



Short communication

Anthelmintic efficacy of trichlorfon and blood parameters of young lambs infected with *Haemonchus contortus*

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ABSTRACT

In this study we evaluated the efficacy of trichlorfon against *Haemonchus contortus*, monitoring its influence on blood parameters and plasma enzymes of lambs with haemonchosis. A lamb group was orally treated with trichlorfon at 100 mg kg⁻¹ while the other group was untreated. Split-plot design analysis was performed with the lamb groups defined as plots while the subplots were the four periods (weeks) of collection. The trichlorfon treatment promoted a significant and effective reduction of fecal egg counts after one week, with efficacies > 99%. After 21 days of treatment, detected blood parameters and serum levels of plasma enzymes were normal. Additionally, serum albumin and urea concentrations increased to normal values, which were not observed in untreated lambs. The treatment with this organophosphate, using a correct oral administration, may represent an effective therapeutic alternative for sheep infected with multi resistant strain of *H. contortus*.

1. Introduction

Gastrointestinal nematodes (GIN) are one of the principal sanitary problems of ruminants, promoting significant economic losses, such as mortality, reduction in meat, milk production, and fertility (Vijayasarithi et al., 2016). The control of these GINs in small ruminants has relied on the frequent and indiscriminate use of broad spectrum anthelmintics as benzimidazoles, macrocyclic lactones and imidazothiazole, which promote a rapid selection of multi-resistant nematodes (Vijayasarithi et al., 2016; Chaparro et al., 2017).

As an alternative, other molecules such as the organophosphate trichlorfon (TCF) have also been used since 1950, and current studies report its anthelmintic efficacies > 95% (Vieira et al., 1992; Lopes et al., 2009; Bichuette et al., 2015). However, reports of intoxication have been described for different groups of animals (Dalton et al., 2011; Lopes et al., 2014).

Therapeutic protocols have evaluated the efficacy of oral treatment with TCF with different species and ages of animals. However, blood parameters and monitoring of plasma enzymes have not been adequately reported. In this study, we evaluated the anthelmintic efficacy of TCF and its effects on blood parameters in lambs with haemonchosis.

2. Material and methods

2.1. Efficacy of trichlorfon against *Haemonchus contortus*

We evaluated 20 crossbred Santa Inês male lambs with a mean body weight (bw) of 26.5 kg. After weaning, the 5-month-old lambs with zero fecal egg counts per gram of feces (FEC) on two exams (day-23 and day-22), were orally infected with 2,500 *H. contortus* infective larvae. The McMaster technique of Gordon and Whitlock (1939) was utilized with the addition of saturated sodium chloride at minimum sensitivity of 25 eggs/g of feces. This isolate of *H. contortus* was obtained from lambs raised on a farm located in Montes Claros, Minas Gerais State, Brazil that were treated with albendazole, ivermectin and levamisole. These anthelmintics had an efficacy of < 60%, and *H. contortus* L3 were produced in cultures inoculated with fecal samples obtained from sheep treated with these three anthelmintics (Duarte et al., 2012). The *H. contortus* strain used in the experiment has been maintained in mono-infected lambs and treated with these anthelmintic, since May 2011.

All procedures were performed according to the principles of animal experimentation approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais, Brazil (protocol 42/2008). After 22 days of infection, lambs were divided into two groups of 10 homogeneous animals showing similar FEC means

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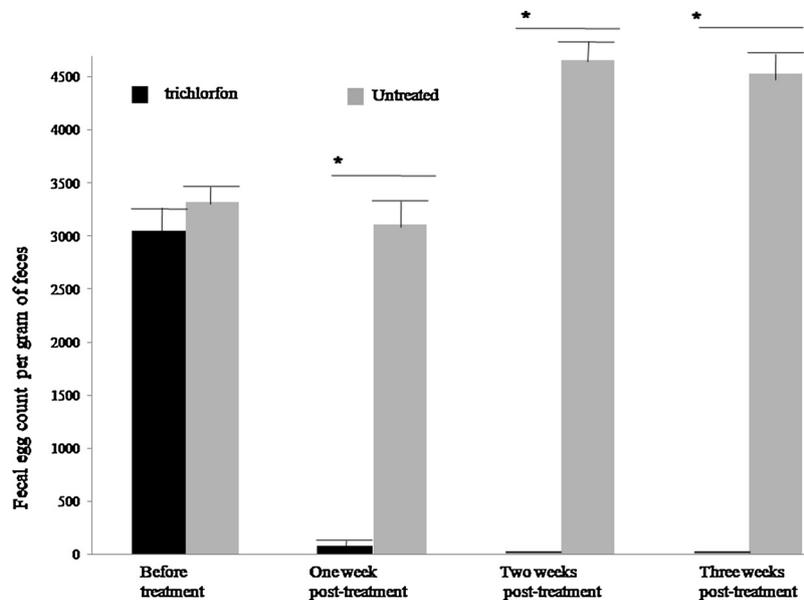


Fig. 1. Mean and standard deviation of fecal egg count (FEC) in lambs infected with *Haemonchus contortus* after oral administration of trichlorfon (100 mg/kg bw) and untreated lambs at different periods of evaluation.

