


# Evaluation of Parasitological Homeopathic Complex in the Control of Gastrointestinal Nematodes in Peripartum Sheep

Poliana Araújo Pacheco<sup>1,2</sup> Michael Nunes Marconato<sup>1,2</sup> Letícia Liepkaln dos Reis<sup>2,3</sup>  
Luciana Morita Katiki<sup>2</sup> Cláudia Cristina Paro de Paz<sup>2</sup> Ricardo Lopes Dias da Costa<sup>2</sup> 

<sup>1</sup> Capes Fellowship

<sup>2</sup> Instituto de Zootecnia (IZ/APTA/SAA), Rua Heitor Penteado 56, Nova Odessa, SP, 13.380-011, Brazil

<sup>3</sup> FAPESP Fellowship

Address for correspondence: Ricardo Lopes Dias da Costa, Instituto de Zootecnia, Rua Heitor Penteado 56, Nova Odessa, SP 13380-011, Brazil (e-mail: rldcosta@iz.sp.gov.br).

Homeopathy

## Abstract

**Background** The appearance of anthelmintic resistance has made it difficult to control verminosis in sheep, leading to increased research to find alternative nematode control. The use of homeopathy in veterinary medicine has been studied as an alternative for the treatment and control of some diseases. In this study, ewes received an anti-parasitic homeopathic complex medicine during the critical peripartum period of increased susceptibility to nematodes.


**Methods** Three randomized groups containing 16 animals each were assigned as follows: 'H10' received 10 g homeopathic complex added to concentrated food per day; 'H20' received 20 g homeopathic complex added to concentrated food per day; 'C' (control group) did not receive a homeopathic complex. Animals were tested to evaluate the effect of homeopathy on several health parameters during a period of 110 days.

**Results** The parasite that prevailed in the copro-cultures of both treatments throughout the experiment was *Haemonchus contortus* (78.26%). Packed cell volume averages did not present statistically significant differences between the treatments (24.5, 24.4 and 23.9% to C, H10 and H20, respectively;  $p < 0.05$ ). For total white cell count, lower mean values ( $\pm$  standard error of mean) were observed for the H20 treatment ( $5,490.9 \pm 0.02/\mu\text{L}$ ;  $p < 0.05$ ), whereas the H10 ( $5,919.4 \pm 0.02/\mu\text{L}$ ) and control ( $6,098.5 \pm 0.02/\mu\text{L}$ ) presented higher and similar averages ( $p > 0.05$ ). The values for erythrocytes, monocytes, and lymphocytes did not show differences between treatments ( $p > 0.05$ ). Body weight was greater in the H10-treated animals compared with control. For the fecal egg count (FEC) of *Trichostrongylidae* and *Strongyloides* spp., respectively, the averages of the H20 treatment (1,523.0 and 30.6) were not different from control (1,616.0 and 31.6) and H10 (1,038.0 and 27.6); for *Trichostrongylidae*, however, H10 presented a lower FEC than the control ( $p = 0.02$ ). For *Cooperia*, H10- and H20-treated animals showed FEC reductions of 97% and 98%, respectively.

**Conclusions** H20 treatment in peripartum sheep resulted in greater body weight and lower leukocyte count. H10 was associated with lower FEC for *Trichostrongylidae*. Both H10 and H20 justify a label of 'effective anthelmintic' for *Cooperia*.

## Keywords

- hatchability
- parasitic infection
- homeopathy
- sheep
- peripartum

 Ricardo Lopes Dias da Costa's ORCID is <https://orcid.org/0000-0001-8888-6915>.

