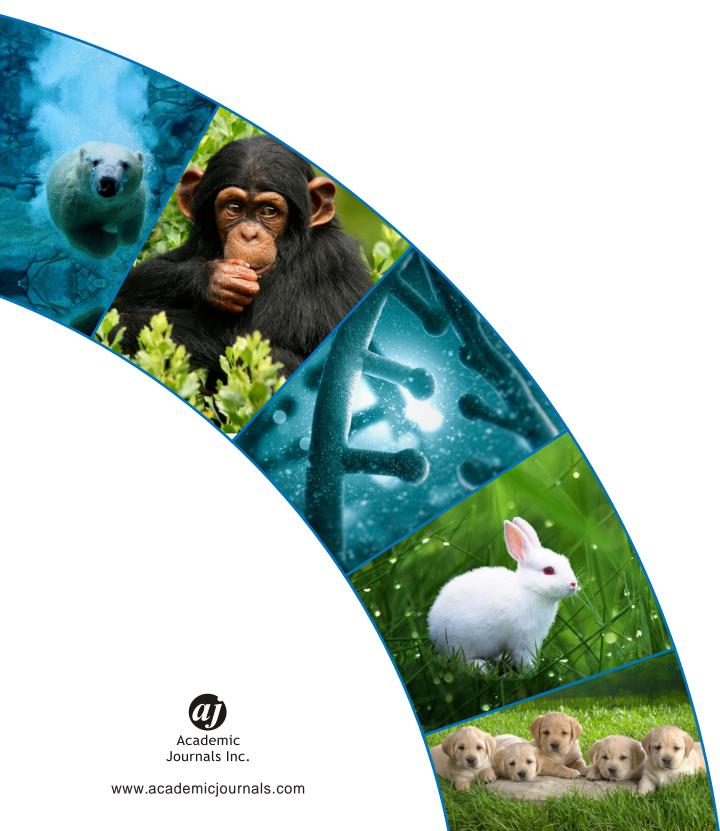
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# Research Article Homeopathy as an Alternative to Control of Verminosis Ewes

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# **Abstract**

**Background:** Verminosis is the main limiting factor in sheep production. Its control has become more and more difficult because of resistance of gastrointestinal helminths to some active ingredients, which has led to search of alternative ways to verminosis control. Based on that, the goal of study was to evaluate the effect of homeopathy for verminosis control in sheep. **Materials and Methods:** Twenty eight sheep in lactation stage without defined breed were evaluated for seven months. The animals were separated into two groups, control and homeopathy, according to counting of helminths Eggs Per Gram (EPG) of feces. Each animal from homeopathy group was treated with Verm® 100 at rate of 10 g day¬¹, mixed at 250 g day¬¹ of corn residue. The control group received 10 g of calcium carbonate mixed at 250 g of corn residue. Helminths eggs per gram of feces, Famacha®, body condition score, hematocrit, hemoglobin, counting of eosinophils, total protein, albumin, globulin and aminotransferase aspartate were evaluated every 21 days. The data were analyzed by variance analysis and Tukey's test at 5% of significance level. **Results:** After the 4th month of treatment, EPG from homeopathy group was significantly lower than the control group (p<0.05) and it had negative correlation with number of eosinophils. There were normal values for both groups regarding to other parameters evaluated: Hematocrit, hemoglobin, globulin, total protein, albumin, aminotransferase aspartate, body condition score and Famacha®. **Conclusion:** These results suggest that homeopathy may be used as an alternative method for control of verminosis in sheep. And using it combined with the traditional system could be a way to minimize the use of allopathic medicines, being a resource in organic farms or for those that aim a more sustainable production system.

Key words: Anthelmintic resistance, biochemical analysis, EPG, eosinophils, Famacha®, *Haemonchus* sp., hematocrit, management practices, nematodes, sheep

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Data Availability: All relevant data are within the paper and its supporting information files.

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## **INTRODUCTION**

Gastrointestinal parasites are the main limiting factor in sheep production around the world, causing considerable economic losses in the herds<sup>1</sup>. It has been an issue mostly due to resistance of helminths to several active ingredients, used as anthelmintic, where the indiscriminate and repetitive use of those products have led to selection of resistant population of helminths<sup>2</sup>. The consequences of resistance ally to crescent concern with environment, food security and animal well-being have had impacts on livestock production. As a result, study for technologies to be implemented in production systems to control of verminosis has become an important topic regarding to animal health<sup>3</sup>.

Many researchers from different regions around the world have been looking for alternatives that can reduce the use of anthelmintic products. Homeopathic medicine is one of these alternatives that has been discussed. It has already been used in treatment of diseases in humans and present its efficiency has been verified in veterinary medicine<sup>4</sup>.

