

Trichlorfon for Treatment of Cutaneous Habronemosis - Evaluation of Intravenous Regional Perfusion in the Distal Equine Limbs

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ABSTRACT

Background: Equine cutaneous habronemiasis is common in the distal regions of the limbs. Organophosphates, applied systemically, one previously used treatment, which is highly effective, but currently in disuse, due to the risks of intoxication. Regional perfusion is a potential technique for distal limb wounds, since, in addition to being used in low doses, it prevents systemic circulation of the drug and possible intoxication, and has a lower treatment cost. The current work aimed to perform clinical, laboratory, and venography evaluations of the use of trichlorfon in regional intravenous perfusion, as a possible form of treatment for cutaneous habronemosis in the distal region of equine limbs.

Materials, Methods & Results: Twelve equines were used, divided into 2 groups, with the left thoracic limb (LTL) being the test limb, and the right thoracic limb (RTL) the control limb. At moment zero (M0), distal radiography and venography were performed. The tourniquet was then loosened and after 5 min, at moment one (M1), the tourniquet was repositioned for 1.25 mg/kg (G1) and 5.5 mg/kg (G2) trichlorfon injections into the left thoracic limb, diluted in 20 mL of Ringer's lactate solution, and 20 mL of Ringer's lactate solution was applied to the right thoracic limb. The tourniquet was maintained for 30 min after infusion in both groups. At moment 2 (M2), 4 days later, blood tests, radiography, and venography were repeated. Every day between M0 and M2, physical examinations were performed, including measurement of the pastern, fetlock, and coronet band, and a lameness examination. There were no significant alterations in clinical parameters, behavior, and appetite. In the blood cell count, there was an increase in leukocytes on D4 in G2, although remaining within the reference values for the species. The biochemical tests showed no alterations. There were no changes in the circumferences evaluated and 4 equines from G2 presented lameness in the LTL and 1 in the RTL. In the venograms, 1 G2 animal was noticed to be hypoperfused in all LTL plexuses.

Discussion: The use of trichlorfon in regional perfusion at a dose of 1.25 mg/kg demonstrated safety, with no clinical, laboratory, and lameness changes in any of the animals. At the highest dose (5.5 mg/kg), individual reactions were observed, such as different degrees of lameness, swelling, and heat in foot. Serial venographies document the response to treatment used, it was decided to perform the second venography 4 days after the trichlorfon perfusion and the first initial venography evaluation, following the recommendations for the repetition time of the examination and evaluation of the use of the drug. The evaluation of radiographic images of venography, in a grading system created considering the range of contrast in 5 regions by the 3 examiners experienced in podiatry and the analysis of radiographic images of limbs of equines, without having participated in the previous procedures was important for the reliability of the assessment. The areas of hypoperfusion observed in the venography were not related to trichlorfon perfusion. It is essential that the application of trichlorfon be performed correctly, intravenously, to avoid reactions such as necrosis, pain, edema, erythema, lameness, and even more severe inflammatory reactions, such as phlegmon and thrombophlebitis. Although a small number of animals were used in this study, trichlorfon regional perfusion of equine limbs, at a dose of 1.25 mg/kg may be a technique appears to be inert to hoof vascularization.

Keywords: horse, venography, trichlorfon, habronemiasis, perfusion.

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INTRODUCTION

Wounds by habronemiasis are common in the distal region of the limbs, with rapid development and intense itching, even reaching self-injury [21]. Trichlorfon was indicated intravenously for the treatment of habronemosis, in doses of 5, 25, and 22 mg/kg respectively [1,12,22] slowly diluted in 1 to 2 L of 0.9% NaCl solution, intravenously. However, depending on the dose administered, organophosphates can cause intoxication, with muscarinic and nervous signs [4].

Regional perfusion is a well-known method to treat and prevent distal infections of the limbs, because it uses low doses of the drug, it prevents systemic circulation and possible intoxication [6,7]. Some researchers used in regional perfusion a third (1/3) [16] of the recommended dose for systemic use and 25% [6] of the recommended systemic dose of amphotericin B, in the treatment for pythiosis.