(*) – Represents significant differences between treated and untreated lambs ($P < 0.001$).

(Fig. 1, initial period). The lambs were housed on sand floors and fed sorghum silage meal; a concentrate of corn, soybean, and cottonseed; mineral premix; and water *ad libitum*. The negative control group received no treatment, while the other group was orally administered 100 mg⁻¹kg TCF (Neguvon®, Bayer, Brazil).

We evaluated FECs and fecal cultures over four time periods, at weekly intervals. Each period covered an average of three days, obtaining two FECs each day, totaling 24 FECs per animal in the experimental period, minimizing the daily variation of FEC (Fig. 1). Days -2, -1, and 0 were denoted as the initial period (before treatment); days 7, 8, and 9 (second period and one week post-treatment); days 14, 15, and 16 (the third period and two week post-treatment), and days 21, 22, and 23 (fourth period and three weeks post-treatment). The McMaster technique was utilized as previously reported and larvae were obtained in coprocultures for identification and confirmation of mono-infection by *H. contortus* (Keith, 1953). Lambs were monitored for clinical signs evaluating the color of mucosa according to the Famacha method (Burke et al., 2007) and weighed in the morning before feeding on the day of treatment and on days 7, 14, and 21 post-treatment. The mean weight gain was compared by Tukey's test and $p < 0.05$ was considered significant.

The FEC data obtained were transformed to $\log_{10}(x + 10)$ and subjected to analysis of variance in a split plot design with respect to the four evaluated periods. Means were compared by Duncan's test and $p < 0.05$ was considered significant. Regression analysis was employed to analyze FECs between the periods using the statistical package System for Statistical Analysis and Genetics (SAEG 9.1). The anthelmintic efficacy was determined by a FEC reduction test and the formula was used to determine the percentage efficacy in FEC reduction (Coles et al., 1992):

$$\% \text{ FEC reduction} = 100 \times (1 - \text{mean FEC of treated group} / \text{mean FEC of control group})$$

2.2. Blood analysis

To evaluate possible toxicity, blood samples were collected on days zero, 7, 14, and 21, in the morning after 12 h of fasting. Five animals showing homogeneous FEC (average 3168.66 \pm 326.8) were selected from each group.

Blood samples were collected from the jugular vein in tubes containing ethylenediaminetetraacetic acid (EDTA) and were evaluated in an automatic analyzer of hematology (BC 2.800 Vet, Mindray Medical International LTD, Shenzhen, China). Serum concentrations of total protein, albumin, creatinine, and urea were analyzed using commercial kits (Biolclin, Quibasa Basic Chemistry Ltd., Belo Horizonte, MG, Brazil). For the blood analyses the data also subjected to analysis of variance in a split plot design and mean values were compared using the Student-Newman-Keuls or Scott-Knott tests, using SAEG 9.1. $p < 0.05$ was considered significant.

Alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (GGT) were quantified using the enzymatic kinetic method with semi-automatic equipment (BioPlus2000® - Bioplus Products LTD Laboratories - Barueri SP, Brazil). These data were non-parametric and were submitted to Kruskal-Wallis using SAEG 9.1. $p < 0.05$ was considered significant.

3. Results

A significant interaction between the treatments and the collection period for FEC analyzes was detected (Fig. 1). The use of TCF promoted a significant and effective reduction of the FEC average after the one, two and three weeks post-treatment, with efficacies $> 99.0\%$ ($p < 0.01$, Fig. 1). Additionally, untreated lambs showed higher FEC means in the second and third week post-treatment than initial period and the first week ($p < 0.05$). Coproculture exams confirmed the mono-infection by *Haemonchus* sp. in all lambs, during all evaluated periods. This treatment did not influence the bw 21 days after treatment (31.15 ± 0.67 kg for treated lambs and 28.13 ± 0.842 kg for untreated lambs, $p > 0.05$).

The sheep were physically healthy, showing no signs of sub-mandibular edema, weakness, or inappetence, and ocular mucosa exams showed Famacha® scores between one and two. The oral administration of TCF increased the number of erythrocytes ($12.83 \times 10^6/\mu\text{l}$ \times $11.52 \times 10^6/\mu\text{l}$) and hematocrit values ($37.24\% \times 33.29\%$) after 21 days of treatment ($p < 0.05$, supplementary data).