Homeopathy has been appointed by several researches as a new method to prevention and control of degenerative diseases, abnormal behavior and pain treatment in company animals<sup>5-7</sup>, infectious and parasitic diseases in small animals<sup>8-10</sup> and farm animals<sup>11-13</sup> and in the improvement of zootechnical indices in animal production<sup>14-18</sup> by collective homeopathy <sup>19</sup>.

There are very few studies about the effectiveness of using homeopathy for verminosis control in sheep, which makes more difficult to analyze, to discuss and to draw conclusion about this theme. Zacharias<sup>20</sup> and Zacharias *et al.*<sup>21</sup> evaluated the homeopathy in lambs and their studies shown a decrease in egg counts per gram feces and higher weight gain of the animals treated with homeopathy. On the other hand, Cavalcanti *et al.*<sup>22</sup>, Chagas *et al.*<sup>23</sup> and Poletto *et al.*<sup>24</sup> did not observe positive effects of homeopathy in their experimental conditions. However, it has to be considered that these studies used different methodologies and active ingredients, what could justify the different results obtained.

Thus, considering that homeopathy use is growing in traditional and organic production systems, the goal of this study was to study the effect of the homeopathic product Verm® 100 for verminosis control in lactating ewes naturally infected by gastrointestinal helminths.

#### **MATERIALS AND METHODS**

**Experimental location:** The experiment was carried out in a commercial production system of ewes, located in

Guarapuava, Paraná State, Brazil. The region's climate is characterized as mesothermal humid subtropical, without dry season, with cool summer and moderate winter according to Köppen's classification. The mean temperature in the coldest month is lower than 18°C and the mean temperature in the warmest month is lower than 22°C. The annual precipitation varies from 1,400-1,200 mm, being April, May and August with the lowest precipitations. The altitude is approximately 1,100 m<sup>25</sup>.

**Animals and experimental procedures:** Twenty eight animals naturally infected by gastrointestinal nematodes were separated into two groups. The first one was the control group and the second one received homeopathy treatment. The animals were randomly assigned into the groups based on the number of eggs of helminths per gram of feces (EPG). The animals did not have defined breed and they were in the third final stage of lactation. The two groups remained separated during the experimental period in a 2 ha area formed by native pasture during summer season and oat (Avena spp.) and common ryegrass (Lollium multiflorum) during winter season. From June, 2012 to January, 2013, the homeopathy group was supplemented with 10 g/animal of Verm® 100 mixed with 250 g/animal/day of corn residue. The control group received 10 g of calcium carbonate mixed with 250 g of corn residue/animal/day.

Evaluations, animal samples collection and statistical analysis: Every 21 days were done evaluation of Famacha<sup>©26</sup>, Body Condition Score (BCS)<sup>27</sup>, collection of feces directly by rectum ampulla to perform EPG<sup>28,29</sup>, coproculture<sup>30</sup> and identification of larvae<sup>31, 32</sup>. The animals that showed EPG above 2,000 or Famacha<sup>©</sup> 3, 4 or 5 were dewormed in the following day of the evaluation with trimix (ivermectine  $200 \,\mathrm{mcg}\,\mathrm{kg}^{-1}$ , levamisole 7.5 mg kg<sup>-1</sup>, albendazole 5 mg kg<sup>-1</sup>). In addition, every 21 days 4 mL of blood were collected by punction of cephalic vein in a 2 mL sterile tube containing anticoagulant ethylene diaminetetraacetic acid potassium (EDTA-K) to perform evaluations of hematocrit (Hct), hemoglobin (Hgb) and counting of eosinophils<sup>33</sup>. Also, 2 mL of blood were collected in a sterile tube without anticoagulant, which were centrifugated to 2,000 rpm for 10 min to obtain serum and then serum dosages of total protein, albumin, aminotransferase aspartate (AST) according to methodology of commercial kits Labtest® to analyzer semi-automatic (Bioplus® 200). The globulin value was obtained by subtraction of albumin from total protein. The data were analyzed by analysis of variance Tukey's test at 5% of significance level with SISVAR software.

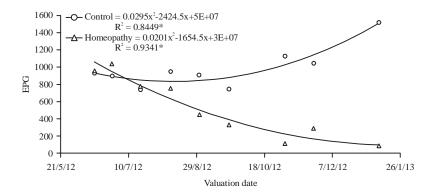


Fig. 1: Number of eggs of helminths per gram of feces (EPG) of homeopathy and control groups during the period of June, 2012 and January, 2013

#### **RESULTS**

The homeopathy and control groups had average of 961 and 932 EPG, respectively. The EPG for the homeopathy group was lower than the control during the experimental period (Table 1), except by the first, second and third evaluations. The groups presented significant difference (p<0.05) after the 4th month using the homeopathic product Verm® 100. During the whole experimental period, eight animals in the control group were dewormed. While only four animals were dewormed in homeopathy group only in the first three months of evaluation.