Considered not very invasive, venography enables evaluation of the vascular perfusion of the distal end of the equine limb, allowing the observation of occlusions or other alterations that may compromise circulation [15,18,19].

Thus, the present study aimed to perform the technique of regional intravenous perfusion with reduced doses of trichlorfon, and clinical, laboratory, radiographic and venography evaluations, before and after application of the drug, for its subsequent use in clinical cases of cutaneous habronemosis in the distal regions of equine limbs.

MATERIALS AND METHODS

Animals and study design

Twelve equines (*Equus caballus*) males (n = 8) and females (n = 4) were used, with an average body weight of 311 ± 75 kg, considered healthy after clinical and laboratory exams. All animals came from the GDF Apprehension Center and were divided into 2 groups: G1 (equines 1 to 6) and G2 (equines 7 to 12). In all animals, the procedure was performed in both thoracic limbs, with the left thoracic limb (LTL) being the test limb and the right thoracic limb (RTL) the control limb.

The equines were evaluated by complete physical examination and laboratory tests (blood count, fibrinogen, total plasma protein), and bio-

chemicals: urea, creatinine, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and albumin. A lameness test and measurement of the circumference of the metacarpophalangeal joints (fetlock), the region of the proximal phalanges (pastern), and the coronet bands of the right and left thoracic limbs were performed to assess the health condition and the possibility of systemic effects of the trichlorfon. Before the experimental procedure, the equine hooves were trimmed in order to achieve foot balance and reduce artifacts due to poor load distribution.

After a 12-h food fast and a 6-h water fast, sedation was performed with detomidine¹ [Detomidin 1%® - 15 µg/kg], a perineural block in the region of abaxial sesamoids, of the digital palmar nerves, using 5 mL of 2% lidocaine without vasoconstrictor² [Xylestesin 2%®] at each point, in both thoracic limbs, and each limb was positioned on a wooden block for radiography.

At moment zero (M0), radiography and venography of the hoof were performed using the tourniquet in the distal third of the metacarpal. For digital venography, the hoof of each equine was sanitized, trichotomy was performed at the level of the pastern of both thoracic limbs on the medial and lateral face, for puncture of the lateral and medial digital vein, and surgical antisepsis at the venipuncture site with povidone-iodine and alcohol, attaching a tourniquet to the distal third of the metacarpal.

The lateral digital veins of each limb were cannulated with a 21-gauge scalpel for the injection of iohexol-based contrast³ [Omnipaque®], totaling 20 mL of contrast, divided into 2 - 10 mL syringes, in each thoracic limb (Figure 1). Immediately after applying the contrast, the carpus was flexed slightly for 1 or 2 s, releasing the tension from the deep digital flexor tendon, in order to relieve the limb load, and allow the complete filling of the digital vasculature, according to the technique described by Rucker [19]. In a continuous action, the limbs were positioned on a wooden block to perform radiographs with lateromedial and dorsopalmar projections in both thoracic limbs, using portable Raio-x portable equipment⁴ [Poskom PXM-40BT® coupled to a CR 30X AGFA® image capture and digitalization system.]



Figure 1. Lateral digital vein of the cannulated right thoracic limb, with 21-gauge scalpel, and injection of 20 mL of contrast based on iohexol.

The tourniquet was then loosened for reperfusion of the limb and after 5 min, at moment one (M1), repositioned. In the medial vein, in the LTL of G1, an injection of 1.25 mg/kg of trichlorfon (Neguvon®)⁵ was performed, which corresponds to 25% of the 5 mg/kg dose used by Lacerda Neto *et al.* [11], diluted in 20 mL of lactated Ringer, with compression maintained for 30 min after the infusion. In G2, 5.5 mg/kg was administered, corresponding to 25% of the IV dose of 22 mg/kg, recommended by Thomassian [22], which was also diluted in 20mL of lactated Ringer, with the tourniquet maintained for 30 min after the infusion. In the RTL of both groups, 20 mL of lactated Ringer was applied to the medial veins, with the tourniquet maintained for half an hour after infusion. Soon after, the catheter was removed and a compressive bandage was applied with topical povidone iodine, gauze, cotton, and a bandage. The dressing was removed the next day and each equine was monitored for 96 h, with a view to possible clinical intervention in the case of phlebitis. Clinical examination, lameness assessment, hoof heat, and measurement of the circumference of the coronet, pastern, and fetlock were performed during the 4-day follow-up (Figure 2).

