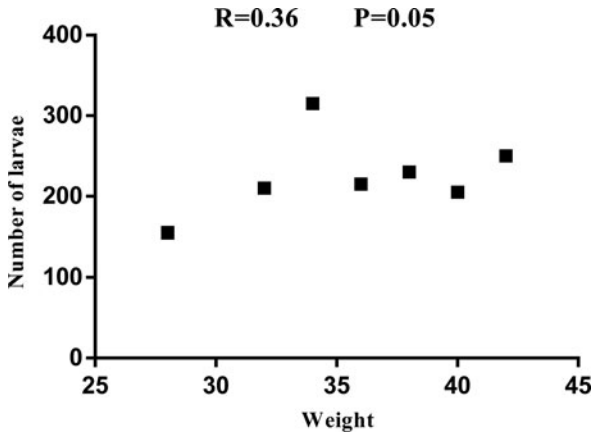


**Fig. 1.** Relationship between the number of larvae recovered and the host body weight (g).

larvae (7 ± 2.64). However, due to high variability this result was not statistically significant.

### In vivo *experiment number 3*

In the third experiment, the mean number of larvae recovered 15 days PI was approximately 225. There was a weak correlation (R = 0.36) between the number of larvae recovered and the body weight of the gerbil. However, no significant differences were found ($P = 0.05$) (fig. 1).

## Discussion

Over the last two decades, a large number of scientific studies on nematodes have been conducted using *M. unguiculatus* as the experimental model. Many protocols for infecting gerbils with *H. contortus* can be found in the literature, and some of them are compiled in table 3. The high number of worms recovered in the present study shows that infection became established in immunocompetent animals, as also demonstrated by Ostlind *et al.* (2006), Rojas *et al.* (2006) and Squires *et al.* (2010, 2011). For unknown reasons, we found significantly more parasites in immunocompetent gerbils (group A) than in those subjected to immunosuppression using dexamethasone (group B). However, using the same *H. contortus* isolate as in the present study, Grando *et al.* (2016) recovered an average of 44.0 worms from gerbils 12 days PI while using the same protocol as used for group B in our study.

We found that the main glucocorticoids (GC) used in gerbils to improve *H. contortus* infection were dexamethasone and hydrocortisone (table 3). Machado *et al.* (2006) evaluated gerbils infected by *H. contortus* and *Trichostrongylus colubriformis* using 4 mg of methylprednisolone per animal (Depo-Medrol®; Pharmacia) every 21 days. The gerbils were sacrificed 58 days PI for recovery of adult worms, which encouraged us to compare the activity of methylprednisolone and dexamethasone. According to Machado

**Table 3.** Protocols used to infect gerbils with *H. contortus* over the last two decades.

| Reference | Sex | Age | Weight (g) | Immunosuppression | Protocol | Number of larvae | Sheathed larvae | Mean number of worms |
|---|---|---|---|---|---|---|---|---|
| Grando *et al.* (2016) | M/F | 8–9 weeks | 35–40 | Yes | A | 2000 | Yes | 44.0 |
| Macedo *et al.* (2015) | M/F | 5 weeks | 25–35 | Yes | B | 5000 | No | 363.2 |
| Ribeiro *et al.* (2013) | M/F | ± 7 weeks | 30–34 | Yes | C | 4500 | No | 171.8 |
| Königová *et al.* (2012) | Not informed | Not informed | 60–65 | Yes | D | 1000 | Yes | 157.1 |
| Squires *et al.* (2011) | Female | ± 5 weeks | 50 | No | _ | 600 | No | 97.1 |
| Squires *et al.* (2010) | Female | ± 5 weeks | 50 | No | _ | 600 | No | 78.25 |
| De Jesús-Gabino *et al.* (2010) | M/F | ± 5 weeks | 20–25 | Yes | F | 40000 | No | 78.0 |
| Königová *et al.* (2008) | Not informed | 6–8 weeks | 60–68 | Yes | E | 1000 | Yes | 92.14 |
| Ostlind *et al.* (2006) | M/F | ± 5 weeks | 30–34 | No | _ | 500 | Yes | 135.08 |
| Rojas *et al.* (2006) | Male | 22 days | 14 | No | _ | 1000 | Yes | 145 |
| Molento & Prichard (1999) | Female | Not informed | 30–35 | Yes | G | 1000 | No | 109.66 |

Protocols for immunosuppression: A, Dexamethasone (Azium®, Coopers Animal Health), 0.2 mg per animal, 3 days before and 2 days after infection; B, Hydrocortisone (Azium®, Schering-Plough Labs), 0.2 mg per animal, 2 days before infection; C, Dexamethasone (Azium®, Coopers Animal Health) 0.2 mg per animal, 3 days before infection; D, Hydrocortisone, 6 mg per animal, 7 days before infection and every day after infection; E, Hydrocortisone, 6 mg/kg, 7 days before infection and every other day after infection; F, Hydrocortisone (Azium®, Shering-Plough Labs), 100 µl per animal, 2 days before infection; G, Hydrocortisone 0.02% in the feed, 5 days before infection and during maintenance of the infection.