Based on the results from the coproculture, the control group had predominance in the first three months (June-August) of the following nematode genera: Trichostrongylus (62.2%), Haemonchus (28%), Strongyloides (5.7%) and Oesophagostomum (4%). However, in September, November, December, 2012 and January, 2013, the prevalent genera were Haemonchus (81.8%), Trichostrongylus (16.4%), Oesophagostomum (1.6%) and Strongyloides Similar pattern was observed for the homeopathy group in June-September 2012 with prevalence of genera Trichostrongylus Haemonchus (65.25%),Strongyloides (4.5%) and Oesophagostomum (3%) and only in November, there was inversion between the genera Haemonchus (90.5%) and Trichostrongylus (9.5%). In December and January, 2012, the animals did not show infection, being the EPG below 500 and then the coprocultures were not performed.

There was negative correlation (R = -0.36) for eosinophils and EPG for the homeopathy group. Regarding to the results for Hct, Hgb, total protein, albumin, globulin, AST, body score evaluation and Famacha $^{\circ}$ , the results were similar and within the reference values for sheep in both groups (Table 1).

Table 1: Mean values of hematocrit (Hct), hemoglobin (Hgb), Total Protein (TP), albumin (Alb), globulin (Glob) and aspartate aminotransferase (AST) of control and homeopathy groups found out in the evaluation during the period of June, 2012 and January, 2013

	Treatments		
Variables	Control group	Homeopathy group	Reference values
Hct (%)	35ª	34ª	27-45 <sup>48</sup>
$Hgb (g dL^{-1})$	11.0 <sup>a</sup>	10.5 <sup>b</sup>	9.0-15.048
TP (g $dL^{-1}$ )	7.8 <sup>b</sup>	7.4 <sup>a</sup>	6.0-7.9 <sup>49</sup>
Alb (g $dL^{-1}$ )	2.7ª	2.6ª	2.4-3.0 <sup>49</sup>
Glob (UI L-1)	5.2 <sup>b</sup>	4.8a	3.5-5.749
AST (UI $L^{-1}$ )	120 <sup>a</sup>	121ª	60-208 <sup>49</sup>

Means followed by the same letter do not differ by Tukey's test (p<0.05). Reference values mentioned by Weiss and Wardrop<sup>48</sup> and Kaneko<sup>49</sup>, reference values were described to comparison of the normal range with the results found out in this study for each variable

#### **DISCUSSION**

Lactating ewes compose one of the most susceptible categories to gastrointestinal parasites<sup>34</sup> because of unspecific immunosuppression from endocrine origin caused by hormonal variation close to parturition and during lactation<sup>35</sup>. The animals are predisposed to the development of larvae in hypobiosis, higher establishment of new larvae or also greater reproduction of existent parasites, resulting in increased number of eggs eliminated by feces<sup>36</sup>.

Seasonal variation of species and infestation by parasites due to environmental conditions has already been observed<sup>37</sup>, in which temperatures below 15°C and low precipitation favor the development of Trichostrongylus rather than Haemonchus and other genera. This inversion of predominance of helminths genera occur in this time of the year in some regions of Parana state because the eggs and larvae of *Trichostrongylus* sp. are able to resist better to cold and wet weather<sup>38</sup>, which is common for the region, where the experiment was carried out. Since prevalence of larvae of *Trichostrongylus* sp. was observed from autumn to the end of

the winter and larvae of Haemonchus genus from the end of winter to the spring, with higher infestations in the summer season, the results of this study agree with data from the studies cited above.

There was difference between the treatments during the period of study (Fig. 1). The homeopathy group had reduction of EPG overtime compared to the control group regarding to parasitic infestation. The negative correlation observed for eosinophils in the homeopathy group suggests that eosinophils are important for control of nematodes. It occurs because they are attracted to sites of invasion of helminths by chemical substances released by mastocytes<sup>20</sup>. The activation of an alternative via of complementation and degranulation of mastocytes promotes increase in the unspecific degranulation of tissue eosinophils that release main basic protein, cationic protein and peroxidase, which are cytotoxics<sup>39</sup>, therefore, representing to be an important mechanism to elimination of helminths.