At moment 2 (M2), 4 days after the infusion of trichlorfon, biochemical tests, blood count, radiogra-

phy, and venography were performed again to assess venous vascularization at the site of application of the drug and the digit.

Evaluation of radiographic images of venography

As performed by Sales [20], in the evaluation of radiographic images of venography, a grading system was created considering the range of contrast in 5 regions: terminal arch, dorsal laminar vessels to the distal phalanx, coronary plexus, circumflex vessels, and heel bulb. Each of the 5 described regions was quantified with values of 0, 1, or 2 according to the filling of the vessels: 0 (zero) absence of regional filling; 1 partial regional filling (< 50%); and 2 good regional filling (> 50%). For the quantification of the filling of the mentioned regions, 3 examiners experienced in podiatry and the analysis of radiographic images of limbs of equines, evaluated the venograms, without having participated in the previous procedures.

Statistical analysis

The data obtained were analyzed for normality using the Shapiro-Wilk test. The fetlock, pastern, and coronet diameter data were subjected to a Student *t*-test to establish whether the values between the left and right limbs were different before applying the solutions, to rule out the possibility of experimental bias. For both analyses of repeated measures over time, the treatment means were compared by the Tukey test at a 5% significance level. All statistical analyses were performed using the SAS program^{®6}.

The values obtained by analyzing the venograms and assigning scores to the thoracic digits of the equines were submitted to ANOVA statistical analysis, complemented by TUKEY, with a significance level $P < 0.05$ for comparison between all groups.

RESULTS

The use of trichlorfon in the regional intravenous perfusion did not cause any systemic alterations in the animals of the experiment, shown through the clinical exams. In the blood count, a significant increase in the number of leukocytes was observed in G2, on D4, although remaining within the reference standards of the species (Table 1). None of the systemic signs of intoxication by trichlorfon were observed in the equines in the experiment, nor changes in biochemical tests. Regarding the circumference of the metacarpal-phalangeal joint (fetlock), pastern, and coronet, there were no significant differences in the observed values, despite the fact that 4 equines presented lameness in G2.