For both lamb groups, the mean leukocyte count was higher on day zero, while the neutrophile concentration decreased on days 14 and 21 post-treatment ($p < 0.05$, Table 1). Lymphocyte concentrations were higher on days zero and 14, while eosinophil concentrations were

Table 1

Mean values for leukocytes, neutrophils, lymphocytes, monocytes and eosinophils in sheep with haemonchosis treated with trichlorfon (100 mg/Kg bw) or untreated at different periods of evaluation.

Blood parameters/ Lambs groups	Initial period	One week Pos-treatment	Two week Pos-treatment	Three week Pos-treatment	Reference ^a
<i>Leukocytes (μL)</i>					
Trichlorfon	10.100Aa	8.580 Ba	8.860 Ba	8.260 Ba	4.000 - 12000
Untreated	8.460 Aa	7.200 Ba	6.560 Ba	6.460 Ba	
<i>Lymphocytes (μL)</i>					
Trichlorfon	5.408 Aa	3.879Ba	6.163 Ba	4.861 Ba	2.000 - 9.000
Untreated	4.144 Aa	3.816 Ba	4.005 Ba	3.621 Ba	
<i>Neutrophils (μL)</i>					
Trichlorfon	4.618 Aa	4.150 Ba	2.527 Ca	3.202 Ca	700 - 6.000
Untreated	4.231 Aa	3.005 Ba	2.407 Ca	2.730 Ca	
<i>Monocytes (μL)</i>					
Trichlorfon	0.000 Ba	0.310 Aa	0.019 Ba	0.000 Ba	0 - 750
Untreated	0.006 Ba	0.124 Aa	0.019 Ba	0.000 Ba	
<i>Eosinophils (μL)</i>					
Trichlorfon	0.071 Ba	0.119 Aa	0.147 Ba	0.187 Ba	0 -1000
Untreated	0.098 Ba	0.246 Aa	0.125 Ba	0.082 Ba	

Different lowercase letters in columns and capital letters in rows indicate significant differences ($P \leq 0.05$ in Student-Newman-Keuls (SNK) test).

^a Reference range for healthy sheep (Pugh, 2004). Variation coefficient for leukocytes = 14.05%.

greater on day 7 (Table 1, $p < 0.05$), despite being within the reference limits. The basophile population was not influenced by treatment or periods, ranging from 0 to 0.043/ μL . The averages of platelet counts were also similar among sheep groups and assessment periods ($p > 0.05$; general mean = $488.60 \pm 40.86 \times 10^3 /\mu\text{L}$).

While initial values of albumin were low, a progressive recovery for treated lambs was detected, returning to baseline levels after three weeks; unlike that of the untreated group (Table 2). The serum urea concentrations increased in treated lambs after seven days ($p < 0.05$), also returning to baseline levels, while serum creatinine values were similar between animal groups and evaluation periods (Table 2).

The sheep treated with TCF did not exhibit behavior changes, weakness, submandibular edema, whitish eye mucous or lack of appetite during the experiment as compared to untreated lambs. No significant differences in plasma concentrations of AST (82.23 ± 3.97 U/L), ALT (10.1 ± 1.74 U/L), and GGT (52.19 ± 4.84 U/L) were detected among the two lamb groups and the four evaluated periods ($p > 0.05$).

4. Discussion

Control of haemonchosis in small ruminants has been performed with multiple anthelmintic classes; thus, favoring the selection of multi-

resistant nematodes. In this study, we observed a significant reduction (up to 99.0% efficacy) of FEC in lambs infected with a multi-resistant strain of *H. contortus* after oral treatment with TCF at 100 mg/kg bw.

The high anthelmintic efficacy in this study may be due to minimal prior use of TCF to control GIN, resulting in lower selection pressure for the development of resistance. Additionally, it has a different mechanism of action when compared with other anthelmintic agents, minimizing the likelihood of cross-resistance (Lopes et al., 2009; Almeida et al., 2010).

This result suggests TCF is an efficient anthelmintic, according Vercruyse et al. (2001). In another study, a similar result was also reported by Vieira et al. (1992) in ovine species experimentally infected with *H. contortus* (99.9% efficacy for TCF). However, the TCF treatment at 100 mg/kg bw promoted significant reduction, but with a lower efficacy (73%), of adult *H. contortus* numbers in sheep infected with multidrug-resistant strains in Brazil (Almeida et al., 2010).

In sheep orally treated with TCF in Argentina, a lower dose 50 mg/kg was effective (> 99%) in lambs infected with GIN that were resistant to the main anthelmintic groups (Fiel et al., 2011). Future studies should evaluate the efficacy of this lower dose (50 mg/kg), to reduce intoxication risks. This analysis should prioritize goats; because, according to Lopes et al. (2014) and Bichuette et al. (2015), this ruminant seems to show a higher susceptibility of intoxication to the

Table 2

Mean value of protein, albumin, globulin, creatinine and urea to sheep treated with trichlorfon (100 mg/Kg bw) or untreated at different periods of evaluation.