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## Introduction

Sheep are directly affected by worms that lead to major losses in productivity. Verminosis is associated with increased anthelmintic resistance due to the indiscriminate use of medicines.<sup>1</sup> In grazing or confinement regimens, sheep are susceptible to infection by helminths that cause large-scale damage.<sup>2</sup> This susceptibility to infection is related to factors such as age, nutritional status, genetic resistance, breed, and what is known as the peripartum phenomenon.<sup>3</sup>

In the peripartum period, which extends from the final third of gestation to the end of lactation, a reduction in immunity causes increased susceptibility of sheep to nematode infections, creating the peripartum phenomenon.<sup>4</sup> This is most evident between the second week before delivery and the second week after delivery and is characterized by an increase in the elimination of eggs in the feces of pregnant and lactating ewes, which is attributed to increased parasite load during this period.<sup>5</sup> This increase is associated with low immunological status and increased nutritional requirements for fetal development and lactation. In all animal phases and categories, the most commonly used strategy to control verminosis is the use of anthelmintics, which are costly and can induce drug resistance.<sup>6</sup>

The control of parasites should be approached considering the diversity that involves animal production, and considering issues that are a growing concern and related to the environment and animal welfare, which imply the search for solutions and implementation of sustainable production systems.<sup>7</sup>

The search for alternative methods to the use of chemical products presents an opportunity for recovery of production, reduction in anthelmintic resistance, and incremental value to products from such systems that seek sustainable alternatives,<sup>8</sup> such as products considered to be healthier by consumers. In this context, homeopathy constitutes a new approach that has been investigated, potentially to increase the options of alternative control of verminosis in sheep.

Research on the performance of homeopathy on verminosis has been the subject of recent studies, and some authors have suggested that homeopathy stimulates the immune system of organisms so that, using their own means of defense, they fight various infections.<sup>9</sup> The use of homeopathy is considered a way to minimize the use of medications in general.<sup>10</sup> In addition, homeopathic therapy used in production animals leaves no residue after treatment,<sup>11</sup> which is an advantage for producers who use it and for consumers' health.

Homeopathic therapy allows all animal species to be treated based on the individualization of cases. In the case of a herd, it may be considered as a single organism and can thus be treated homeopathically according to the principles of individual treatment<sup>10</sup>; that is, in this case the 'individual' would be the herd or flock. Studies on the use of homeopathy for parasitic control are scarce, but a large portion of existing work demonstrates positive results for the herd.<sup>10–12</sup>

The objective of this study was to evaluate, through measurement of indices of their general health status and parasite load, whether the supply of commercial homeopathic supplementation to sheep would provide an increase

in their ability to resist the detrimental effect of gastrointestinal helminths.

## Materials and Methods

### Ethical Aspects

The experiment was registered with the Ethics Committee on Animal Experimentation (CEUA/IZ—no. 222/15; **—Supplementary File 1**, available in online version only). This committee is institutional, but it is governed and inspected by the National Council for the Control of Animal Experimentation, under the responsibility of the Ministry of Science, Technology and Innovation, at the national level.

The present research was veterinary in nature. All animals used in the experiment are part of the Sheep Unit of the Animal Science Institute and, after the project, they returned to the breeding system according to the management of the farm.

The animals were housed in clean, dry, collective stalls with good ventilation and sufficient space to move freely through the pen. Good food, clean water, and mineral salt were offered before, during, and after the experiment. As a health check, all animals were clinically examined periodically.

### Animals and Location

In the present study, 48 females of the Santa Inês ( $n = 21$ ) and Morada Nova ( $n = 27$ ) breeds, in the final third of gestation and lactation, naturally infected, were kept in confinement at the Sheep Unit of the Institute of Zootecnics in Nova Odessa/SP (22° 27' S, 47° 10' W; 560 m mean altitude) from December 2015 to March 2016. The climate type is tropical at altitude and semi-humid, with dry winters and a south-east wind, and average annual rainfall of 1370 mm.

The animals were grouped into blocks (three blocks of 16 animals each), according to the initial values of weight, packed cell volume (PCV) and eggs count per gram of feces (fecal egg count, FEC). The treatments were assigned to animals randomly and this randomization was done within each block (**—Table 1**).

The sheep were kept in 3 m × 15 m collective stalls and received fresh water in automatic drinking fountains, mineral salt for sheep, sorghum silage, and commercial food concentrate in troughs.

The homeopathic complex, a powder formulation, was added to the concentrate in the amounts of 10 g/ewe/day for the 'H10' treatment and 20 g/ewe/day for the 'H20' treatment. The control group received the concentrate without the homeopathic complex. The components of the anti-parasitic and anti-anemic Minerphós homeopathic complex (Minerphós, Paraná/Brazil) are listed in **—Table 2**.