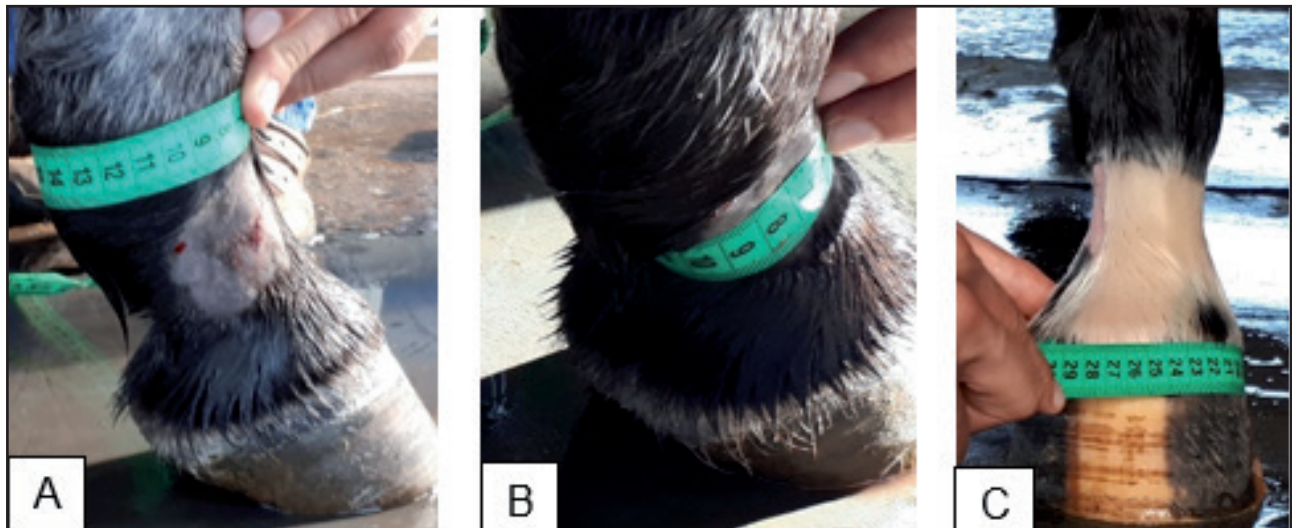


Figure 2. Measurements of the circumferences of A- Fetlock. B- Pastern and C- Coronet, before (D0) and after (D1, D2, D3, and D4) trichlorfon infusion in the LTL and lactate Ringer in the RTL.

Table 1. Mean $\pm$ standard deviation values of hematological data, before (D0) and 4 days after (D4), in 12 equines submitted to regional trichlorfon infusion, using the doses of 1.25 mg/kg (G1) and 5.5 mg/kg (G2).

Variables	Groups	D0	D4
Erythrocytes ($\times 10^6/\mu\text{L}$)	G1	6.33 ± 0.80	6.51 ± 0.79
	G2	5.23 ± 0.80	4.87 ± 0.79
Hemoglobin (g/dL)	G1	11.40 ± 1.33	11.57 ± 1.32
	G2	9.50 ± 1.33	9.35 ± 1.32
Hematocrit (%)	G1	30.17 ± 3.34	30.67 ± 3.30
	G2	28.00 ± 3.34	26.00 ± 3.30
MCV $\ddagger$ (fL)	G1	47.67 ± 2.12	48.00 ± 2.07
	G2	55.00 ± 2.12	55.00 ± 2.07
MCHC $\ddagger$ (%)	G1	37.67 ± 0.86	37.67 ± 0.75
	G2	34.17 ± 0.86	35.17 ± 0.75
Total Protein (g/dL)	G1	7.00 ± 0.22	7.02 ± 0.21
	G2	7.55 ± 0.22	7.75 ± 0.21
Fibrinogen (mg/dL)	G1	366.67 ± 37.92	433.33 ± 31.29
	G2	400.00 ± 41.54	440.00 ± 34.27
Platelets ($\times 10^3$)	G1	225.67 ± 21.21	230.17 ± 20.52
	G2	150.00 ± 23.23	171.20 ± 22.47
Leukocytes ($\times 10^3$)	G1	8.22 ± 0.73	8.07 ± 0.71
	G2	7.26 ± 0.73	$8.41 \pm 0.71^*$

$\ddagger$ MCV: mean corpuscular volume; $\ddagger$ MCHC: mean corpuscular hemoglobin concentration. *Value statistically different from D0.

Venography and intravenous regional perfusion are techniques that require skill from the veterinarian as well as which the equine needs to be well contained, sedated, and a perineural block should be performed. In all animals, these procedures were carried out, however, some setbacks were observed: in equines number 4 and 6 (G1) and number 8 (G2), during the application of the RTL contrast, the scalpel left the lateral vein, it being necessary to perform contrast and lactate Ringer application in the medial vein, with no clinical alterations observed in these animals. In G2, equine number 9 presented heat and sensitivity to touch in the LTL perfusion region and grade 3 (3/5) lameness (according to the American Association of Equine Practitioners classification), between D1 and D3, and grade 4 (4/5) on D4. The RTL presented heat in the region of perfusion and lameness grade 2 (2/5) on D4. Equine number 10 showed sensitivity to touch, heat in the region, lameness of grade 5 (5/5) in the LTL on D1 and sternal decubitus, grade 4 (4/5) on D2 and grade 2 (2/5) on D3, being medicated with phenylbutazone 4.4 mg/kg, via IV, for 2 days and 2.2 mg/kg for 3 more days, as well as heat in the RTL, on D1 and D4. Equines 11 and 12 also presented lameness in the LTL, grade 2 (2/5) on D3 and D1 to D3 respectively, in addition to heat in equine 11 in the LTL, on D1 to D4.