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*et al.* (2006), methylprednisolone reduced the bio-nutritional efficiency of the gerbils, such that treated animals showed significantly lower performance than untreated animals. This finding corroborates the reduction in mean body weight observed in the animals treated with methylprednisolone in our study.

Larval exsheathment is a critical part of the process of experimental infection (Macedo *et al.*, 2015). However, only a few studies have investigated the establishment of *H. contortus* infection in gerbils by means of larvae exsheathed using sodium hypochlorite (Conder *et al.*, 1990; De Jesús-Gabino *et al.*, 2010; Squires *et al.*, 2010; 2011; Ribeiro *et al.*, 2013; Macedo *et al.*, 2015). Conder and Johnson (1996) reported that none of the *in vitro* exsheathing media, including sodium hypochlorite, was optimal for parasites, and that they appeared to reduce larval viability. However, among the exsheathment media used, the best infection rate was achieved using carbon dioxide for exsheathment. To standardize a larval migration inhibition test, Demeler *et al.* (2010) used exsheathed *Cooperia oncophora* larvae and found significantly fewer viable larvae, and migration rates as low as 50%, compared with the use of sheathed larvae. We observed that exsheathed larvae of *H. contortus* showed lower motility. Even though we selected the ones with highest motility to infect the gerbils, it was not possible to have a satisfactory rate of parasite recovery in both experiments. The use of exsheathed larvae resulted in decreased establishment of worms in experiment 2, thus supporting the results of the first experiment.

In relation to the immune response, GC caused lymphopenia, affecting T lymphocytes through inhibiting the Th1 response and, especially, the Th2 recruits and activating cells responsible for IgE production (Larini, 2008). Furthermore, GC induces neutrophilia and eosinopenia and reduces the number of macrophages (Pereira *et al.*, 2007). Because of the inhibitory effect of GC, it facilitates dissemination and establishment of infectious agents, including parasites such as *H. contortus*. In our study, infected gerbils that had undergone immunosuppression using methylprednisolone showed higher numbers of leukocytes, despite also showing lymphocytosis and neutropenia. Concerning the haematological and biochemical parameters, strong thrombophilia was observed even with increased total globulins, due to increased synthesis of hepatic proteins as an adverse effect of GC administration (Freitas & Souza, 2007).

It is known that the effect of corticosteroids on mucosal mast cells, mast cell proteinases and eosinophils, and on the antibody response, is capable of influencing B cell and T-helper cell responses (Ziam *et al.* 1999). According to Amorim *et al.* (2010), gerbils infected by *Giardia duodenalis* showed specific IgA faecal antibodies and serum levels of $IgG_1$ and IgM, 7 days PI. However, the antibody levels decreased as soon as immunosuppression induced by methylprednisolone acetate was started (Amorim, 2008).

In addition to IgE, other immunoglobulins may perform important functions towards protecting the host against larvae (Tizard, 2014), thereby hampering larval establishment in gerbils. Therefore, one explanation for the higher number of larvae recovered from methylprednisolone-immunosuppressed gerbils is that this occurred through reduction of the humoral response due to increased plasma levels after GC administration, as a result of high doses of methylprednisolone administered over a short period of time (Pereira *et al.*, 2007).

Our study provided additional evidence that weaned gerbils at an age of approximately five weeks appear to be an acceptable alternative for use as an experimental model, as they showed body development, which contributes towards immunosuppression and/or infection. The decision regarding which methodology should be used may be influenced by other factors, such as the parameters that will be analysed in the research and the influence of GC on them. We highlight that in the first and second experiments it was possible to recover a satisfactory number of worms using sheathed larvae and immunocompetent animals. Use of methylprednisolone increased the number of parasites recovered, compared with untreated gerbils or those receiving dexamethasone. Infected gerbils that had been subjected to immunosuppression using methylprednisolone showed alterations to haematological and biochemical parameters, along with poor performance. A considerable larval burden was recovered from the immunosuppressed gerbils 15 days PI, and body weight did not influence establishment of larval infection.

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**Conflict of interest.** None.

**Ethical standards.** This study was approved by the Ethics Committee for Animal Research (CARE) of the Federal University of Santa Maria (UFSM), under protocol numbers 3768260515/2015 and 3787160917/2017.

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