Among the compounds used in the complex formulation, *Bunostomum* sp., *Haemonchus* sp., *Nematodirus* sp., *Oesophagostomum* sp., *Ostertagia ostertagi*, *Strongyloides* sp., *Trichostrongylus* sp., and *Trichuris* sp. were of particular interest because they are gastrointestinal nematodes, with special attention to *Haemonchus* sp., *Strongyloides* sp., and *Trichostrongylus* sp., as they are the most prevalent in the region where the study took place.

**Table 1** Distribution of experimental groups and respective values (means  $\pm$  SD) of weight, packed cell volume (PCV), and egg count per gram of feces (FEC) at start of experiment in relation to the amount of homeopathic complex provided

Group	Homeopathy (g/sheep/day)	Weight (kg)	PCV (%)	FEC
C	0	38 $\pm$ 8.5	26.3 $\pm$ 3.20	384 $\pm$ 279.7
H10	10	39 $\pm$ 11.0	26.4 $\pm$ 2.80	400 $\pm$ 316.2
H20	20	40 $\pm$ 13.7	26.4 $\pm$ 3.01	381 $\pm$ 237.2

Abbreviation: SD, standard deviation.

Means followed by distinct letters in the same column differed by Tukey's test ( $p < 0.05$ ).

**Table 2** Composition of the anti-parasitic and anti-anemic Minerphós homeopathic complex, in 100 g

<i>Artemisia abrotanum</i>	CH9	0.04 mL
<i>Anidrido arsenioso</i>	CH12	0.04 mL
<i>Calcarea carbonica</i>	CH9	0.04 mL
<i>Boophilus microplus</i>	CH9	0.04 mL
<i>Bunostomum</i> sp.	CH6	0.04 mL
<i>Damalinia ovis</i>	CH6	0.04 mL
<i>Dermatobia hominis</i>	CH6	0.04 mL
<i>Ferrum metallicum</i>	CH9	0.04 mL
<i>Haematobia irritans</i>	CH6	0.04 mL
<i>Haemonchus</i> sp.	CH6	0.04 mL
<i>Linognathus stenopsis</i>	CH6	0.04 mL
<i>Musca domestica</i>	CH6	0.04 mL
<i>Nematodirus</i> sp.	CH6	0.04 mL
<i>Oesophagostomum</i> sp.	CH6	0.04 mL
<i>Oestrus ovis</i>	CH6	0.04 mL
<i>Ostertagia ostertagi</i>	CH6	0.04 mL
<i>Strongyloides</i> sp.	CH6	0.04 mL
<i>Trichostrongylus</i> sp.	CH6	0.04 mL
<i>Trichuris</i> sp.	CH6	0.04 mL
<i>Sulfur</i>	CH9	0.04 mL
Calcitic limestone vehicle	q.s. 100 g	

Abbreviations: CH, Hahnemannian centesimal (dilution scale used); <http://www.minerphos.com.br/>; q.s., quantum satis or quantum sufficit.

A pre-blend of the homeopathic complex, mineral salt, and concentrate was made for better homogenization. After this preparation, the pre-mix was placed in the feed mixer along with the concentrate for 30 minutes. The concentrate with the homeopathic complex was prepared to last no more than a week.

Feeding was performed twice a day (9 AM and 4 PM), with daily feed and left-overs being weighed. The mixture (concentrate + homeopathic complex) was offered in the amount of 500 g/ewe/day, before the silage was offered. The bulky food was offered in quantities calculated to allow 10% to 20% left-over; whenever less than 10% or more than 20% remained, the quantity offered was readjusted for this interval: that is, the food offered was increased or decreased,

respectively, according to the consumption of the previous day.

During the experiment, samples of the food offered and left-overs were used for bromatological analysis, in which the percentage of total dry matter (DM), crude protein (CP), and crude fat (CF) of silage and concentrate were measured.<sup>13</sup>

The mean values of DM, CP, and CF of the silage were 28.12%, 7.69% and 2.15% and of the concentrate were 88.86, 16% and 1.94%, respectively, according to chemical analyses<sup>14</sup> performed at the Food Science Laboratory of the Animal Science Institute.