One equine was removed from the experiment, as there was an extravasation of trichlorfon in the LTL

during the infusion, showing lameness of grade 4 (4/5) on D1 and D2 that increased on the following days, and significant effusion and heat in the region in the first days, evolving to distal phlegmon 6 days after the application.

Regarding the circumference of the metacarpophalangeal joint (fetlock), pastern, and coronet, there were no significant differences in the observed values, despite the fact that 4 equines presented lameness in G2.

The venography clarified, on D4, in G1: equine 1: coronet and medial laminar hypoperfusion in both limbs (Figure 3A & 3B). Equine 2 maintained the coronet and medial laminar hypoperfusion observed on D0 in the LTL and in the lateromedial aspect of the RTL, there was a decrease in perfusion in the bulbar, circumflex, and soleal plexuses (Figure 3C & 3D). Equine 3 maintained the minimal alterations in the coronet and medial laminar perfusion in both thoracic limbs observed on D0. Equine 4 presented a decrease in medial and dorsal coronary perfusion on D4 in the RTL (Figure 3E & 3F) and in the LTL coronary medial, dorsal, and in the dorsal laminar vessels. Equine 5 presented no alterations and in equine 6, there was a decrease in perfusion in the bilateral dorsal coronary plexus (Figure 3G & 3H).

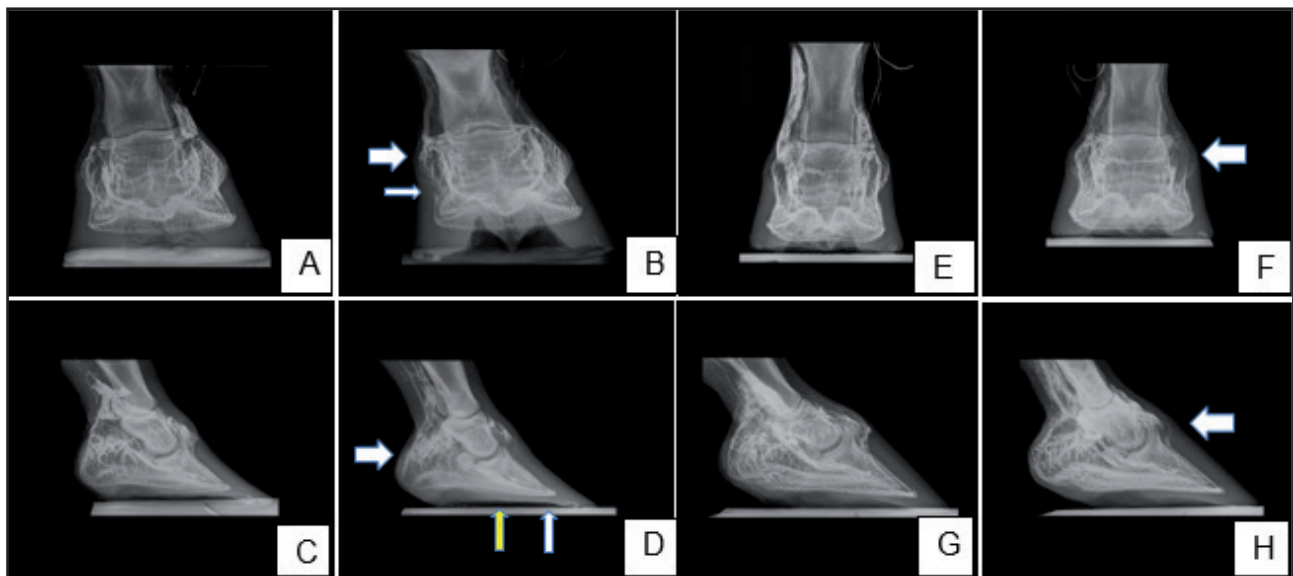


Figure 3. Group G1: (A- D0) and (B- D4) Equine 1: coronary (thick arrow) and laminar (thin arrow) medial hypoperfusion in the LTL; (C- D0) and (D- D4) Equine 2: decreased perfusion in bulbar (thick arrow), circumflex (thin arrow), and soleal plexuses (yellow arrow) in the RTL; (E- D0) and (F- D4) Equine 4: decreased medial coronary perfusion (thick arrow) on D4 in the RTL; (G- D0) and (H- D4) Equine 6: decreased perfusion in the dorsal coronary plexus (thick arrow) in the RTL.