Blood parameters/ Lambs groups	Initial period	One week Pos-treatment	Two week Pos-treatment	Three week Pos-treatment	Reference ^a
<i>Protein (g/dL)</i>					
Trichlorfon	6.654 Aa	5.819 Aa	6.312 Aa	6.072 Aa	6 - 7.9
Untreated	6.516Aa	6.283 Aa	6.352 Aa	6.338 Aa	
<i>Albumin (g/dL)</i>					
Trichlorfon	1.09 Db	1.11 Cb	2.19 Ba	2.55 Aa	2.4 - 3
Untreated	2.91 Aa	1.99 Ba	1.86 Cb	1.9 Db	
<i>Globulin (g/dL)</i>					
Trichlorfon	5.567 Aa	4.709 Aa	4.223 Aa	3.531 Aa	3.50 - 5.70
Untreated	3.593 Aa	4.301 Aa	4.496 Aa	4.753 Aa	
<i>Urea (mg/dL)</i>					
Trichlorfon	15.20 Da	23.41Ca	26.54 Ab	24.01 Ba	17 - 42.8
Untreated	14.00 Da	25.90 Aa	20.01 Bc	16.76 Cb	
<i>Creatinine (mg/dL)</i>					
Trichlorfon	1.086 Aa	1.017Aa	0.988 Aa	1.080 Aa	1.2 - 1.9
Untreated	1.053 Aa	1.023 Aa	0.939 Aa	0.979Aa	

Different lowercase letters in columns and capital letters in rows indicate significant differences ($P \leq 0.05$) in Scott-Knott test.

^a Reference range for healthy sheep (Pugh, 2004; Payne and Payne, 1987).

recommended dose of TCF (100 mg/kg).

Despite the high initial FEC in lambs, the erythrocyte count was within normal limits (Pugh, 2004). The erythrocyte average was greater at 21 days after TCF treatment, which could be because by reduction of the adult hematophagous nematodes, since TCF showed high anthelmintic efficacy (99.8–100%). The high leukocyte count in the initial period for both lamb groups can be justified by abomasitis caused by the nematode. The animals had been infected for 21 days before the start of the experiment, corresponding to the acute phase of haemonchosis. However, the leukograms were all within the reference values (Pugh, 2004).

The neutrophil concentration decreased over the evaluated periods for both groups and the means were within the normal values. For lymphocyte counts, the reduction occurred in the first and third weeks after treatment, while monocyte and eosinophil values increased significantly after seven days. The mean counts of these defensive cells were within the normal range, suggesting that TCF did not promote immune sensitivity or toxicity of treated young lambs.

In the initial period, the lambs with acute haemonchosis showed hypoalbuminemia, which may be associated with abomasitis, promoting the reduction of protein digestion, and consequently, intestinal absorption of amino acids (Bricarello et al., 2004). Surprising, the TCF-treated lambs recovered albumin levels in the third week after treatment and this regularization may be attributed to FEC reduction, associated with an elimination of adult *H. contortus* and improving the health of the abomasum mucosa. In contrast, after the seventh day, untreated lambs presented significant reduction of serum albumin values, concomitantly with an increase of FEC.

We detected that the serum concentration of urea was close to the lower reference limit for healthy sheep. The low concentration of serum urea directly reflects the low amount of protein nitrogen ingested, digested or absorbed may be associated with injuries in the gastrointestinal mucosa (Ribeiro et al., 2004). However, as with albumin levels, the animals treated with TCF recovered normal urea levels after three weeks of treatment, which was not observed for untreated lambs, which could show injuries to the gastrointestinal mucosa.

Treated lambs did not alter plasma concentrations of AST, ALT, or GGT after oral administration with TCF. AST and GGT concentrations were within the normal range for sheep, while the ALT concentration was below the reference value, as reported by Pugh (2004), for both lamb groups.

The use of TCF as an anthelmintic has been limited due to the risk of toxicity (Lopes et al., 2014). However, intoxication by organophosphate in production animals has been associated with errors in dosing, administration in categories of animals not indicated (very young animals), failures in dilution of the product, or administration in doses and routes which are not indicated by the manufacturers (Lopes et al., 2014; Santos et al., 2014).

5. Conclusion

This study points that when a *H. contortus* strain is resistant to conventional anthelmintics, TCF could be an appropriate and effective therapeutic alternative for incorporation into control programs for this nematode in sheep, considering the special care with the correct oral administration.

Statement on the welfare of animals

All procedures were performed according to protocol 42/2008 by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais, Brazil.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetpar.2019.06.015>.

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