The dates of birth (lambing) were recorded to provide more information on the peripartum period and possible physiological changes related to the immunity of the ewes.

The ewes were followed for 110 days from the beginning to the end of the experiment. Data collection was performed every 14 days, when the ewes were weighed, body condition was assessed, blood was collected, and feces were collected. Those responsible for clinical examinations of the animals for measuring the clinical outcome were blinded to the original group allocation of the animals. The analyses were performed at the Clinical Analysis and Parasitology Laboratory of the Animal Science Institute, where parasitological and hematological examinations were performed.

All weighings were done using electronic scales, and the body condition score was determined by palpating the lumbar region, assigning scores from 1 to 5, where 1 represents a cachectic animal and 5 represents an obese animal.<sup>15</sup> To allow for variations, intervals of 0.5 points were admitted (1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5; 5).<sup>16</sup>

Individual stool specimens were obtained directly from the rectal ampulla in plastic bags, identified, and packed in polystyrene boxes until the sample was sent to the laboratory for parasitological examination. Copro-parasitological examinations comprised FEC, according to the modified Gordon and Whitlock technique,<sup>17</sup> as described by Ueno and Gonçalves<sup>18</sup>; culture of feces and obtaining nematode larvae for later identification of genera<sup>19</sup>; and larval hatchability tests (see also below), performed according to Coles et al,<sup>20</sup> modified by Bizimenyera et al.<sup>21</sup> These examinations were performed on days 54, 68, 82, 96, and 110 of the experiment.

The eggs recovered from the feces were transferred to a beaker and measured into 30  $\mu$ L aliquots containing approximately 100 eggs, which were placed in each well of the plate,

totaling six wells per treatment. The plates were incubated for 24 hours at 27°C and relative humidity > 80%, after which the egg and larvae counts (L1) were performed under an inverted microscope, with the results of the quantifications being the percent inhibition of hatchability for each treatment.

The quantification of the genera *Moniezia* and *Eimeria* was achieved by assigning crosses to the presence or absence of the genus in the feces.

Blood samples were collected, following jugular venipuncture of all animals, in vacuum tubes (5 mL) containing potassium ethylene-diamine-tetra-acetic acid to perform a complete blood count and to make blood smears for identification and differential count of neutrophils, lymphocytes, eosinophils, monocytes, basophils, and rods.<sup>22</sup>

The experiment was conducted with repeated measures (date of measurement for the variables weight, PCV, FEC) and a treatment factor (doses of homeopathic complex: control [0 g], H10 [10 g] and H20 [20 g]). The other variables were analyzed using a logistic model considering the Poisson distribution. As no basophils were found in the three treatments, the data are not presented in the results. To test the comparison of averages, the Tukey test was used, considering 5% probability as significant.

Egg counts per gram of feces (FEC) and copro-culture were analyzed using RESO FECRT analysis software, version 2.0,<sup>23</sup> using the Eq.  $100(1 - T/C)$ , where T is FEC count in the treated group and C is FEC count in the control group at the same time, to determine the efficacy of the tested homeopathic product. Although this program is frequently used in the evaluation of medications, the purpose of the software is to evaluate the reduction in egg counts in feces, which allows it to be used to evaluate the efficacy of a homeopathic formulation. In this program, the product is considered effective if there is a minimum of 95% reduction in the FEC of the treated group in relation to the control, and if the confidence level is greater than 90%: that is, the product is effective if it reduces FEC by 95%, with  $p < 0.10$ .<sup>23</sup>

## Results

The means for live weight of the ewes after 110 days did not present statistical differences ( $p = 0.51$ ) between the H10 treatments ( $40.6 \pm 1.04$  kg) and control ( $39.0 \pm 1.04$  kg), whereas in the H20 treatment ( $44.2 \pm 1.04$  kg) the means were higher than the other treatments (control,  $p = 0.0012$ ; H10,  $p = 0.04$ ) (►Table 3).

The animals' body condition scores were similar throughout the experiment. PCV averages did not present statistically significant differences between treatments ( $p = 0.38$ ).