In G2, equine 7 presented lateral coronary hypoperfusion in the RTL and medial in the LTL and equine 8 generalized hypoperfusion in the LTL (Figure 4A & 4B). Equine 9 did not present venography alterations. In equine 10, there was a decrease in dorsal and medial coronary and laminar vascularization in the RTL (Figure 4C & D). There was medial coronary hypoperfusion in the LTL in equine 11. Equine 12 presented coronary and laminar medial and bulbar hypoperfusion in the RTL.

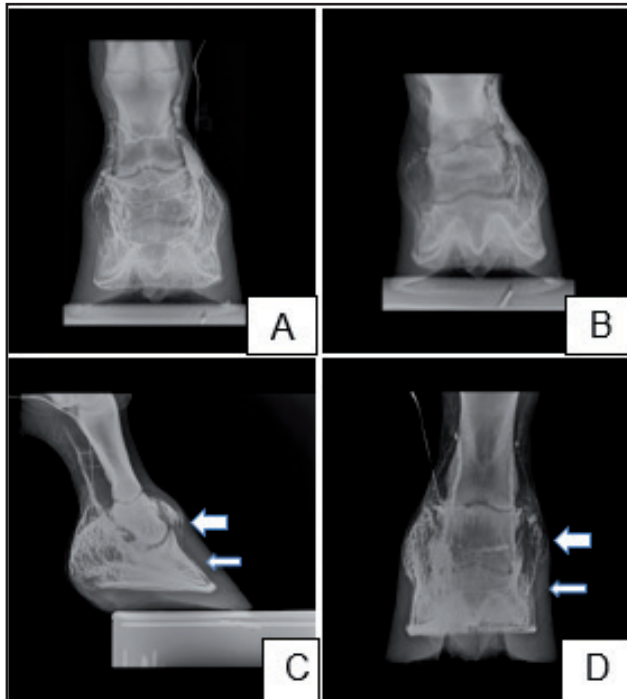


Figure 4. Group G2: (A- D0) and (B- D4) Equine 8: hypoperfusion in all plexuses in the LTL; (C- D4) and (D- D4) Equine 10: there was a decrease in coronary vascularization (thick arrow) and dorsal and medial laminar (thin arrow) in the RTL.

The evaluation of radiographic venography images, using the grading system that considered the range of contrast in each region, did not demonstrate significant differences between groups, limbs, and moments in the analyzed regions, and there were also no differences between the scores awarded between the evaluators.

DISCUSSION

The treatment and healing of distal habronemosis wounds in the limbs of equines is difficult, requiring the study of different treatment modalities and protocols in order to reduce the rate of morbidity and complications.

Regional perfusion favors the effects of the drug, reducing systemic effects, thus, the use of 25% of the systemic dose of trichlorfon, as performed with amphotericin [6], did not cause any systemic alterations in clinical, hematological, and biochemical exams in the animals of the experiment. We chose to use a quarter of the intravenous doses of trichlorfon reported of 5 mg/kg [11] and 22 mg/kg [22], for the treatment of habronemosis, due lower doses reduce the potential risk of systemic toxic effects [14].

Intoxication from formulations belonging to this chemical group have been reported in cattle, buffaloes, sheep, and even in humans, being considered toxic to vertebrates, when they absorb excess organophosphates. Therefore, these formulations are used with restrictions, and should not be administered to pregnant females, animals under 3 months of age, or even animals that are consuming a diet rich in proteins, so that there is no liver overload [13].

Organophosphate poisoning causes systemic signs [13], who used the therapeutic dose of trichlorfon in goats (100 mg/kg orally), and observed ataxia, decubitus, sialorrhea, tremors, pupil constriction, dyspnoea with noise, urinalysis and involuntary defecation, spastic paresis, tympanism, and lacrimation. Organophosphates are anticholinesterase agents, with fat-soluble substances that are easily absorbed throughout the body surface [8].