Lambs treated for six months with a homeopathic product composed of ferrum phosphoricum, arsenicum album and calcarea carbonica had negative correlation for eosinophils, which led to suggest that probably the homeopathy would act stimulating the immune response from the host, increasing the number of circulating eosinophils<sup>21</sup>. However, in another study performed for 18 months with one-year-old Morada Nova ewes, no effect was verified for the control of gastrointestinal parasites with a homeopathic product compose of *Bunostomum* sp., *Haemonchus* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Trichuris* sp., *Oestrus ovis*, *Eimeria* sp., *Fasciola hepatica*, *Dermatobia hominis*, *Damalinia caprae*, *Linognathus* sp., *Musca domestica* and *Bixa orellana*<sup>23</sup>.

Lambs evaluated in semi-confined system from the birth until 218 days during the experimental period, demonstrated significant reduction in EPG and number of larvae of *Haemonchus contortus* in coproculture for those animals treated with the homeopathic product ferrum phosphoricum and arsenicum album compared to the control group<sup>20</sup>.

The homeopathic treatment with sulphur, ferrum phosphoricum, arsenicum album and mercurius solubilis, each one applied to a different group test for three months and having a non-treated group as control did not promote reduction in the EPG in the crossbred lambs Santa Inês<sup>22</sup>.

It is important to take into consideration, that the product in this study (Verm® 100) contains in its formulation: *H. contortus* ( $10^{-30}$ ), *Cooperia* sp. ( $10^{-30}$ ), ferrum metalicum ( $10^{-30}$ ), sulphur ( $10^{-30}$ ), *Artemisia maritime* ( $10^{-30}$ ), *Apis mielifera* ( $10^{-30}$ ), China officinalis ( $10^{-30}$ ) and *Spigelia* 

anthelmintica ( $10^{-24}$ ) all of these products are different from those used in the studies cited above, making difficult a direct comparison and analysis of the results.

Despite of that, in the homeopathic medicine the principle of similarity is used<sup>40</sup>, where a complete report of all symptoms of each patient is necessary to individual therapy, indicating that in distinct individuals with the same disease, the homeopathic treatment might be different<sup>41</sup>. Thus, perhaps this is the main challenge in veterinary homeopathic medicine, in which a treatment applied on the herd with only one organism, which could influence the results reported.

The Famacha® method consists in individually evaluating the sheep eye conjunctiva color, which may vary in the scale from 1-5. It correlates the level of anemia due to parasitism by *H. contortus* with Hct and EPG<sup>26,42</sup>. While a Famacha® degree 1 refers to a red mucous and consequently to a healthy animal, a degree 5 refers to almost white mucous, which infers high degree of anemia and high parasitism. Considering that the nematode *H. contortus* is the most common and aggressive parasite in sheep production this method makes possible to treat only those individuals who are in high danger of haemonchosis<sup>26</sup>.

Considering the Famacha® method, where the coloration of the ocular conjunctivas observed and then the degree of anemia is correlated with Hct and EPG42, no significant difference between the groups was observed. Both groups presented average of Famacha® 2.0, which is justified based on the fact that in the coprocultures the prevalence of Trichostrongylus genus were verified. This parasite cause lesions in the anterior small intestine, which are frequently assintomatic, but when in high amounts can cause watery diarrhea, prostant and debilating<sup>43, 44</sup>. Thus, the main clinical symptom is diarrhea due to enteritis and not due to anemia. Similar pattern was verified to Hct in both groups, which average values remained within the normality during the period of evaluation (Table 1). The anemia caused by verminosis may cause lesion on liver because of tissue hypoxia, thereby increasing the values for hepatic enzymes as AST<sup>45</sup>, however, it was not observed in this study.

In homeopathy, heal this seen as a maintenance of "Vital force" and the disease occurs when this force has been compromised. On the other hand, environmental factors can also exert positive or negative influence on the patient<sup>46</sup> and they could interfere in the results found out in the different study previously cited. Considering that hemoglobin, total protein, albumin and globulin summed up to evaluation of corporal score (average of BCS 2.5 in both groups) are important tools to monitoring of nutritional status of the

animals, in this study was verified that all of them had similar values for both groups and within the normal reference values to the specie. It shows that the conditions in the diet of the animals did not differ and therefore, the nutritional situation was not variable to the response to verminosis in those animals.

Since veterinary homeopathic medicine is still very little studied, there are many divergences about it. A systematic review by meta-analysis was performed based on the results of papers referent to alternative control of verminosis in sheep and only two papers were mentioned regarding to homeopathy, suggesting that any conclusion about its efficacy would be precipitated<sup>47</sup>.

## **CONCLUSION**

The homeopathic product controlled the verminosis after the 4th month of evaluation when there was significant difference in the EPG counting combined with the negative correlation in the eosinophils counting. In this study, the homeopathy may be an alternative method for control of verminosis in sheep and it could be used combined with the traditional system as a way to minimize the use of allopathic medicines, mainly in organic farms or for those that aim a more sustainable production system.

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