The averages of hematological variables are presented in ►Table 4. The neutrophils, lymphocytes, eosinophils, and monocytes are within the reference standards of the species; only erythrocytes had averages below the reference values.<sup>24</sup> The values of erythrocytes ( $p = 0.16$ ), monocytes ( $p = 0.20$ ), and lymphocytes ( $p = 0.23$ ) did not show differences between treatments.

**Table 3** General mean and standard errors, of the experimental period, of weight and packed cell volume (PCV) and means and standard deviation of body condition score (BCS)

Treatment	Weight (kg)	BCS	PCV (%)
C	$39.0 \pm 1.04^a$	$2.0 \pm 0.14$	$24.5 \pm 0.33$
H10	$40.6 \pm 1.04^a$	$2.0 \pm 0.13$	$24.4 \pm 0.33$
H20	$44.2 \pm 1.04^b$	$2.0 \pm 0.15$	$23.9 \pm 0.33$

Abbreviations: H10: 10 g homeopathic complex, H20: 20 g homeopathic complex, and C: control.

Note: Means followed by distinct letters (<sup>a</sup>, <sup>b</sup>) in the same column differed by Tukey's test ( $p < 0.05$ ).

For the white cell count (total leukocytes), a lower mean was observed for H20 treatment ( $p = 0.0007$  and  $p = 0.04$ , compared with controls and H10, respectively), whereas H10 and the control presented similar averages ( $p = 0.42$ ). However, the mean number of eosinophils in the H20 treatment group was lower in relation to that in the other groups ( $p = 0.03$ ), whereas H20 supplementation decreased ( $p = 0.03$ ) the mean for neutrophils ( $3313.5 \pm 0.03/\mu\text{L}$ ) in relation to the control ( $3650.8 \pm 0.03/\mu\text{L}$ ). The mean neutrophils for H10 ( $3468.9 \pm 0.04$ ) did not differ from the other treatments ( $p = 0.30$ ).

All three groups had the same baseline levels of FEC (*Trichostrongylidae*) at the start of the experiment (►Table 1). In FEC of *Trichostrongylidae* and *Strongyloides* spp., presented in ►Table 5, the averages of the H20 treatment were not different from control ( $p = 0.74$  and  $p = 0.93$ , respectively) and H10 ( $p = 0.07$  and  $p = 0.76$ , respectively); however, H10 presented a lower mean to *Trichostrongylidae* than the control ( $p = 0.02$ ). The same cannot be verified for the egg count of *Strongyloides* spp., which had similar mean FEC in the control treatment as in the H10 treatment ( $p = 0.65$ ). Although the supplementation of H10 for sheep in peripartum decreased the egg count of *Trichostrongylidae* combined by 35% (all genera together—*Haemonchus*, *Trichostrongylus*, *Strongyloides*, *Oesophagostomum* and *Cooperia*), it was not effective against *Haemonchus*.

The average of the copro-cultures in all collections showed that the genus *Haemonchus* was the most prevalent for all treatments (77–80%). The genera that appeared in smaller amounts, compared with *Haemonchus*, were *Trichostrongylus*, *Oesophagostomum*, *Strongyloides*, and *Cooperia* (►Table 6).

In the hatchability test, the mean percentages for the H10 and H20 treatments and the control to all collections were 82.3%, 80.7%, and 89.5%, respectively. ►Figure 1 shows the hatchability percentages of eggs collected from the H10, H20, and control groups.

The analysis using the RESO software made it possible to determine the efficacy expressed in percentage (►Fig. 2). The results obtained showed that there were reductions in the counts of *Cooperia* eggs in the H10 and H20 treatment animals of 97% and 98%, respectively, with percentages of efficacy comparable to effective conventional formulations

**Table 4** Means, standard errors, and reference values of erythrocytes, leukocytes, neutrophils, lymphocytes, monocytes, and eosinophils of the animals during the experiment