None of the systemic signs of intoxication by trichlorfon were observed in the equines of the experiment, nor alterations in biochemical tests after the perfusion, thus demonstrating, that regional perfusion prevents systemic circulation of the drug and possible intoxication [9,17].

Tissue distension, due to the extravasation of intravascular drugs in adjacent tissues, depends on the volume of the drug and its properties. To cause harm, the compound must leave its initial area of extravasation and can cause inflammation, heat, erythema, and pain, in addition to the cytotoxic capacity of the drug itself, the wounds can also be related to their physical-chemical properties, such as the pH. The more acidic drugs induce protein denaturation, and coagulation necrosis, and the alkalis are usually linked to liquefaction necrosis, associated with deeper wounds [1,10].

Organophosphates also have myotoxic properties, which can cause necrosis of muscle fibers [3]. Thus, it is essential that the application of trichlorfon be performed correctly, intravenously, to avoid reactions such as necrosis, pain, edema, erythema, lameness, and even more severe inflammatory reactions, such as phlegmon and thrombophlebitis.

The digital blood vessels supply nutrients and remove residues from the digits, in addition to providing the perfusion of substances in the hoof tissues [5]. Venography is a minimally invasive, practical diagnostic method showing the conditions of the vascular system, with good prognostic value, and for monitoring patients, which can be repeated in 3 to 14 days [15,18].

The venous involvement or occlusion observed in the venography results in a reduction or absence of contrast in the affected area. The causes of venous impairment include changes in the load distribution within the hoof, and masses such as keratomas and thrombosis, the extent of vascular alterations being decisive to assess the severity of the condition, assisting in the treatment and prognosis. Serial venographies document the response to treatment used [18,19]. Thus, it was decided to perform the second venography 4 days after the trichlorfon perfusion and the first initial venography evaluation, following the recommendations for the repetition time of the examination and evaluation of the use of the drug.

It was observed that the main areas of hypoperfusion were the coronary plexus and dorsal laminar vessels. However, the decrease in irrigation was not only related to the regional perfusion of trichlorfon in the LTL. In many animals, areas of hypoperfusion were also observed in the RTL, and 2 equines already had areas of hypoperfusion before application (D0). Another fact to be analyzed is that some animals that limped in the LTL, with the highest dose of trichlorfon, such as equines number 9 and 12, did not present venography changes in the digits.

During the injection of contrast into the digital vein, if the limb is fully supported, the vascular filling of the laminar cortex is limited. However, by relieving tension in the deep digital flexor tendon, through slight flexing of the equine's carpus during contrast injection, normal laminar corium filling is observed [2]. It is pos-

sible the observation of many dorsal coronary alterations, without actually having wounds, only artifacts, may be related to this limitation of filling, despite the flexing of the carpus.

The decrease in coronary perfusion results from the uneven weight distribution of the limb [18]. Due to the origin of the equines and the promiscuity in handling the hooves, all had some degree of medial-lateral imbalance that could not be corrected with trimming due to chronicity. For this reason, the concentrations of medial loads generated some level of artifact in the ipsilateral vascular filling. Taking this fact into account, comparisons between D0 and D4 were based on this assumption.

Thus, the observed venography alterations are not elucidating lesions caused by the infusion of trichlorfon, but are possibly due to technical artifacts, irregular filling, imbalance of support in the hooves, and even the injection of perivascular contrast.

CONCLUSIONS

The use of trichlorfon in the regional infusion at a dose of 1.25 mg/kg demonstrated safety, with no clinical, laboratory, or lameness alterations in any of the equines. At the highest dose (5.5 mg/kg), individual reactions were observed, such as different degrees of lameness, swelling, and heat. The hypoperfusion areas observed in the venography were not related to trichlorfon perfusion. Thus, the use of trichlorfon in the regional perfusion of equine limbs, at a dose of 1.25 mg/kg may be a technique that can be indicated in clinical cases of cutaneous habronemiasis, in the distal regions of the equine limbs.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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