Treatment	Red cells (x10 <sup>6</sup> /μL)	Leukocytes (/μL)	Neutrophils (/μL)	Lymphocytes (/μL)	Monocytes (/μL)	Eosinophils (/μL)
C	7.7 ± 0.018	6098.5 ± 0.021 <sup>a</sup>	3650.8 ± 0.03 <sup>b</sup>	1756.3 ± 0.03	98.0 ± 0.08	499.9 ± 0.06 <sup>a</sup>
H10	8.1 ± 0.014	5919.4 ± 0.023 <sup>a</sup>	3468.9 ± 0.04 <sup>ab</sup>	1727.0 ± 0.05	107.8 ± 0.12	520.8 ± 0.06 <sup>a</sup>
H20	7.8 ± 0.015	5490.9 ± 0.022 <sup>b</sup>	3313.5 ± 0.03 <sup>a</sup>	1630.0 ± 0.03	85.5 ± 0.08	423.2 ± 0.06 <sup>b</sup>
Reference values	9–15	4000–12000	400–6100	1600–9000	0–750	0–1000

Note: Means followed by distinct letters (<sup>a</sup>, <sup>b</sup>) in the same column differed by Tukey's test ( $p < 0.05$ ). Reference values.<sup>6</sup>

**Table 5** Mean and standard errors of egg count per gram of feces of *Trichostrongylidae* and *Strongyloides* spp. observed in treatments H10, H20, and control

Treatment	<i>Trichostrongylidae</i>	<i>Strongyloides</i> spp.
C	1616 ± 0.11 <sup>b</sup>	31.6 ± 0.21
H10	1038 ± 0.14 <sup>a</sup>	27.6 ± 0.23
H20	1523 ± 0.13 <sup>ab</sup>	30.6 ± 0.25

Abbreviations: H10 and H20, 10 g and 20 g of homeopathic complex added to the concentrate, respectively.

Note: Means followed by distinct letters in the same column differed by Tukey's test ( $p < 0.05$ );.

**Table 6** Mean percentage of infective larval genera (L3) of nematodes obtained after fecal culture and identification in all collections of treatments H10 and H20 (10 g and 20 g of homeopathic complex added to concentrate, respectively) and control

Larvae (L3)	H10 (%)	H20 (%)	C (%)
<i>Haemonchus</i>	77.22	77.55	80
<i>Trichostrongylus</i>	14	15.44	11.66
<i>Cooperia</i>	3.44	1.88	1.55
<i>Oesophagostomum</i>	3.11	2.55	3.55
<i>Strongyloides</i>	2.22	2.55	3.22

to reduce FEC of this species. Regarding the *Haemonchus* species, no efficacy was observed in any of the groups treated with the homeopathic complex.

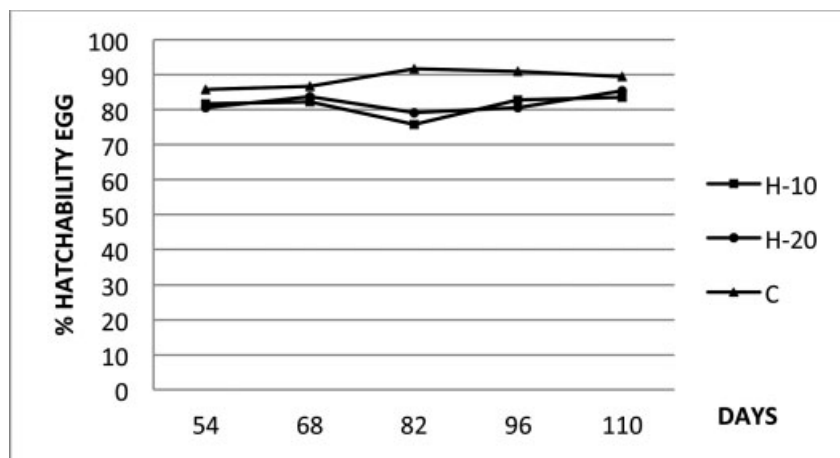
Regarding *Strongyloides* and *Oesophagostomum*, 49% efficacy rates were observed for the H10 treatment and 28% for the H20 treatment. For the *Trichostrongylus* genus, the H10 treatment showed a 27% egg reduction capacity. When all species were considered together, there was a reduction of 35% and 6%, respectively, for H10 and H20.

## Discussion

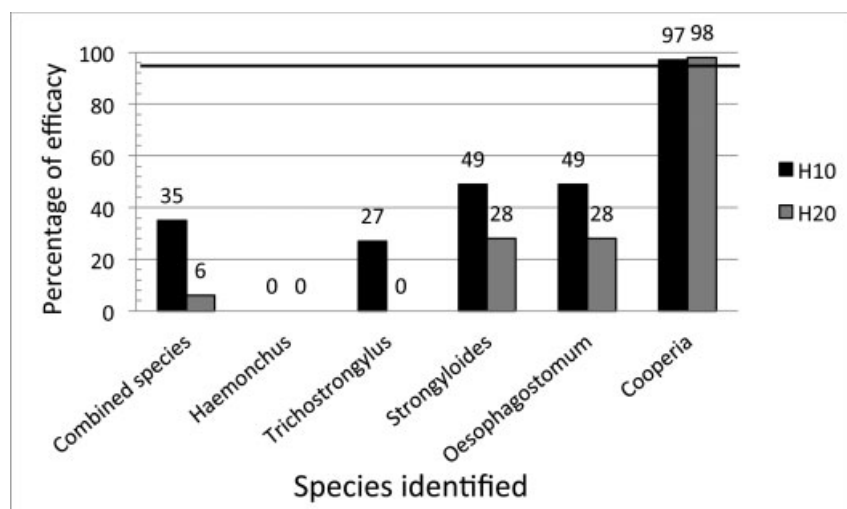
One of the biggest problems limiting sheep production in tropical regions is infection by gastrointestinal parasites, mainly *Haemonchus contortus*, especially during the peripartum period. When animals' resistance decreases, problems with verminosis are even more pronounced, resulting in a decrease in production and high mortality.<sup>25</sup>

The increased susceptibility to gastrointestinal nematodes in sheep in the final third of gestation and lactation (peripartum period), promoted by the peripartum phenomenon, leads to the alteration of some physiological parameters, identified through fecal and blood tests that are performed simultaneously to guarantee more accurate diagnosis of the degree of parasite infection.<sup>4,12</sup>

During the experimental period, all sheep had a PCV lower than reference values for the species.<sup>26</sup> This fact

**Fig. 1** Percentage of hatchability of eggs collected from H10 (10 g of homeopathic complex), H20 (20 g of homeopathic complex) and control groups (C).





**Fig. 2** Percentage of efficacies of homeopathic treatments H10 and H20 (H10: 10 g homeopathic complex, H20: 20 g homeopathic complex) obtained through analysis using RESO software.

can be related to several factors such as the gestational period in which the sheep were included in the experiment, since, in the period corresponding to peripartum (start of experiment), the increase in the nematode parasite load (including hematophagous worms) is more pronounced,<sup>4</sup> causing detrimental effects such as losses in blood volume.

In addition to the PCV, the erythrocyte values for both treatments remained below the reference values for the species and may be associated with the hormonal changes that influence erythropoiesis.<sup>27</sup> In the present study, *Haemonchus* spp. showed a high frequency of hemorrhagic parasites, with a loss of approximately 0.05 mL of blood per day, either by direct ingestion or extravasation of blood by lesions in the abomasum. This was identified in the copro-culture of both treatments, and is a relevant factor to clarify the values of hematocrit and erythrocytes below the reference values.

The supply of homeopathy in cases of parasitic infection may assist the response of the animal body to the effects of nematodes. In a study<sup>12</sup> investigating the effects of administering homeopathic medication to sheep, the results showed a mean weight gain in treated animals. This weight gain<sup>12</sup> was higher than control animals treated with anthelmintic, but the weight difference was not statistically significant.<sup>12</sup> This is contrary to the results of the present study, which showed that the mean weight of H20-treated animals was statistically different from the mean weight of control animals.

Regarding the values for white blood cells, no differences were observed in the variables, lymphocytes and monocytes, but there was a difference observed in neutrophil means between the H20 treatment and control: there was a higher number of circulating neutrophils in the blood of the animals of the group that did not receive the homeopathic complex medicine. This indicates more pronounced immune responses in untreated animals, since neutrophils are cells involved in non-specific immune responses and are the first to migrate to injured sites<sup>28</sup>: i.e., abomasum and intestine. The literature also reports that helminths are able to activate<sup>28</sup> neutrophils on initiation of the inflammatory response, thus inducing the

activation and proliferation of other leukocytes.<sup>29</sup> However, the immune defense is not performed exclusively by neutrophils, since eosinophils also act on helminths directly through the degranulation and release of products such as superoxide and hydrogen peroxide.<sup>30</sup>

In this study, the mean eosinophils of the H20 group were lower than the average of the other groups. Eosinophilia is related to infections caused by parasites that cause tissue invasion,<sup>31</sup> as is the case of *H. contortus*, which was the parasite most frequently observed in copro-cultures of all treatments throughout the experiment. The lower frequency of the parasites *Trichostrongylus* spp. and *Oesophagostomum* spp. compared with *H. contortus* in the co-cultures of all treatments during the experiment is in agreement with other studies in the literature.<sup>8</sup>

The observed difference in FEC of trichostrongylids between the control treatment and H10, for which the H10 group was lower, shows an effect of this level of homeopathy on this species (trichostrongylids). The present work did not evaluate whether the effect was on the number of adult worms or on the oviposition. In addition to the lower FEC counts observed for the treatment with the lowest level of homeopathy, in the hatch test the H10 treatment presented the lowest percentage of eggs incubated, also indicating a possible treatment action on the potential of hatching eggs.

The routine use and improper dosage of anthelmintics has induced resistance to several active ingredients available on the market,<sup>32</sup> making it necessary to use new control systems, as well as better handling techniques.<sup>33</sup> Thus, homeopathy could be a solution. However, one of the great challenges of this work was to select sheep in the peripartum period, which has already been described as the most complicated phase in relation to gastrointestinal parasites in sheep. Likewise, *H. contortus* is the most pathogenic and frequent parasite found in tropical environments, as was the case with this project. Thus, it is possible to consider that lower FEC values (even small differences), or a decrease in hatchability of eggs, would be promising for ovine production, since it is known that more

than 95% of these parasites are present in pastures. Therefore, a decrease in the frequency of *H. contortus*, lower counts of trichostrongylids eggs, and lower hatchability could reduce pasture infestation, which could bring benefits to long-term sheep rearing.

The efficacy observed in the H20 and H10 treatments for *Cooperia* has led to their classification as anthelmintic formulations that are effective against this parasitic genus. Paradoxically, however, this species is not represented in the formulation of the homeopathic complex used. One of the possible explanations is that the species studied belong to the same phylogenetic group. According to the results, none of the treatments was effective against the genus *Haemonchus* (►Fig. 2), which was the most prevalent from the beginning to the end of the experiment (77%) compared with the other parasites.

Reasons for the differing findings between our H10 and H20 groups are not clear. The variable weight of the animals, for example, which was higher for the H20 treatment, was probably due to a lower milk production in that group: that is, a lower nutritional requirement resulting in a faster weight recovery. However, since we did not evaluate this parameter, it remains only a hypothesis. Regarding the decrease in total leukocytes and eosinophils in H20, the cause may be related to a greater migration of these defense cells to the site of infection and, consequently, a decrease in their general blood circulation.<sup>25</sup>

## Conclusion

Administration of 20 g homeopathic complex per day to peripartum sheep (H20 group) led to greater body weight and lower leukocyte count, whereas 10 g of complex (H10 group) resulted in lower FEC for *Trichostrongylidae*. Both H10 and H20 were effective anthelmintics for *Cooperia*.

### Highlights

- The appearance of anti-helminthic resistance has made it difficult to control verminosis in sheep and has raised interest in alternative nematode control research.
- Research on the performance of homeopathy in verminosis is the subject of recent studies.
- The supplementation of 10 g of a homeopathic complex (H10) in peripartum sheep decreased the egg count of *Trichostrongylidae* spp.
- The efficacy observed in the H10 and H20 treatments for *Cooperia* justifies the classification of these homeopathy formulations as effective against this parasitic genus.

## Supplementary File 1 Ethics Committee letter

Conflict of Interest  
None declared.

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